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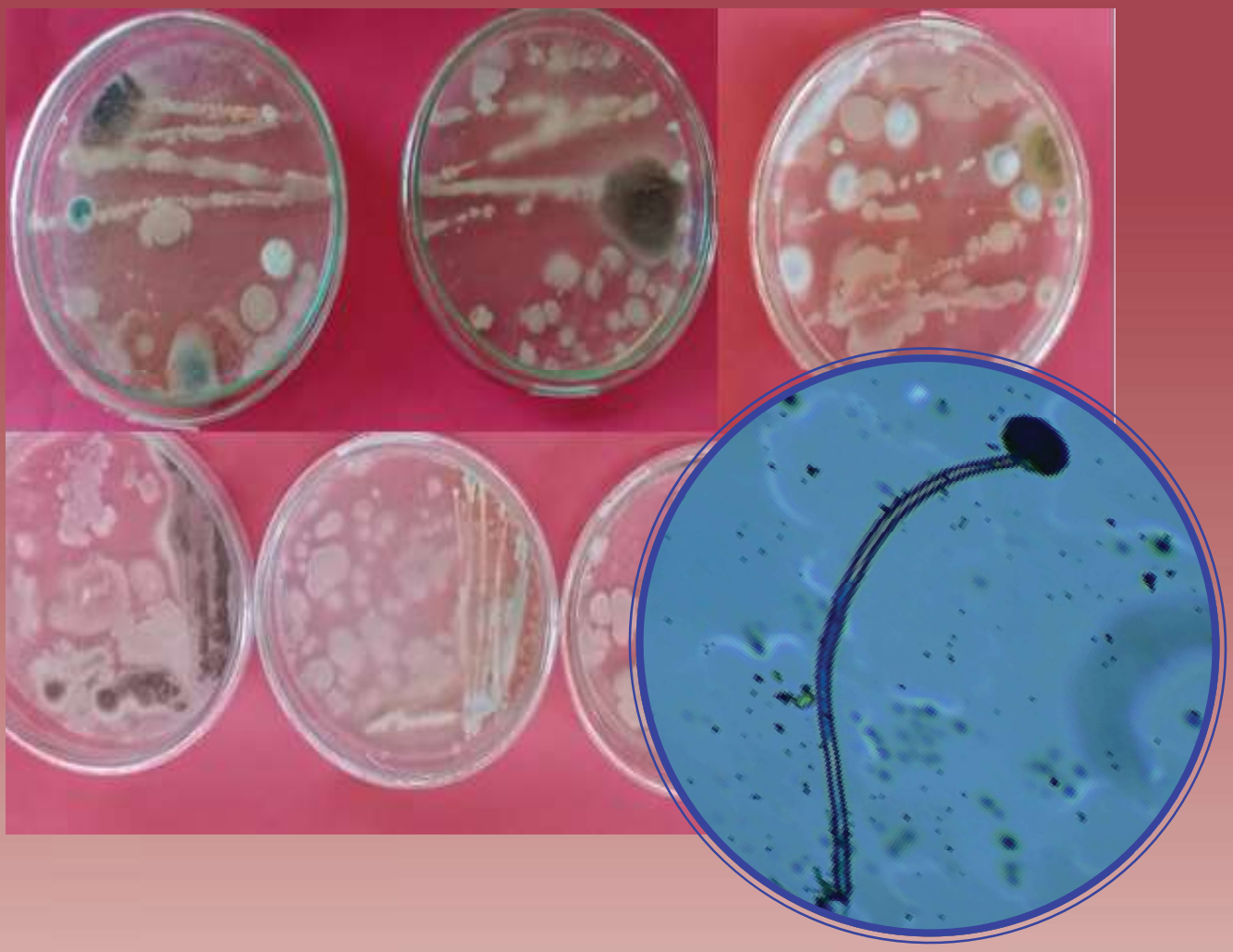

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In the Name of God, the Compassionate, the Merciful

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ON THE COVER

Aspergillus flavus colonies on Potato Dextrose Agar, Incubated at 30oC for 3 days.
Morphology of Aspergillus flavus using a phase-contrast microscope at 10x100 magnification (see page 75).

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TABLE OF CONTENTS

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REVIEW ARTICLE

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Foodborne Bacteria in Iran: A 23-year Systematic Review of High-risk Foods

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ABSTRACT

Foodborne diseases are a significant global public health concern due to their high prevalence, mortality, and economic losses. The aim of this study was to conduct a systematic review of identified foodborne pathogens and outbreaks in Iran over the past 23 years to provide an overview of the risk assessment and prevention approaches in the country. Using appropriate keywords and searching major databases, such as ScienceDirect, Scopus, PubMed, Google Scholar, and the Iranian Scientific Knowledge Database, we initially identified 4,740 articles. Finally, 328 articles were selected for evaluation. Among these articles, publications on *Salmonella, Staphylococcus aureus*, and *Listeria* were the most numerous. Poultry meat was found to be the main source of major foodborne pathogens in Iran, including *Campylobacter* (46.21%), *Listeria monocytogenes* (38.45), *Salmonella* (24.83%), and *Yersinia enterocolitica* (16.81%). Given the high prevalence of foodborne bacteria in Iranian foods, it is crucial to implement effective control measures to reduce the risk and burden of foodborne diseases. In particular, poultry meat, which poses a high risk for the occurrence of foodborne diseases in Iran, should be subjected to further risk assessment and control measures throughout the food chain.

Keywords

Foodborne bacteria, Salmonella, Staphylococcus aureus, Listeria, food, prevalence

Number of Figures: 13
Number of Tables: 12
Number of References: 369
Number of Pages: 47

Abbreviations

EWHO: World Health Organization
CDC: Center for Disease Control and Prevention
RTE: Ready-To-Eat
EFSA: European Food Safety Authority

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Introduction

Foodborne diseases typically result from the consumption of food or water contaminated with pathogens or their toxins [1]. These illnesses often present as acute health problems with diverse symptoms, such as gastrointestinal distress (e.g., diarrhea, vomiting, nausea, and abdominal cramps) or neurological symptoms (e.g., headache, paralysis, and paresthesia) [2, 3]. The bacterial pathogens most commonly associated with foodborne illness worldwide include *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus, Salmonella enterica, Bacillus cereus, Vibrio spp., Campylobacter jejuni,* and *Clostridium perfringens* [2, 4, 5]. The food products most frequently implicated in outbreaks include poultry, ground meat, seafood, dairy products, as well as fruits and vegetables [6].

The food industry faces significant challenges in ensuring the safety and nutritional quality of food products for consumers due to various sources of contamination, such as animals, soil, water, air, and food handlers during production and storage [7, 8]. However, the implementation of proper cold preservation methods (e.g., refrigeration and freezing) and appropriate thermal processing of foods can effectively prevent foodborne diseases [3].

In the contemporary era, regulatory frameworks and directives pertaining to food safety have been fortified and intensified. Nevertheless, foodborne diseases continue to represent a significant threat to global public health and an economic burden, particularly in developing countries [9]. In its inaugural estimation of the global burden of foodborne diseases in 2015, the WHO attributed 600 million cases of foodborne diseases, 420,000 deaths, and a loss of 33 million years of healthy life worldwide to unsafe food consumption [9].

In 2018, the United States documented 25,606 cases of foodborne infections, resulting in 5,893 hospitalizations and 120 deaths [10]. The burden of foodborne diseases is particularly significant in low- and middle-income countries. Identifying the source of contamination and transmission route is of paramount importance for preventing foodborne illnesses and implementing effective interventions in food safety. However, attributing an infection to specific food and identifying foodborne transmission is challenging and requires source attribution methodologies. Consequently, there is a dearth of studies identifying the sources of foodborne infections, particularly in developing countries [11].

In this study, we aimed to conduct a systematic review of the prevalence of foodborne pathogens in different types of foods in Iran. As a result, we can gain an overview of the role of food in the transmission of infections and emphasize the importance of food safety in controlling foodborne diseases and reducing their health and economic burden on society.

Materials and Methods

Search strategy

A comprehensive and systematic search was conducted in various databases, including ScienceDirect, Scopus, PubMed, Google Scholar, and local Iranian databases, namely the Iranian Scientific Information Database (www.sid.ir). The literature review was limited to studies published during 2000-2023. The keywords used for searching included "prevalence", "detection", and "identification" in conjunction with terms, such as "food", "Iran", "foodborne pathogen", "food infection", "food poisoning", "food illness", "food disease", "foodborne bacteria", "*Campylobacter*", "*Listeria*", "*Salmonella*", "*Helicobacter pylori*", "Vibrio", "*Clostridium botulinum*", "*Clostridium difficile*", "*Clostridium perfringens*", "*Mycobacterium tuberculosis*", "*Coxiella burnetii*", "*Staphylococcus aureus*", "*Shigella*", "*Pseudomonas*", "*Bacillus cereus*", "*Brucella*", and "*Yersinia enterocolitica*".

Eligibility criteria

This systematic review included articles that focused on the prevalence of foodborne pathogens in any type of food in Iran. Duplicate reports and articles without a clear sample size or other essential data were excluded.

Data extraction

Data collection included extracting information, such as the year of publication, types of foods tested for pathogen contamination, sample size, and number of positive samples contaminated with foodborne pathogens.

Results and Discussion

Results and Discussion

Figure 1 illustrates the study selection process presented in the PRISMA diagram. A systematic literature search using Scopus, ScienceDirect, Google Scholar, SID, Magiran, and cross-references yielded an initial total of 4740 articles. After removing duplicates, 1719 articles remained for title/abstract screening. Following this screening, 655 articles were selected for full-text review. Finally, 328 eligible studies were included in the systematic review.

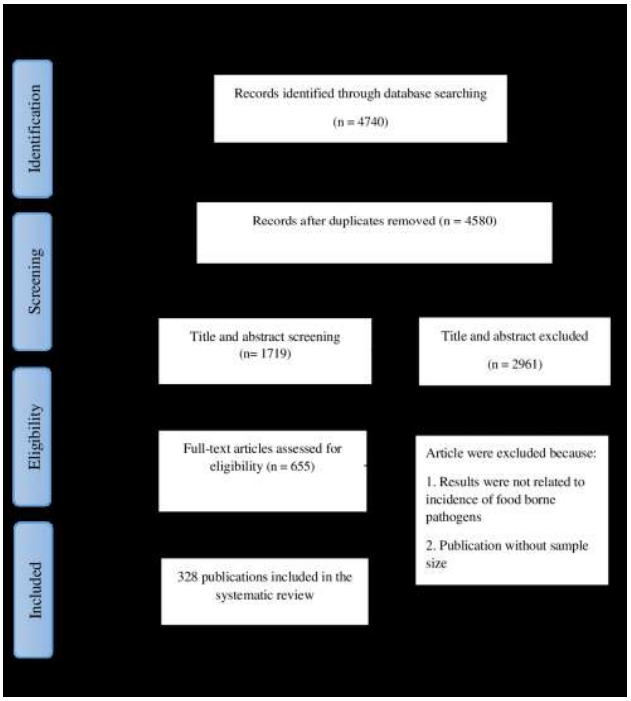

Figure 1.
Flow diagram showing the results of search

1) *Salmonella spp.* prevalence in food

Salmonella (S.) enterica enterica has more than 2300 serotypes, with *S. Enteritidis* and *S. Typhimurium* being the most commonly reported serotypes. Symptoms of salmonellosis include abdominal pain, vomiting, nausea, diarrhea, and fever [12]. Raw meat, particularly poultry, and egg products, are the main sources of foodborne salmonellosis. Other reported foods that transmit *Salmonella* to humans include fish, peanuts, unpasteurized juice, and milk. It is important to cook raw foods thoroughly to a safe minimum internal temperature to prevent foodborne salmonellosis, as *Salmonella* is heat-sensitive. However, processed foods, such as RTE meats and salads can become contaminated through cross-contamination during processing [12]. In Europe in 2020, 0.15% of RTE food samples and 2.4% of non-RTE food samples were positive for *Salmonella* [13].

Table 1 presents the prevalence of *Salmonella* in different foods in Iran based on our review. The highest levels of contamination were found in poultry meat (23.03%), followed by red meat (14.13%), dairy products (11.66%), RTE foods (11.34%), eggs (9.93%), vegetables (7.8%), fish and shrimp (5.93%), raw milk (3%), and water (2.25%) (Figure 2). In a study conducted in China in 2019, out of 1035 different food samples, a total of 147 samples (14.2%) were positive for *Salmonella*. In their study, the highest prevalence of *Salmonella* was found in fresh meat samples (28%), followed by RTE foods (9%), frozen foods (7.1%), and fresh produce (4.5%) [14]. Fresh meat is a common source of *Salmonella* contamination due to the nature of its production and processing [15]. During the slaughter and processing of animals, there is a high risk of cross-contamination with various bacteria, such as *Salmonella* [16]. In addition, fresh meat products consumed raw or undercooked increase the risk of foodborne illness [17]. The handling and storage of fresh meat products can also contribute to *Salmonella* contamination [18]. In contrast, RTE foods and frozen foods undergo processing and packaging that can reduce the risk of *Salmonella* contamination [19]. However, it is still possible for *Salmonella* to be introduced during the processing or packaging of these products [20]. Fresh produce, while less likely to be contaminated with Salmonella compared to fresh meat, can still pose a risk if not properly handled and washed before consumption [18].

2) *Staphylococcus aureus* prevalence in food

Although *Staphylococcus* (S.) *aureus* is the primary causative agent of hospital and community-acquired infections, it has also been associated with foodborne diseases. *S. aureus* can cause various gastrointestinal illnesses, which are characterized by nausea, vomiting, abdominal cramps, weakness, and diarrhea [21]. Table

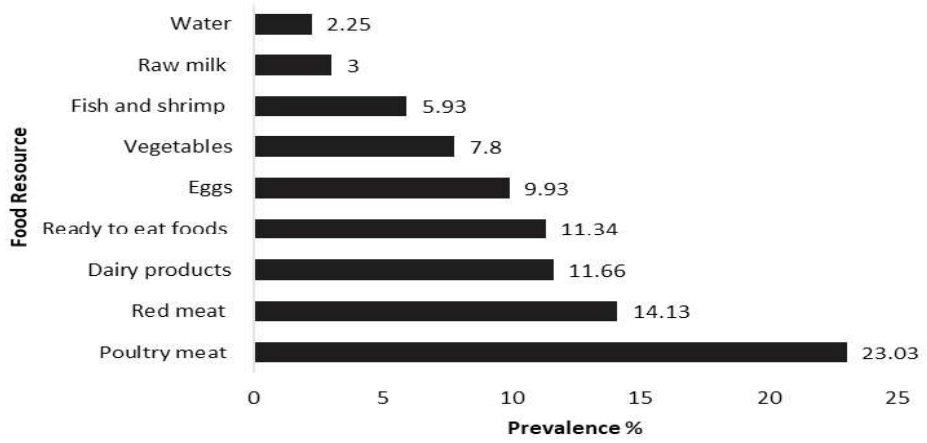

Figure 2.
Prevalence of *Salmonella spp.* in different foods in Iran.

Table 1.
Summary of the studies reporting the prevalence of *Salmonella spp.* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	Type of *Salmonella spp.*	References	City
1	2023	Eggs	40	4	10	*Salmonella Enteritidis*	[72]	Qazvin
2	2023	poultry meat	440	39	9	*Salmonella enteritidis and Salmonella typhimurium*	[73]	Shahrekord
3	2022	Eggs	500	405	81	*Salmonella spp*	[74]	Lahijan
4	2022	retail raw meat	60	16	26	*Salmonella spp.*	[75]	Urmia
5	2022	poultry products	80	11	13.75	*Salmonella spp.*	[76]	Ardabil
6	2022	Eggs duck	130	21	16.6	*Salmonella spp*	[77]	Qazvin
7	2022	Chicken meat	150	4	2.7	*Salmonella spp.*	[78]	Zahedan
8	2022	cattle raw milk	100	2	2	*Salmonella spp.*	[79]	Mazandaran
9	2022	Red meat	300	35	11	*Salmonella spp*	[80]	Shahrekord
10	2021	Chicken meat	100	6	6	*Salmonella spp.*	[81]	Ardabil
11	2021	poultry and egg	3125	250	8	*Salmonella spp.*	[82]	six provinces of Iran
12	2021	chicken meat, beef	450	40	8	*Salmonella enterica*	[83]	Tehran
13	2019	Olivier salad	26	6	23	*Salmonella spp.*	[84]	Mashhad
14	2019	Raw chicken meat Egg yolk	60 30	29 0	48.3 0	*Salmonella spp.*	[85]	Karaj
15	2019	Eggs (shell & contents)	525	0	0	*Salmonella spp.*	[86]	Isfahan
16	2019	Traditional cheeses	100	0	0	*Salmonella spp.*	[87]	Mohabad
17	2018	Pastry	75	5	6.6	*Salmonella spp.*	[88]	Mashhad
18	2018	Chicken Meat	100	7	7	*Salmonella spp.*	[89]	Mashhad
19	2018	Industrial eggs (shell & contents)	60	0	0	*Salmonella spp.*	[90]	Zanjan
		Local eggs shell	60	0	0			
		Local egg contents	60	2	1.6			

Table 1 cont.

20	2018	Industrial eggs (shell & contents)	34	0	0	*Salmonella spp.*	[91]	Sanandaj
		Local eggs shell	42	6	4.2			
		Local egg contents	42	2	4.7			
		Bulk eggs shell	40	3	7.5			
		Bulk egg contents	40	0	0			
21	2018	Industrial eggs (shell & contents)	60	0	0	*Salmonella spp.*	[92]	Qazvin
22	2018	Shrimp	245	33	13.4	*Salmonella spp.*	[93]	Khuzestan
23	2017	Pizza	90	8	8.8	*S. arizonae*	[94]	Ilam
		Frankfurter	90	22	24.4			
		Sausages	90	19	21.1			
24	2017	Camel meat	150	0	0	*S. Typhimurium*	[95]	Kohgiluyeh & Boyerahmad/ Isfahan
25	2017	Hamburgers	100	2	2	*Salmonella spp.*	[96]	Kashan
26	2016	beaf meat	190	7	3.6	*S. Typhimurium*	[97]	Gilan
		Poultry meat	190	15	7.8	*S. Typhimurium*		
27	2016	Poultry meat	183	52	28.4	*S. Typhimurium*	[98]	Zanjan
28	2016	Industrial Olovier salad	48	0	0	*Salmonella spp.*	[99]	Isfahan
		Traditional Olovier salad	54	11	20.4			
29	2016	Traditional ice cream	90	62	68.8	*Salmonella spp.*	[100]	Zabol
30	2015	Pastry cream	120	0	0	*Salmonella spp.*	[101]	Arak
31	2015	Olivier salad	50	9	18	*S. Ttypimurium*	[102]	Shahrekord
32	2015	Poultry meat	625	35	5.6	*S. Enteritidis*	[103]	Tehran
		Eggs	625	25	4			
33	2015	Eggs	50	5	10	*S. Enteritidis*	[104]	Shiraz
34	2015	Native eggs	64	0	0	*Salmonella spp.*	[105]	Yasuj
35	2015	Traditional & Industrial cheese	200	34	17	*Salmonella spp.*	[106]	Shahrekord
				11	5.5			
36	2015	Eggshells	150	2	1.3	*S. Enteritidis &* *S. Typimurium*	[107]	Tabriz
		Egg contents	150	0	0			

Table 1 cont.

37	2014	Meat Products (Burgers, Sausages, Kababs, Cutlets	42	0	0	*Salmonella spp*	[108]	Urmia
38	2014	Unpasteurized cream	100	2	2	*S. Paratyphi B*	[109]	Tehran
39	2014	Chicken	190	86	45	*Salmonella spp.*	[110]	Tehran
		Beef meat	189	38	20.2	*Salmonella spp.*		
		Beef meat	189	19	10	*S. thompson*		
		Chicken	190	65	34.2	*S. thompson*		
40	2014	Chicken meat	200	58	29	*Salmonella spp.*	[111]	Alborz
		Liver	120	26	21.6			
		Heart	120	17	14.1			
41	2014	Poultry meat	89	28	31.6	*Salmonella spp.*	[112]	Shahrekord
		Beef meat	98	26	26.1			
42	2013	Olovier salad	50	9	18	*S. Typhimurium*	[112]	Shahrekord
43	2013	Tap water	144	5	3.4	*Salmonella spp*	[113]	Isfahan
		304	304	5	1.1			
44	2013	Poultry slaughtered	250	7	2.8	*S. infantis*	[114]	Brijand
45	2013	Local eggs	210	14	66.6	*Salmonella spp.*	[115]	Kohgiluyeh & Boyerahmad
46	2013	Industrial egg contents	100	0	0	*Salmonella spp.*	[116]	Talesh
		Industrial eggshells	100	19	19			
		Local egg contents	100	0	0			
		Local eggshells	100	4	4			
		Local chicken meat	100	21	21			
		Industrial chicken meat	100	5	5			
		Red meat	150	5	3.3			
47	2013	Industrial Olovieh salad	200	0	0	*Salmonella spp.*	[117]	Yazd
48	2012	Seafood	384	19	5	*Salmonella spp.*	[118]	Bushehr, Hormozgan, Khuzestan
49	2012	Chicken meat	150	14	9.3	*Salmonella spp.*	[119]	Isfahan & Shahrekord
		Turkey meat	105	7	6.7			
		Ostrich meat	45	1	2.2			
50	2012	Beef meat	60	7	11.6	*Salmonella spp.*	[120]	Sanandaj
				4	6.6	*S. Typhimurium*		
51	2012	Packed chicken meat	96	19	19.7	*Salmonella spp.*	[121]	Mazandaran
		Unpacked chicken meat	104	24	23			

Table 1 cont.

52	2012	Salt water fish	70	2	2.9	*Salmonella spp.*	[122]	Ahvaz
		Shrimp	70	3	4.3			
		Shrimp burge	10	1	10			
53	2011	Raw cow's milk	350	14	4	*Salmonella spp.*	[123]	Shahrekord
54	2010	Egg	100	0	0	*Salmonella spp.*	[124]	Shahrekord
55	2010	Chicken meat	190	86	45	*Salmonella spp.*	[125]	Tehran
		Beef meat	189	38	20			
56	2010	Turkey meat	144	14	9.7	*Salmonella spp.*	[126]	Isfahan
		Ostrich meat	65	3	4.6			
		Partridge meat	40	0	0			
57	2009	Eggshells	250	4	1.6	*S. Typhimurium*	[127]	Mashhad
		Egg contents	250	0	0	*Salmonella spp.*		
58	2009	Poultry carcasses	60	5	8.3	*Salmonella spp.*	[128]	Mashhad
			60	1	1.6	*S. Typhimurium*		
59	2009	Egg contents	120	0	0	*Salmonella spp.*	[129]	Zanjan
		Eggshells	120	68	56.6			
		Chicken meat	120	104	86.6			
60	2009	Chicken meat	67	32	47.7	*Salmonella spp.*	[130]	Tehran
		Beef meat	66	19	28.7			
61	2008	Local egg contents	500	1	0.2	*Salmonella spp.*	[131]	Birjand
		Local eggshells	500	2	0.4			
62	2008	Raw poultry	134	24	17.9	*Salmonella spp*	[132]	Isfahan
		Cooked poultry	56	3	5.3			
		Turkey	3	1	33.3			
		Quail	5	2	40			
		Red meat	101	8	7.9			
		Cooked meat	118	2	1.6			
		Vegetables	38	3	7.8			
		Fish	15	0	0			
		Yogurt	32	0	0			
		Olovieh salad	20	0	0			
		Hamburger	5	0	0			
		Mayonnaise souse	8	0	0			
63	2007	Poultry carcasses	132	92	69	*Salmonella spp.*	[133]	Tehran
64	2007	Traditional cheeses	200	0	0	*Salmonella spp.*	[134]	Jahrom
65	2006	Liver	145	12	8.1	*Salmonella spp.*	[135]	Yazd
		Meat (before chiller)	145	28	18.4			
		Meat (after chiller)	145	50	34.4			
66	2006	Local eggs	500	3	0.6	*Salmonella spp.*	[133]	Birjand

Table 2.
Summary of the studies reporting the prevalence of *S. aureus* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	City
1	2023	Poultry meat	94	16	17	Shahrekord
2	2022	Sausages and Bologna	100	31	31	Tehran
3	2022	Raw and ready-to-eat green leafy vegetables	366	134	36.6	Tehran
4	2022	Retail raw meat	60	23	39	Urmia
5	2022	Raw Milk And Traditional Dairy	150	23	15.33	Alborz
6	2022	Ready To Eat Food	320	10	3.12	Tehran
7	2022	Raw Milk	380	42	11.05	Alborz
8	2022	Raw Cow Milk	90	35	38.88	Shahrekord
9	2021	Baghlava	112	3	2.67	Qazvin
10	2021	Raw milk	250	46	18.4	Mashhad
11	2021	Cheese, raw and pasteurized milk	100	10	10	-
12	2019	Meat Products	160	26	16.25	Shahrekord
13	2021	Milk and Cheese	200	23	11	Khuzestan
14	2021	Meat retail	90	31	34.5	Zanjan
15	2021	Ready-to-eat food	415	64	15.42	Tehran
16	2021	Chicken meat	24	6	25	Tehran
17	2019	Zoolbia & Bamieh	75	21	28	Mashhad
18	2019	Fowl meat	240	22	9.6	Tehran
19	2019	Traditional cheese	100	21	21	Maragheh
20	2018	Traditional cheese	100	45	45	Mahabad
21	2017	Raw foods with animal origin	84	20	23.8	Isfahan
		Cooked foods with animal origin	132	12	9	
		Cooked foods without animal origin	269	15	5.7	
22	2017	Season salad	18	0	0	Bandar abbas
		Pasta salad	5	2	40	
		Lettuce	16	0	0	
		Shirazi salad	7	0	0	

Table 2 cont.

23	2017	Pizza	90	11	12.2	Ilam
		Frankfurter	90	25	27.7	
		Sausages	90	22	24.4	
24	2016	Cheese	*120*	*18*	*41.6*	Hamedan
25	2016	Meat	*380*	*78*	*20.5*	Gilan
26	2016	Shrimp	*300*	*84*	*28*	Persian Gulf, Caspian Sea
		Fish	*300*	*122*	*47*	
27	2015	Red meat	*379*	*36*	*9.4*	Hamadan
		Dairy products	*671*	*62*	*7.2*	
28	2015	Raw milk	*320*	*88*	*27.5*	Chaharmahal va Bakhtiyari
		Dairy products	*350*	*87*	*24.8*	
29	2015	Shrimp	*300*	*74*	*24.6*	Persian Gulf, Tehran
30	2015	Raw milk	*1930*	*248*	*12.8*	Mazandaran
		Dairy products	*720*	*80*	*11.1*	
31	2015	Bovine milk	*92*	*44*	*47*	Maku
		Sheep milk	*86*	*32*	*37*	
32	2015	Industrial Olivier salad	*30*	*15*	*50*	Shahrekord
		Traditional Olivier salad	*20*	*8*	*40*	
33	2015	Cheese	*80*	*80*	*100*	Marand
34	2015	Chicken nuggets	*420*	*24*	*5.7*	Isfahan
35	2015	Different food	*606*	*12*	*1.9*	Gilan
36	2014	Cream pastry	*450*	*194*	*43.3*	Gorgan
37	2014	Milk	*100*	*9*	*9*	Tabriz
		Cheese	*100*	*45*	*45*	
38	2014	Traditional ice cream	*30*	*2*	*6.7*	Yasuj
		Olovier salad	*4*	*0*	*0*	
		Cream suit	*30*	*9*	*30*	
39	2014	Raw milk	*300*	*125*	*41.6*	Ahwaz
40	2014	Dairy product	*460*	*127*	27.6	Marand
41	2014	Cheese	*80*	*80*	100	Tehran, Gilan
42	2014	Doogh	*126*	*86*	68	,Mazandaran

Table 2 cont.

43	2014	Raw milk	*120*	*49*	40.8	Kurdistan	
44	2014	Meat products	*150*	*19*	12.6	Tonekabon	
45	2014	Traditional cheeses	100	16	16		East- Azerbaijan
46	2013	Dairy products	347	20	5.8		Isfahan, Chaharmahal va Bakhtyari, Khuzestan,
47	2013	Industrial Olivier salad	200	40	20	[117]	Yazd
48	2013	Milk	200	22	11	[175]	Fars
49	2012	Traditional white cheese	100	26	26	[176]	Tabriz
		butter	150	24	16		
50	2012	Ground-meat kebab	72	72	100	[177]	Shahrekord
		Bakkhtiyari Kebab	72	72	100		
		Fish	72	72	100		
		Salad	72	72	100		
51	2012	Seafood products	245	22	8.9	[122]	Different markets of Iran
52	2012	Packaged hamburger	256	64	25	[178]	Tehran
53	2012	Raw milk	100	50	50	[176]	Urmia
		Pasteurized milk	100	2	2		
		Ice cream	100	26	26		
54	2012	Raw milk	348	46	13.2	[179]	Shahrekord
55	2010	Fruit juice	360	32	8.8	[180]	Shahrekord
56	2008	Different food	216	30	55.6	[181]	Tehran
57	2006	Fresh fish	67	15	22.3	[182]	Gilan, Caspian Sea

2 presents the findings of studies conducted in Iran regarding the prevalence of this pathogen in different food categories, including seafood (38.51%), meat products (35.47%), dairy products (31.70%), red meat (25.85%), RTE foods (23.59%), raw milk (23.32%), and poultry meat (14.32%) (Figure 3). Seafood and fish are conducive to microbial growth due to their abundant protein and water content. *S. aureus* is not typically found in the natural microflora of fish, therefore, its presence can indicate poor personal hygiene, new contamination, or potential disease in the fish [22]. Improper conditions in the fishery, storage, and non-standard transportation provide conditions for pathogens to grow [23]. Furthermore, the hot climate in Iran can facilitate the growth and proliferation of *S. aureus* bacteria in food products, such as meat and dairy items, particularly if they are not stored and refrigerated correctly [24].

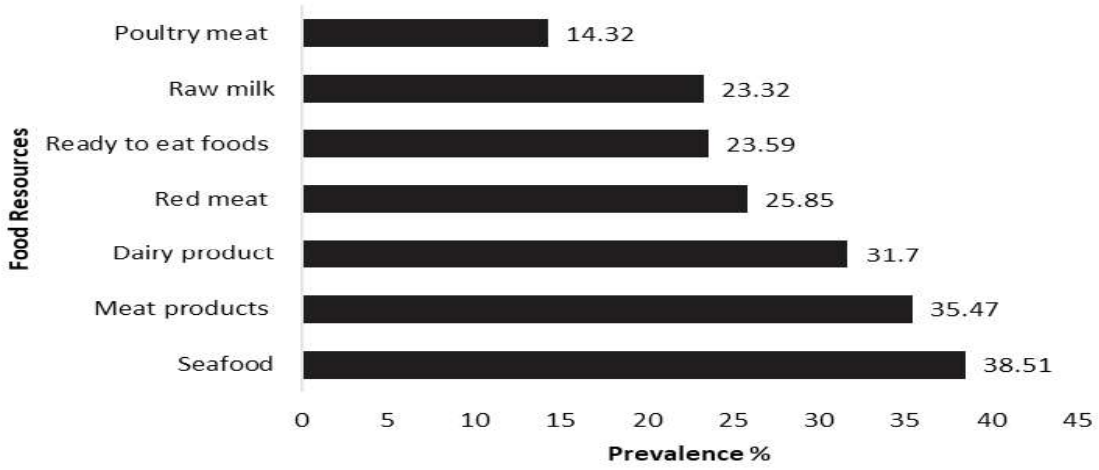

Figure 3.
Prevalence of *S. aureus* in different foods in Iran.

Table 3.
Summary of the studies reporting the prevalence of *Listeria* spp. in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	*Type of Listeria spp.*	References	Area
1	2023	Various Food	900	136	15.1	*L. monocytogenes*	[183]	Mazandaran and Golestan
2	2022	Retail raw meat	60	25	42	*L. monocytogenes*	[75]	Urmia
3	2022	Curd and cheese	150	14	9.33	*L. monocytogenes*	[184]	-
4	2021	Non-Pasteurized Milk	50	30	60	*L. monocytogenes*	[185]	Kerman
5	2021	Traditional cheeses	60	1	1.6	*L. monocytogenes*	[186]	Tehran
6	2021	Raw Milk	100	10	10	*Listeria spp.*	[187]	Tehran
7	2021	Seafood	350	40	11.42	*Listeria spp.*	[188]	Genaveh port
8	2020	Beef and chicken meat	90	45	50	*L. monocytogenes*	[189]	Zanjan
9	2019	Eggs	525	0	0	*Listeria spp.*	[86]	Isfahan
10	2019	Chicken meat retailers	811	257	30.5	*Listeria spp.*	[190]	Mashhad
11	2018	Traditional dairy products	545	64	11.7	*Listeria spp.*	[191]	Yazd
				22	4.3	*L. monocytogenes*		
12	2017	Food (sausage, milk, cheese, chicken and meat)	267	8	2.9	*Listeria spp.*	[192]	Urmia
13	2017	Fresh chicken carcasses	200	80	40	*Listeria spp.*	[193]	Mashhad
14	2016	Dairy products	107	9	8.4	*L. monocytogenes*	[194]	Tehran
		Processed meat	210	11	5.2	*Karaj &*		
15	2016	Seafood	237	7	2.9	*L. monocytogenes*	[195]	Tehran

Table 3 cont.

16	2016	Argyrosomus	240	30	12.5	*Listeria spp.*	[196]	Isfahan& Bandaranzali
		hololepidotus		5	16.6	*Bandaranzali*		
17	2015	Koozeh cheeses	100	3	3	*L. monocytogenes*	*[197]*	Urmia
18	2015	Minced beef	150	4	2.7	*Listeria spp.*	*[198]*	Ahvaz
				1	0.6	*Mazandaran*		
19	2015	Raw fish	488	104	21.3	*Listeria spp.*	*[199]*	Mazandaran
20	2015	Raw milk	60	0	0	*L. monocytogene*	*[200]*	Zanjan
21	2015	Traditional dairy products	292	21	19.7	*Listeria spp.*	*[201]*	Isfahan
22	2015	Raw milk	100	5	5	*L. monocytogene*	[202]	Kerman
23	2014	Ready to eat food (olovier salad, Yogurt stew, macaroni salad and meat salad)	235	20	8.5	*Listeria spp.*	[203]	Shahrekord
24	2014	Meat products	98	12	32.4	*L. monocytogene*	[204]	Qazvin
		Milk products	84	25	29.7	*Fars & Khuzestan*		
25	2014	Bulk milk	260	27	10.4	*Listeria spp.*	[205]	Fars & Khuzestan
				7	2.7	*Bandar anzali*		
26	2014	Smoked fish	80	7	8.8	*Listeria spp.*	[206]	Isfahan & Bandar anzali
				2	2.5	*L. monocytogene*		
		Salted Fish	40	6	15	*Listeria spp.*		
				1	2.5	*L. monocytogene*		
27	2013	Meat and meat products	60	8	13.3	*Listeria spp.*	[207]	Khoramabad & Tehran
				2	6.6	*L. monocytogene*		
28	2013	Crayfish meat	40	3	7.5	*L. monocytogene*	[208]	Aras
29	2013	Raw cow milk	986	25	2.5	*Listeria spp.*	[209]	Isfahan
				20	2	*L. monocytogene*		
30	2013	Vegetables and ready mayonnaise salads	300	26	8.7	*Listeria spp.*	[210]	Tehran
				21	7	*L. monocytogene*		
31	2013	Raw seafood products	331	16	4.8	*L. monocytogene*	*[211]*	Shahrekord
		RTE seafoods	321	46	14.5	*L. monocytogene*		
32	2013	Raw milk	466	83	18.6	*Listeria spp.*	*[212]*	Tehran
33	2013	Dairy products	185	7	3.8	*Listeria spp.*	*[213]*	Kermanshah
		Meat products	187	51	27.2			
		Ready-to-eat foods	158	8	5.1			
34	2013	Seafood	300	24	8	*Listeria spp.*	*[214]*	Isfahan & Shahrekord
				18	6	*L. monocytogene*		
35	2013	Quail products	150	10	6.6	*Listeria spp.*	*[215]*	Isfahan
				1	0.6	*L. monocytogene*		
36	2013	Lamb	200	5	2.5	*L.ivanovii*	*[216]*	Shahrekord
37	2012	Different types of raw meat	1107	141	12.7	*Listeria spp.*	*[217]*	Shahrekord, Isfahan, Ahvaz ,Shiraz,Yazd,
				27	2.4			
38	2012	Poultry product	402	134	33.3	*Listeria spp.*	*[218]*	Shahrekord
39	2012	Seafood	264	20	7.6	*Listeria spp.*	[219]	Isfahan & Shahrekord

Table 3 cont.

40	2012	Various seafood products	245	2	0.8	*L. monocytogene*	[122]	Different markets of Iran
41	2011	Eggs	100	0	0	*L. monocytogene*	[124]	Shahrekord
42	2011	Fish	194	24	12.3	*Listeria spp.*	[220]	Urmia
				5	2.5	*L. monocytogene*		
43		Raw cow milk	45	5	1.1	*Listeria spp.*	[221]	Shiraz
				2	4.4	*L. monocytogene*		
		Raw goat milk	32	1	3.1	*Listeria spp.*		
				1	3.1	*L. monocytogene*		
		Traditional cheese	41	10	24.4	*Listeria spp.*		
				4	9.7	*L. monocytogene*		
		Traditional ice-cream	60	8	11.7	*Listeria spp.*		
				2	3.3	*L. monocytogene*		
44		Raw milk	100	4	4	*L. monocytogene*	[222]	
45		Dairy products	360	6	1.6	*L. monocytogene*	[223]	
46		Chilled ready to eat foods	41	3	7.3	*L. monocytogene*	[224]	
		Meat, meat products	332	4	1.2	*L. monocytogene*		
		Milk and dairy products	88	0	0	*L. monocytogene*		
47		Cattle carcasses	203	6	3	*L. monocytogene*	[225]	

3) *Listeria monocytogenes* prevalence in food

Listeria (L.) *monocytogenes* represents a significant public health concern due to its ability to be transmitted from the environment to food, which can lead to foodborne listeriosis in humans [25]. In 2020, the EFSA reported a total of 1876 cases of listeriosis, with 97.1% of these cases necessitating hospitalization [26]. Moreover, the EFSA indicated an increase in the case fatality rate and hospitalization rate associated with *L. monocytogenes* infections in 2020. Among all the reported zoonoses in Europe in 2020, listeriosis had the highest case fatality rate of 13% [26]. Those at the greatest risk of developing listeriosis include pregnant women, the elderly, newborns, and patients with compromised immune systems [27]. Moreover, a multitude of food items were identified as potential sources of listeriosis outbreaks during this period. Specifically, 4.8% of RTE meat products and 0.44% of

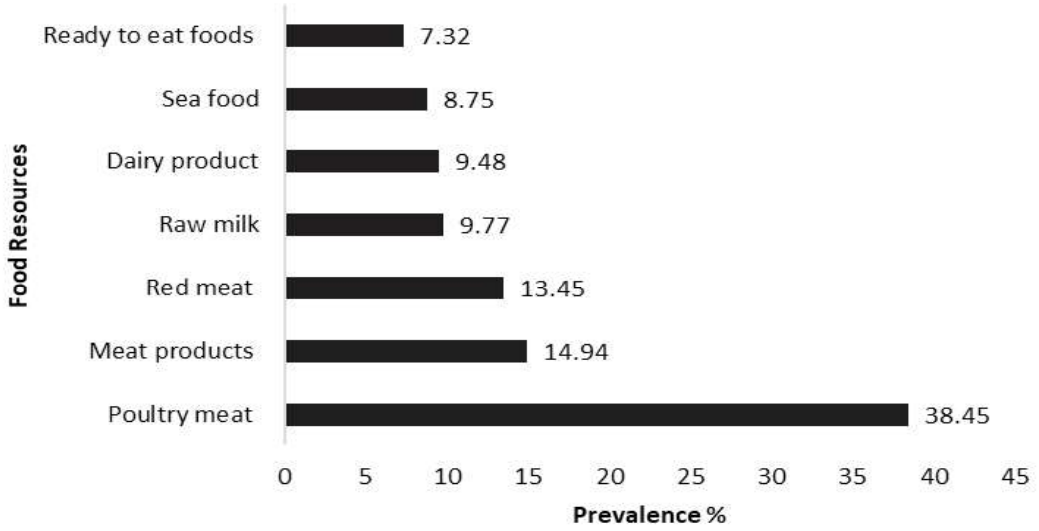

Figure 4.
Prevalence of *Listeria* in different foods in Iran.

Table 4
Summary of the studies reporting the prevalence of C. *burnetii* in Iran..

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	References	Area
1	2022	Unpasteurized Camel Milk	100	6	6	[226]	Mashhad
2	2022	Kope cheese and cattle milk	800	103	12.87	[227]	West Azerbaijan
3	2021	Raw Milk	162	23	14	[228]	Tehran, Hamadan, and Mazandaran
4	2021	Raw milk	100	27	27	[229]	Mazandaran
5	2020	Raw milk	204	21	10.2	[230]	Gilan
6	2020	Sheep and goats milk	420	51	12.1	[231]	West Azerbaijan
7	2019	Milk	126	44	34.9	[232]	Qom
8	2019	Milk	840	14	16.9	[233]	West Azerbaijan
		Cattle milk			14.4		
		Buffalo milk			19.3		
9	2019	Cream & butter	200	6	3	[234]	Shahrekord
		Traditional bovine cream,	69	4	5.7		
		Traditional sheep butter,	20	1	5		
		Traditional bovine butter	39	1	2.5		
10	2018	Raw milk	500	9	1.8	[235]	Khorramabad
		Sheep milk		3			
		Goat milk		6			
11	2018	Bulk milk	100	3	3	[236]	Shiraz
12	2018	Non-pasteurized dairy products	238	20	8.4	[29]	Shiraz
13	2018	Raw milk	100	10	10	[237]	Tehran
		Traditional unpacked cheese	40	3	7.5		
14	2016	Raw sheep milk	72	15	20.8	[238]	Khorramabad
15	2015	Bovine bulk milk	70	12	17.4	[239]	Jahrom
				7	10		
				7	10		
16	2015	Individual raw milk	60	7	11.6	[200]	Zanjan
		Bovine	38	5	8.3		
		Ovine	22	2	3.3		
17	2015	Cow milk	150	18	12	[240]	Tehran
18	2015	Goat milk	31	5	16.1	[241]	Kerman
19	2015	Cow milk	80	20	25	[242]	Ajabshir
20	2014	Cheese	28	2	7.1	[243]	Mashhad
		Yoghurt	26	2	7.6		
		Sheep milk	23	8	34.7		
		Cow milk	60	2	3.3		
21	2014	Bovine bulk tank	100	5	5	[244]	Mashhad
22	2014	milk	51	21	41.1	[245]	Khoramabad.
23	2013	Goat milk	100	14	14	[246]	Qom

Table 4 cont.

24	2013	Bovine bulk milk	100	11	11	[247]	Jahrom
25	2011	Bovine milk	247	8	3.2	[248]	Kerman
		Ovine bulk milk	140	8	5.7		
		Caprine bulk milk					
		Camel bulk milk	110	5	4.5		
26	2010	Bulk milk	296	6	2	[249]	Fars, Ghom, Kerman, Yazd Khuzestan
27	2010	Cow milk	210	13	6.2	[250]	Chaharmahal va Bakhtiari
		Sheep milk	110	0	0		
		Goat milk	56	1	1.8		

milk and milk products were found to be contaminated with *L. monocytogenes* [26]. Table 3 and Figure 4 present the findings of studies conducted in Iran regarding the prevalence of *L. monocytogenes* in various food types. As illustrated in Figure 4, poultry meat exhibited the highest contamination rate of 38.45%, followed by meat products (14.94%), red meat (13.45%), raw milk (9.77%), dairy products (9.48%), seafood (8.75%), and RTE foods (7.32%) (Figure 4). A previous review study conducted in Iran until 2015 yielded comparable results regarding the contamination of food with Listeria. The highest prevalence of *L. monocytogenes* was approximately 9.2%, which was observed in RTE foods [25]. Therefore, RTE foods should be considered a potential hazard to consumers [25]. Similarly, other developing countries have also yielded comparable results. For example, a study conducted in Ethiopia revealed that 28.4% of raw milk and milk products were contaminated with Listeria spp., with 5.6% of these samples testing positive for L. monocytogenes [23].

4) *Coxiella burnetii* prevalence in food

Coxiella burnetii is a zoonotic pathogen that causes Q fever in humans and coxiellosis in livestock. Cattle, goats, and sheep serve as the primary reservoirs for the pathogen, facilitating its transmission to humans [28]. The primary routes of human infection are through the inhalation of contaminated aerosols or the consumption of unpasteurized milk and dairy products [29]. In Europe, 523 cases of Q fever were identified in 2020, resulting in a case fatality rate of 2.1% [30]. Table 4 presents the results of studies conducted in Iran concerning the prevalence of *C. burnetii* in different food items. As illustrated in Figure 5, the foods with the highest contamination rates were raw milk (12.36%) and dairy products (6.40%). *C. burnetii* is a bacterium that causes Q fever, a zoonotic disease that can be transmitted from animals to humans. In numerous rural regions of Iran, milk is still produced and processed using traditional methods that fail to meet the requisite modern hygiene standards [31]. The absence of adequate hygiene protocols in milk

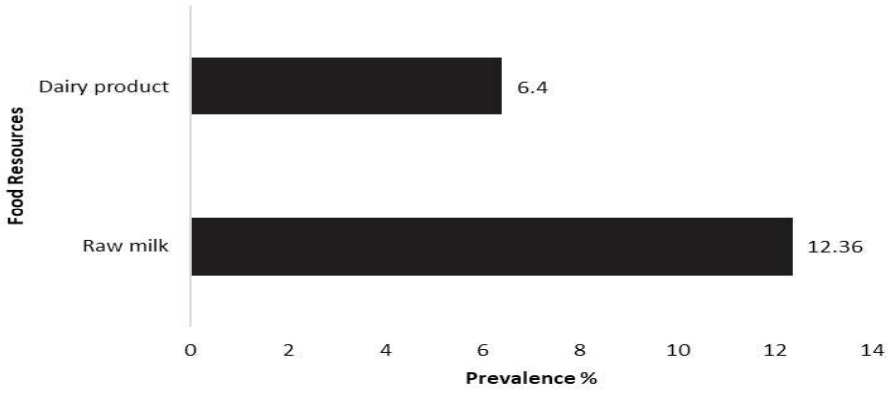

Figure 5.
Prevalence of *C. burnetii* in different foods in Iran.

Table 4

production and processing facilities may result in the contamination of milk with *C. burnetii*. Moreover, the proximity of animals to humans in the rural areas of Iran contributes to the high levels of contamination of raw milk and dairy products with *C. burnetii* [32]. Animals, such as cows and goats, can carry the bacterium and shed it in their milk, which can then be transmitted to humans through consuming contaminated dairy products [33].

A study conducted in Italy in 2017 reported that 15% of milk samples were contaminated with *C. burnetii*, with a higher prevalence of contamination in bovine milk (41%) compared to sheep milk (12%) [34]. In Brazil, in 2020, 9.43% of cheese samples (out of 53 samples) were positive for *C. burnetii* DNA [35]. Another research in the United States reported that 94% of bulk milk samples from dairy herds were contaminated with *C. burnetii* [36]. Our review indicates that the data from Iran align with the reports from other countries. However, it should be noted that the prevalence of *C. burnetii* contamination varies depending on the type of dairy products, including specific variations within milk.

5) *Bacillus cereus* prevalence in food

Bacillus cereus spores are a well-documented contaminant of food that can survive high temperatures during cooking and pasteurization [37]. This bacterium is associated with two distinct types of gastrointestinal diseases: the emetic (vomiting) syndrome and the diarrheal syndrome [38]. In Europe, 835 cases of foodborne illness caused by *B. cereus* were reported in 2020, with a hospitalization rate of 1.2% and a mortality rate of 0.1% [30]. The diarrheal syndrome is typically attributed to the consumption of contaminated foods, including raw and cooked beef, meat products, fish, poultry, soups, sauces, stews, milk, and vegetables. In contrast, the emetic syndrome is associated with the consumption of a toxic dose of the pre-formed emetic (cereulide) toxin produced by *B. cereus* in starchy foods, such as rice, pasta, noodles, potatoes, bread, pastries, and sesame products [39]. Table 5 presents the results of studies conducted in Iran regarding the prevalence of *B. cereus* in different food items. As illustrated in Figure 6, the highest prevalence of *B. cereus* contamination was observed in rice (100%), followed by raw milk (48.8%), poultry meat (42.17%), spices (42%), infant food (32.62%), dried vegetables (31.42%), meat products (11.16%), red meat (9.33%), and dairy products (8.9%) (Figure 6). In Australia, B. cereus contamination was identified in a variety of food samples, including uncooked pizza bases (1.58%), cooked pizzas (4.57%), processed meats (0.28%), cooked meat pies (4.45%), cooked sausage rolls (3.26%), and raw diced chicken (5.45%) out of 1,263 retail food samples [40]. In China, *B. cereus* contamination was observed in 50% of rice and noodle samples, 34% of cooked meat samples, and 22% of cold vegetable dishes [41]. In Poland, the highest prevalence of *B. cereus* contamination was found in herbs and spices, with a rate of 63.3%. Moreover, other food items, including breakfast cereals, pasta, rice, pasteurized milk, infant formulas, as well as fresh and ripening cheeses, were also found to be contaminated with *B. cereus* [37].

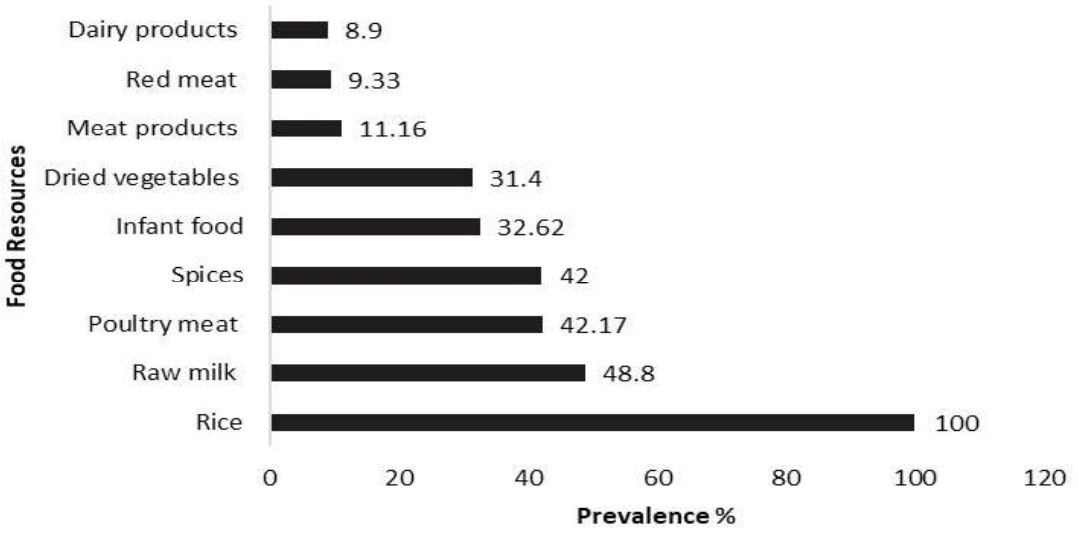

Figure 6.
Prevalence of *B. cereus* in different foods in Iran.

Table 5.
Summary of the studies reporting the prevalence of *B. cereus* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	References	Area
1	2023	Various Spices	200	84	42	[251]	Isfahan
2	2020	Individual meat	200	29	14.5	[252]	Zanjan
		Raw lamb					
		Raw beef	60	7	3.5		
		Cooked beef	60	10	5		
			80	12	6		
3	2020	Traditional dairy products	150	16	10.6	[253]	Tabriz
4	2019	Different rice	10	10	100	[254]	Zanjan
5	2018	Cow's raw milk	120	13	10.8	[255]	Tabriz
6	2018	Dried vegetable	140	44	31.4	[256]	Tehran
7	2018	Cream	62	0	0	[257]	Zanjan
8	2017	Different types of beef burgers	80	18	22.5	[258]	Tehran
9	2017	Milk-based infant food	300	9	3	[259]	
10	2017	Powdered infant formula milk	125	84	67.2	[260]	
11	2017	Cow milk	42	41	97.6	[261]	
12	2016	Cheese	200	10	5	[262]	
13	2016	Beef meat and poultry	380	44	11.8	[97]	
14	2016	Dairy products	230	46		[263]	
15	2015	Poultry meat foods Frozen	104	80	76.5	[264]	Tehran
		Semi cooked	39	9	24.2		
		Refrigerated	46	26	[97]		
16	2014	Rice	408	408	100	[265]	Urmia
17	2013	Infant foods	200	84	42	[266]	Isfahan
18	2012	Kefir type drinks	32	9	28	[267]	
		Pasteurized milk	32	12	Tehran		
19	2007	Infant formula	60	11	18.3	[268]	Tehran

6) *Yersinia enterocolitica* prevalence in food

In Europe, 236 cases of foodborne yersiniosis were reported in 2020, with 4.7% of cases necessitating hospitalization [30]. *Yersinia enterocolitica* contamination has been documented in a variety of foods in Europe, including red meat (beef, pork, and lamb), poultry, seafood, eggs, milk and milk products, bean sprouts, vegetables, tofu, and stewed mushrooms [42]. Table 6 presents the results of studies conducted in Iran regarding the prevalence of *Y. enterocolitica* in different food items. As illustrated in Figure 7, poultry meat exhibited the highest contamination rate of 16.81% in Iran. This was followed by raw milk (11.93%), red meat (11.63%), and dairy products (10%) (Figure 7). In Europe, 5.2% of RTE meat was found to be positive for *Yersinia* in 2020, which is a relatively high and concerning rate [30]. A study conducted in Argentina in 2019 reported chicken (12.4%) and bovine-originated foods (10.2%) as the most contaminated foods with *Y. enterocolitica* [43], which aligns with the findings in Iran. However, the latter study reported a lower prevalence of contamination in dairy products (0.7%) compared to the findings in Iran [43]. The elevated contamination rates of *Y. enterocolitica* in poultry meat observed in Iran and Argentina can be attributed to several factors, including the hygiene practices employed during the processing, transportation, and

Table 6.
Summary of the studies reporting the prevalence of *Y. enterocolitica* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	References	Area
1	2022	Raw Milk	360	3	0.83	[269]	Tehran
2	2021	red meat	200	26	13	[270]	Shiraz
3	2021	Bovine Raw Milk	100	33	33	[271]	Mashhad
4	2021	Traditional Cheeses	200	38	19	[272]	Khorasan Razavi and Golestan
5	2020	Raw milk	360	3	0.8	(Soltan Dallal, 2020)	Tehran
6	2019	Cheeses	200	38	19	[273]	Khorasan Razavi and Golestan
		Raw milk	100	33	33		
7	2018	Chicken meat	100	25	25	[274]	Mashhad
8	2018	Raw milk (sheep & goats)	100	9	9	[275]	Shahrekod
9	2018	Turkey meat	300	55	18.3	[276]	Shahrekord
10	2015	Meat	450	56	12.4	[277]	Tehran
		Chicken meat	226	35	15.4		
		Beef meat	224	21	9.3		
11	2015	Raw milk	446	19	4.3	[278]	Varamin
12	2014	Dairy products	552	28	5	[279]	Isfahan
13	2014	Raw chicken meat	300	65	21.6	[280]	Shahrekod
14	2014	Unpasteurized cream	100	3	3	[281]	Tehran
15	2013	Chicken meat	720	132	18.3	[282]	Shahrekod
16	2012	Bulk raw milk	354	8	2.6	[283]	Eastern Azerbaijan
		Cheeses	200	8	4		
17	2012	Chicken meat	200	18	9	[121]	Mazandaran
18	2012	Broiler meat	120	19	15.8	[284]	Tabriz
19	2011	Beef and chicken meat	379	48	12.6	[285]	Tehran

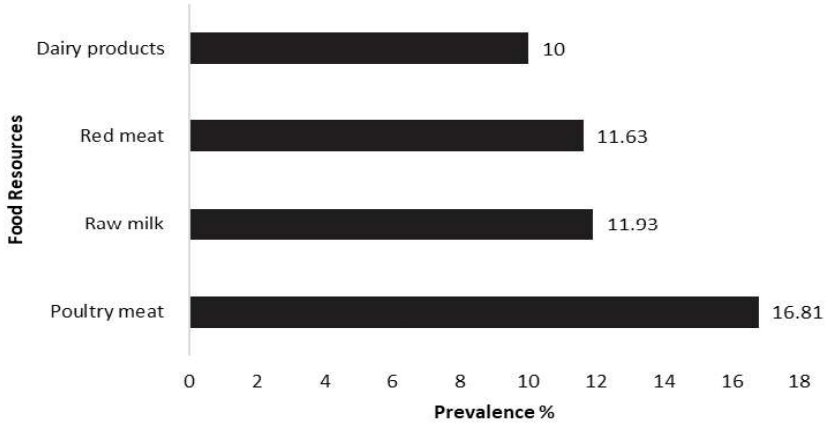

Figure 7.
Prevalence of *Y. enterocolitica* in different foods in Iran.

storage of these products [44]. Poultry meat has been identified as a significant source of *Y. enterocolitica* contamination due to the presence of the bacterium in the intestinal tracts of birds [45]. Inappropriate handling and processing of poultry can result in the cross-contamination of the meat with *Y. enterocolitica*. In addition, raw milk, red meat, and dairy products can serve as reservoirs for *Y. enterocolitica* if not properly pasteurized or handled [46].

7) *Campylobacter* prevalence in food

Campylobacter spp. has been identified as the leading cause of foodborne gastroenteritis in Europe since 2005 [30]. In addition to acute gastroenteritis, *Campylobacter* infections can also result in chronic manifestations in humans [47]. Among the various species within the genus Campylobacter, *C. jejuni* and *C. coli* are the most commonly reported causes of Campylobacteriosis in humans [48]. Table 7 presents the results of studies conducted in Iran regarding the prevalence of *Campylobacter* in different food items. As illustrated in Figure 8, the most prevalent occurrence of *Campylobacter* contamination in Iran was observed in poultry meat (46.21%), followed by red meat (40%) and eggs (28.06%). The contamination of dairy products and raw milk was observed in 2.36% and 2.5% of samples, respectively (Figure 8). A study conducted in

Table 7.
Summary of the studies reporting the prevalence of *Campylobacter* spp. in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	*Campylobacter spp.*	References	Area
1	2023	chicken meat	100	81	81	*Campylobacter spp.*	[286]	Hamedan
2	2023	chicken meat	255	64	25.09	*Campylobacter spp*	[287]	Shahrekord
3	2023	Mushrooms	740	74	10	*Campylobacter spp*	[288]	-
4	2022	poultry meat	380	24	6.25	*Campylobacter spp*	[289]	Shahrekord
5	2022	poultry meat	100	35	35	*Campylobacter spp*	[290]	Tehran
6	2022	raw meat	200	27	13.5	*Campylobacter jejuni*	[291]	-
7	2022	cattle raw milk	100	7	7	*Campylobacter jejuni*	[292]	Mazandaran
8	2021	Poultry Carcasses	370	203	54.8	*Campylobacter spp*	[293]	south of Iran
9	2021	Camels meat	40	5	12.5	*Campylobacter spp.*	[294]	Chaharmahal and Bakhtiari
10	2019	poultry meat	328	217	66.7	*Campylobacter spp.*	[295]	Jahrom
11	2019	Industrial chicken meat		1	0.6	*Campylobacter spp.*	[295]	Ahvaz
				0	0			
		Traditional chicken meat		8	16	*C. jejuni*		
				3	37.5	*Campylobacter spp*		
		Fresh packed chicken meat		0	0	*C. jejuni* *Campylobacter spp*		
		Beef meat		7	14	*Campylobacter spp.*		
				7	100			
		Mutton meat		12	24	*C. jejuni*		
				12	100			
		Water buffalo meat	50	4	8	*Campylobacter spp.*		
				4	100	*C. jejuni*		
12	2019	Packed chicken meat		26	28.9	*Campylobacter spp.*	[296]	Shiraz

Table 7 cont.

13	2016	Red meat	90	21	23	*Campylobacter spp.*	[297]	Zanjan
				13	61.9			
		Chicken-meat	120	33	27.5	*C. jejuni*		
				22	66.6	*Campylobacter spp.*		
		Eggshells	120	38	31.6	*C. jejuni*		
				20	52.6	*Campylobacter spp.*		
14	2015	Chicken wing	96	37	38.5	*Campylobacter spp.*	[298]	Urmia
15	2015	Meat	360	227	63.1	*Campylobacter spp.*	[299]	Mashhad
				200	88.1	*C. jejuni*		
16	2015	Raw ovine milk	38	0	0	*C. jejuni*	[300]	Zanjan
		Raw bovine milk	22	0	0	*C. jejuni*		
17	2014	Chicken	250	110	44	*Campylobacter spp.*	[300]	Tehran
				87	79			
18	2013	Pasteurized milk	30	0	0	*Campylobacter spp.*	[301]	Isfahan & Chaharmahal va Bakhtyari
		Camel milk	37	0	0			
		Commercial dairy	290	0	0			
		Raw cow milk	80	5	6.2	*Campylobacter spp.*		
		Raw sheep milk	60	1	1.6	*Campylobacter spp.*		
		Raw goat milk	60	2	3.3	*Campylobacter spp.*		
		Traditional cheese	60	3	5	*Campylobacter spp.*		
		Traditional ice-cream	35	1	2.8	*Campylobacter spp.*		
		Traditional butter	25	1	4	*Campylobacter spp.*		
19	2012	Packed chicken meat	96	22	22.9	*Campylobacter spp.*	[121]	Mazandaran
		Unpacked chicken meat	104	31	28.8	*Campylobacter spp.*		
20	2011	Eggs	100	0	0	*C. jejuni*	[124]	Shahrekord
21	2011	Raw bovine milk	120	3	2.5	*Campylobacter spp.*	[302]	Isfahan
22	2011	Chicken	200	94	47	*Campylobacter spp.*	[303]	Shahrekord
				91	96.8	*C. jejuni*		
		Turkey	49	49	49	*Campylobacter spp.*		
				41	83.7	*C. jejuni*		
		Quail	33	37	43	*Campylobacter spp.*		
				33	89.2	*C. jejuni*		
		Partridge	6	6	35.3	*Campylobacter spp.*		
				6	100	*C. jejuni*		
		Ostrich	21	1	4.8	*Campylobacter spp.*		
				1	100	*C. jejuni*		
23	2010	Chicken meat	60	37	61.7	*Campylobacter spp.*	[304]	Ahvaz
				35	94.6	*C. jejuni*		
		Turkey meat	50	18	83.3	*Campylobacter spp.*		
				15	36	*C. jejuni*		
		Sheep meat	50	3	6	*Campylobacter spp.*		
				1	33.3	*C. jejuni*		
		Goat meat	45	17	4.4	*Campylobacter spp.*		
				17	100	*C. jejuni*		

Table 7 cont.

24	2010	Raw camel meat	107	1	0.9	*Campylobacter spp.*	[305]	Isfahan & Yazd
				0	0	*C. jejuni*		
		Beef meat	190	5	2.4	*Campylobacter spp.*		
				3	60	*C. jejuni*		
		Lamb meat	225	27	12	*Campylobacter spp.*		
				23	92	*C. jejuni*		
		Goat meat	180	17	9.4	*Campylobacter spp.*		
				16	94.1	*C. jejuni*		
25	2008	Raw chicken meat	280	157	56.1	*Campylobacter spp.*	[306]	Isfahan
				140	89.2	*C. jejuni*		
		Quail meat	248	68	27.4	*Campylobacter spp.*		
				53	77.9	*C. jejuni*		
		Turkey meat	212	145	68.4	*Campylobacter spp.*		
				92	63.4	*C. jejuni*		
		Ostrich meat	60	7	11.7	*Campylobacter spp.*		
				3	42.9	*C. jejuni*		

the United States in 2020 reported that while various broiler products carry the risk of *Campylobacter spp.* contamination, the highest prevalence of contamination was observed in chicken carcasses [49]. Similarly, in the European Union, *C. jejuni* has been identified as the most prevalent species (51%) in broiler meat, followed by *C. coli* (35.5%) [47]. Consequently, poultry meat represents the greatest risk of *Campylobacter* transmission to humans worldwide. The consistent reporting of the highest prevalence of *Campylobacter* contamination in poultry meat in multiple studies, including those conducted in Iran, the United States, and the European Union, underscores the importance of addressing this issue [47, 49]. This finding highlights the necessity of implementing rigorous food safety measures and regulations in the poultry industry to prevent the transmission of *Campylobacter* to consumers.

8) *Helicobacter pylori* prevalence in food

Helicobacter pylori is associated with several digestive diseases, including peptic ulcer, mucosa-associated lymphoid tissue lymphoma, gastritis, and an increased risk of gastric cancer [50]. It is estimated that approximately 50% of the global population is infected with *H. pylori* [51]. The prevalence of *H. pylori*

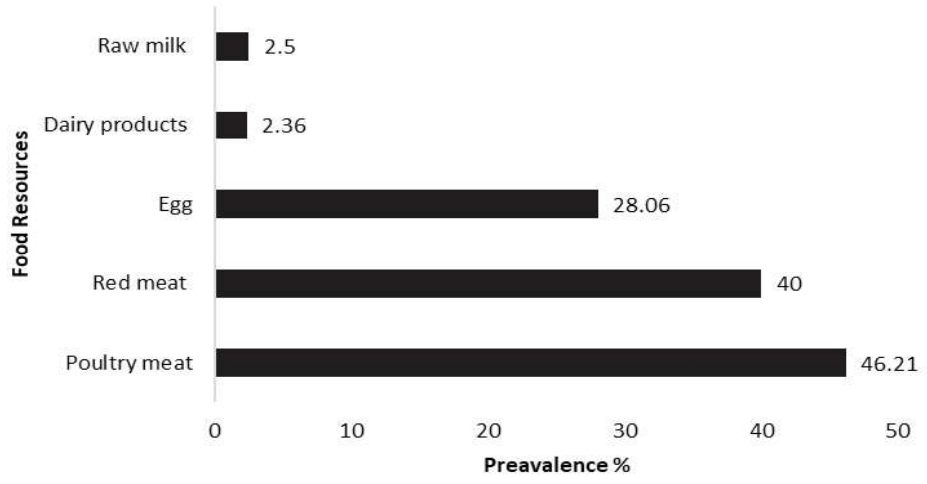

Figure 8.
Prevalence of *Campylobacter* in different foods in Iran.

infection is observed to be higher in developing countries, with rates ranging from 70% to 90%, compared to developed countries, where rates are reported to be 25%-50%. Iran is considered a high-risk region for *H. pylori* infection due to the high prevalence (60%-90%) among its population [52]. *H. pylori* can be found in a variety of animal-derived foods, vegetables, and water sources, which contribute to its transmission [50]. Table 8 presents the findings of studies conducted in Iran regarding the prevalence of *H. pylori*

Table 8.
Summary of the studies reporting the prevalence of *H. pylori* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	References	Area
1	2023	Raw Poultry Meat	320	20	6.25	[307]	Shahrekord
2	2020	Red meat	*600*	*52*	8.6	[308]	Tehran
3	2018	Traditional dairy products	800	31	3.8	[309]	Isfahan
4	2017	Red meat	220	11	5	[310]	Isfahan
5	2017	Meat	150	11	7.3	[311]	Alborz
		Milk	150	24	16		
		Vegetable	40	5	12.5		
6	2016	Cream-candy	50	9	18	[312]	Isfahan
		Traditional bread	50	3	6		
		Sausage	50	0	0		
		Salami	50	0	0		
		Hamburger	50	1	2		
		Soup	50	11	22		
		Restaurant salad	50	15	30		
		Falafel	50	3	6		
		Olivier salad	50	18	36		
		Chicken nugget	50	0	0		
		Fruit salad	50	14	28		
7	2016	Milk	420	92	21.9	[313]	Shahrekord
		Meat	400	105	26.2		
8	2016	Meat products	150	11	7.3	[314]	Isfahan
9	2016	Drinking water (total)	120	5	4.2	[315]	Isfahan
			110	2	1.8		Shiraz
			100	3	3		Yazd
							Shahrekord
10	2016	Ready to eat fish	70	2	2.8	[316]	Shiraz
		Ham	60	9	15		
		Chicken sandwich	60	5	8.3		
		Vegetable sandwich	40	2			
		Meat sandwich	40	18	5		
		Minced meat	50	10	45		
		Minced meat	50	16	20		
					32		
11	2015	Raw milk	210	28	13.3	[317]	Tehran
12	2015	Bovine milk	120	20	16.6	[318]	
		Traditional cheese	80	10	[318]		
		Traditional cream	40	3	7.5		
		Total	240	33	[319]		

Table 8 cont.

13	2014	Vegetable and salad	460	44	9.5	[319]	Shahrekord
14	2014	Vegetable and salad	430	59	13.7	[320]	Isfahan
15	2013	Water	200	14	7.2	[51]	Isfahan
16	2012	Milk	447	56	12.5	[321]	Isfahan, Fars, Chaharmahal & Bakhtiari, Khuzestan

in various food items. As illustrated in Figure 9, the highest prevalence of *H. pylori* in food samples in Iran was observed in RTE foods (25.5%) and vegetables (22.14%), followed by raw milk (16.06%), red meat (15.82%), dairy products (7.93%), meat products (6.26%), and water (3.8%) (Figure 9). In other countries, studies have also identified the presence of *H. pylori* in a variety of food sources. In Japan, the ureA gene of *H. pylori* was found in 72.2% of raw milk samples and 55% of pasteurized milk samples [53]. In Italy, the glmM gene of *H. pylori* was identified in 34.7% of raw milk samples [54]. In the United States, *H. pylori* was detected in 44% of RTE raw tuna meat and 36% of raw chickens using a multiplex PCR assay [55]. These findings underscore the potential presence of *H. pylori* in various food sources and the significance of food as a potential route of transmission.

9) *Clostridium* prevalence in food

Clostridium botulinum

Clostridium botulinum is a gram-positive, anaerobic bacterium that is capable of producing spores. It is known to cause botulism, a severe illness characterized by the production of a potent neurotoxin. Table 9 presents the findings of research conducted in Iran on the prevalence of *C. botulinum* in various food items. As illustrated in Figure 10, the most prevalent contamination of *C. botulinum* in Iran was observed in seafood (12.56%), followed by red meat (12.23%), dairy products (9.02%), and honey (2%) (Figure 10). Honey is recognized as a reservoir for *C. botulinum* spores, particularly types B and A, and has been implicated in cases of neonatal botulism [30]. Studies conducted in various countries, including Turkey, Brazil, Denmark, Sweden, and Norway, have demonstrated the presence of *C. botulinum* spores in honey samples, with prevalence rates ranging from 2% to 26% [30]. In Iran, the prevalence of *C. botulinum* contamination in honey samples was reported to be 2% (Figure 10), indicating a relatively lower level of contamination compared to some other regions.

While *C. botulinum* spores may be present in certain foods, the risk of botulism is contingent upon the conditions that facilitate the germination of spores and toxin production, such as inadequate food processing, storage, or handling. Proper food safety practices, including adequate cooking, storage at appropriate temperatures, and hygienic handling, can help prevent the growth and toxin production of *C. botulinum* in food.

Clostridium perfringens

C. perfringens is a significant contributor to foodborne gastrointestinal illnesses in both humans and animals. The spores of *C. perfringens* exhibit remarkable resilience to external influences. In Europe in 2020, there

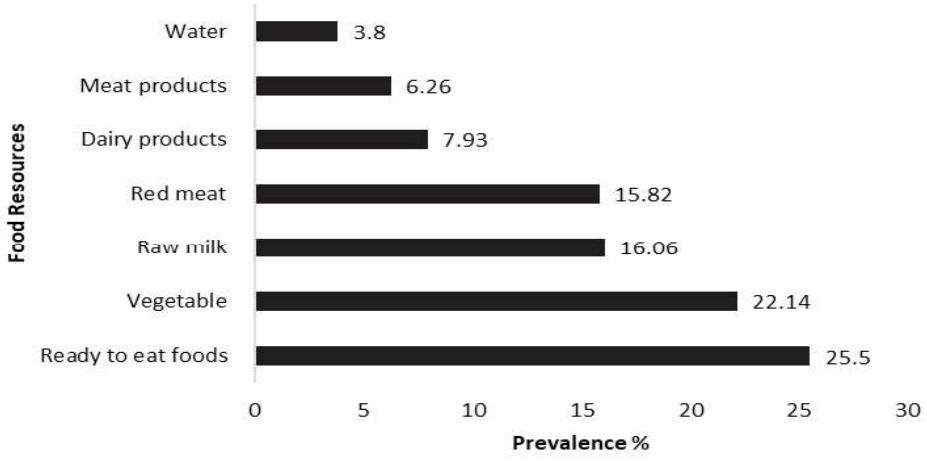

Figure 9.
Prevalence of *H. pylori* in different foods in Iran.

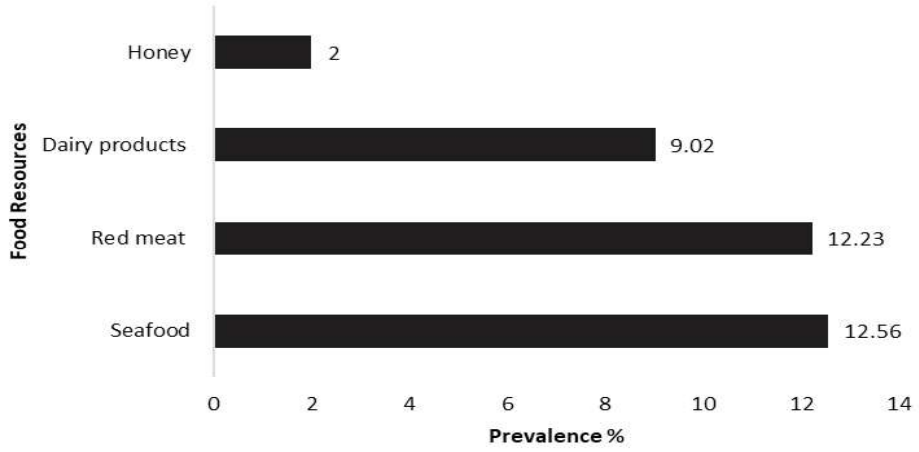

Figure 10.
Prevalence of *Clostridium* in different foods in Iran.

were 682 reported cases of food poisoning caused by *C. perfringens* toxins, with a hospitalization rate of 1.5%. Conversely, there were fewer cases (n = 34) of food poisoning due to *C. botulinum* toxins, yet the hospitalization rate for botulism cases was 100%. It is noteworthy that no fatalities were reported in these cases. Early diagnosis, hospitalization, and treatment are essential for reducing the severity of botulism [30]. Table 9 presents the findings of studies conducted in Iran regarding the prevalence of *C. perfringens* in various food items. *C. perfringens* type A is the most prevalent cause of food poisoning associated with this bacterium. The available data indicate that *C. perfringens* was most commonly isolated from red meat in Iran. It is of paramount importance to ensure that meat is cooked and handled properly to minimize the risk of contamination with *C. perfringens* and subsequent foodborne illnesses. In Europe in 2019, two outbreaks were associated with pig meat and products, one caused by toxins produced by *C. perfringens* and the other by *C. botulinum*. Conversely, vegetables, juices, and other related products were linked to a greater number of outbreaks, with two outbreaks reported for each category during the same period [30]. Nevertheless, only one study has been conducted in Iran regarding the presence of *C. perfringens* in vege-

Table 9.
Summary of the studies reporting the prevalence of *Clostridium spp*. in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	Clostridium spp.	References	Area
1	2023	Meat native birds	300	35	11.6	*C. perfringens*	[322]	Shahrekord
2	2023	Meat Nuggets	600	7	1.17	*C. perfringens*	[323]	Isfahan
3	2023	Ground Beef	133	24	18.04	*C. perfringens*	[324]	Qazvin
			94	3	3.22			
4	2022	Raw Meat	240	7	2.91	*C. perfringens*	[325]	Mazandaran
5	2022	Raw Beef Meats	133	18	13.53	*C. perfringens*	[326]	Qazvin
6	2022	raw and ready-to-eat green leafy vegetables	366	66	18	*C. perfringens*	[139]	Tehran
7	2022	Olivier Salad	26	0	0	*C. perfringens*	[327]	Mashhad
8	2021	Cattle and sheep carcasses	200	61	30.5	*C. perfringens*	[328]	Shiraz

Table 9 cont.

9	2021	Broiler chickens	122	95	77.8	*C. perfringens*	[329]	Kerman
10	2019	Broiler chickens	400	169	42.2	*C. perfringens*	[330]	Chaharmahal & Bakhtiari
11	2019	Honey	130	0	0	*C. perfringens*	[331]	
12	2017	Traditional curds	50	12	25	*C. perfringens*	[332]	Shahrekord
		Commercial curds	50	5	10			
		Beef meat	20	1	6			
		Lamb meat	23	3	13			
13	2015	Broiler meat	200	31	15.5	*C. perfringens*	[333]	Mashhad
14	2015	Minced meat	200	25	12.5	*C. perfringens*	[334]	Mashhad
15	2013	Honey	100	2	2	*C. perfringens*	[335]	Shiraz
16	2013	Fish	80	4	5	*C. perfringens*	[336]	Shiraz
		Honey	50	2	4			
		Kashk	80	2	2.5			
		Dough	80	1	1.2			
17	2013	Dairy products	57	12	21	*C. perfringens*	[337]	Gilan, Tehran, Golestan, Hamedan
		Fish	68	18	26.4			
		Meat	14	1	7.1			
18	2010	Cheese	57	2	3.5	*C. perfringens*	[338]	Gilan
		Kashk	11	0	0			
		Salted fish	63	4	6.3			

tables and juices, and other related products. Further research and surveillance are necessary to gain a more comprehensive understanding of the prevalence and sources of *C. perfringens* in various food items in Iran.

10) *Brucella* prevalence in food

Brucella spp. are the causative agents of brucellosis [56], an infectious disease of humans that presents with chronic and recurring febrile symptoms that can be life-threatening [57]. The primary etiological agent of the disease is *B. melitensis*, although other species, including *B. abortus, B. canis,* and *B. suis*, can also result in human brucellosis [58]. The infection can be transmitted to humans from various animals, including buffalo, cattle, yak, elk, camel, domestic pig, and rodents [58]. Globally, approximately 500,000 cases of human brucellosis are reported annually, with animals and animal-derived foods serving as the primary sources of infection [57]. A global systematic review conducted in 2020 revealed that the Southeast Asia region exhibited the highest prevalence of *Brucella spp.* at 25.55% [57]. The consumption of unpasteurized dairy products plays a significant role in the transmission of *Brucella spp*. to humans [57]. Table 10 presents the results of studies conducted in Iran on the prevalence of *Brucella spp*. in food. As illustrated in Figure 11, the primary sources of reported contamination with *Brucella spp.* are dairy products (34.28%) and raw milk (16.64%). Dairy products, particularly unpasteurized or inadequately pasteurized ones, can serve as reservoirs for *Brucella* contamination [57]. This can occur due to infected dairy animals shedding the bacteria in their milk. Raw milk, in particular, has been identified as a common source of *Brucella* infection in various parts of the world, including Iran. Improper handling and processing of raw milk can contribute to the transmission of *Brucella spp.* to humans [59].

In Iran, where dairy products hold cultural and dietary significance, ensuring the safety of these products from *Brucella* contamination is crucial for public health [60]. Implementing stringent control measures in dairy production, processing, and distribution can help mitigate the risk of *Brucella* transmission through dairy products and raw milk [57, 59, 60].

11) *Vibrio* prevalence in food

Vibrio spp. are halophilic marine bacteria. Some species, including *V. cholerae, V. parahaemolyticus,* and *V. vulnificus*, have the potential to cause gastroenteritis or septicemia in humans. The primary mode of transmission for this foodborne illness is the ingestion of raw, undercooked, or mishandled seafood contaminated by bacteria [61]. Table 11 presents the re-

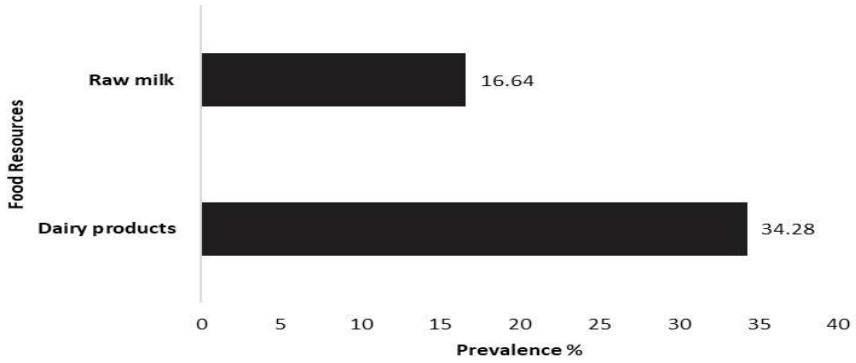

Figure 11.
Prevalence of *Brucella* in different foods in Iran.

Table 10.
Summary of the studies reporting the prevalence of *Brucella spp.* in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	*Brucella spp.*	References	Area
1	2022	Unpasteurized Milk and Dairy	291	12	4.1	*Brucella spp.*	[339]	Hamadan
2	2021	Bovine Milk	240	16	6.66	*Brucella spp.*	[340]	Kurdistan
3	2020	Dairy products	227	9	4	*Brucella spp.*	[341]	Hamadan
		Non boiling milk	43	1	2.3			
		Fresh cheese Cream	21	2	[341]			
4	2019	Raw camel milk	96	3	3	*Brucella spp.*	[342]	Isfahan, Semnan
			51	2	[342]			
			45	1				
5	2018	Dairy Products	208	60	28.8	*Brucella spp.*	[58]	Tehran
		Goat raw milk	33	15	45.5			
		Non-pasteurized cheese	23	9	39.1			
		Sheep raw milk	33	9	27.3			
		Cow raw milk	57	15	26.3			
		Pasteurized cheese	28	7	25			
		Pasteurized milk	34	5	14.7			
6	2017	Dairy Products	14	11	78.6	*Brucella spp.*	[343]	Tehran
				8	72.7	*B. melitensis*		
				3	27.3	*B. abortus*		
7	2017	Sheep raw milk Goat raw milk	530	41	8.1	*Brucella spp.*	[344]	Kerman
8	2017	Raw milk	700	9	1.28	*Brucella spp.*	[345]	Kerman
		Sheep's raw milk	300	3	1			
		Goats raw milk	400	6	1.5			
9		Unpasteurized milk	132	4	3	*Brucella spp.*	[346]	Isfahan
		Dairy products	65	1	1.5			
10	2016	Cow's raw milk	48	4	8.3	*Brucella spp.*	[347]	Kerman

Table 10 cont.

11	2016	Milk	225	20	8.9	*Brucella spp.*	[348]	Shahrekord & Isfahan
		Sheep milk	125	12	9.6			
		Goat milk	100	18	18			
12	2016	Raw goat milk	470	51	10.8	*Brucella spp.*	[344]	Southeast region of Iran
		Raw sheep milk	330	18	5.4			
13	2015	Raw milk	60	32	53.3	*Brucella spp.*	[200]	Zanjan
14	2014	Raw cow milk	57	19	33	*Brucella spp.*	[349]	Tehran
		Pasteurized cow milk	34	10	29			
		Pasteurized cheese	28	8	28			
		Traditional cheese	23	14	60			
		Raw goat milk	33	21	63			
		Raw sheep milk	33	19	57			
15	2013	Cattle milk	1117	18	1.6	*Brucella spp.*	[350]	Urmia
		Sheep milk	598	99	16.5			

sults of studies conducted in Iran on the prevalence of Vibrio spp. in different types of food. *Vibrio spp*. were predominantly detected in seafood, including lobster, fish products, crayfish, fish, and shrimp, as well as drinking water. As illustrated in Figure 12, the prevalence of Vibrio spp. was highest in seafood, with fish exhibiting the greatest incidence (49.33%), followed by lobster (21.53%), crayfish (8.63%), shrimp (8.12%), fish products (7.8%), and drinking water (1.3%) (Figure 12). The findings from Iran are in alignment with those from other countries. For instance, a comprehensive systematic review conducted in 2016 revealed that V. parahaemolyticus contamination was observed in 63.4% of oysters, 52.9% of clams, 51% of fish, and 48.3% of shrimps [62]. A similar study in China in 2020 reported that 15.34% of shrimp samples, 14.17% of fish samples, and 3.67% of RTE food were contaminated with *V. parahaemolyticus* [63]. However, there are no reports available from Iran regarding the prevalence of *V. parahaemolyticus* in RTE foods.

12) *Shigella* prevalence in food

The *Shigella* genus encompasses four known species: *S. dysenteriae, S. boydii, S. flexneri,* and *S. sonnei,* which have also been classified as subgroups A to D, respectively [64]. While *S. flexneri* has traditionally been reported as the main cause of shigellosis in developing countries, recent studies have shown that *S. sonnei* has become the predominant species of Shigella in Iran [64]. According to the WHO, *Shigella*

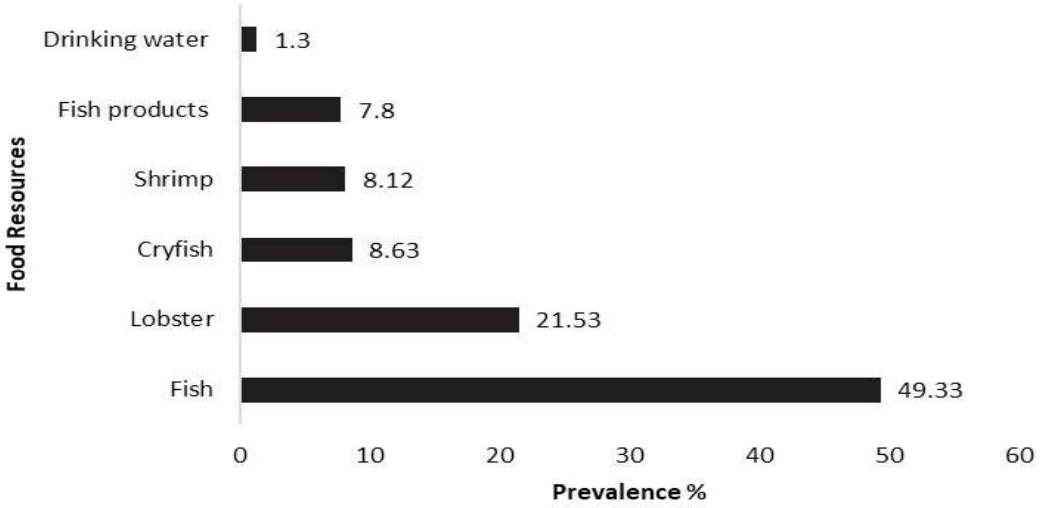

Figure 12.
Prevalence of *Vibrio* in different foods in Iran.

Table 11.
Summary of the studies reporting the prevalence of *Vibrio spp*. in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	Type of *Vibrio spp.*	References	Area
1	2021	Fish	64	61	95	*Vibrio species*	[351]	Isfahan
2	2020	Frozen fish	*200*	*0*	*0*	*V. parahaemolyticus*	[352]	Mazandaran
3		Shrimp	70	12	17.1	*V. parahaemolyticus*	[353]	Zanjan
4	2018	Cold Smoked Salted Fishes	200	46	23	*Vibrio spp.*	[354]	Mazandaran
5	2016	Fish	58	18	31	*V. parahaemolyticus*	[355]	Persian Gulf
		Shrimps	55	7	12.7			
6	2015	Fresh shrimps	30	2	6.6	*V. parahaemolyticus*	[356]	Genaveh seaport
		Salted shrimps	30	2	6.6			
7	2014	Shrimps	36	7	19.4	*Vibrio spp.*	[357]	South coast of Iran
8	2014	Fish	100	22	22	*V. parahaemolyticus*	[358]	Bushehr, Persian Gulf
		Lobster	60	13	21.6			
		Crab caught	40	7	17.5			
9	2014	Crayfish	97	11	11.3	*V. vulnificus*	[357]	Aras
				7	7.2	*V. harveyi*		
				2	2	*V. alginolyticus*		
				1	1	*V. mimicus*		
10	2013	Tap-water	144	3	2	*V. cholerae*	[360]	Isfahan
		Bottled mineral water	304	3	0.6			
11	2012	Fresh shrimp	70	5	7.1	*V. parahaemolyticus*	[122]	
		Salted fishes	70	2	2.9			
		Fish nugget	10	0	0			
		Shrimp burger	10	0	0			
12	2012	Lobsters	100	40	40	*Vibrio spp.*	[361]	Persian Gulf
			100	3	3	*V. parahaemolyticus*		
		Crab	32	4	12.5	*Vibrio spp.*		
			32	1	3.1	*V. parahaemolyticus*		
13	2010	Fresh shrimp	300	29	9.6	*V. parahaemolyticus*	[361]	Bohsher. Hormozgan,Khoozcstan
14	2004	Fresh shrimp	770	16	2.1	*Vibrio spp.*	[361]	Bohsher. Hormozgan, Khoozestan

spp. cause approximately 165 million cases of *bacillary* dysentery and 1 million deaths worldwide each year [64]. In general, *Shigella spp*. are among the most prevalent causes of acute diarrhea in Iran, with a particularly high incidence among children and young adults. A diverse array of foods, encompassing meat, dairy products, and vegetables, have been identified as potential sources of shigellosis outbreaks worldwide [64]. Table 12 presents the results of studies conducted in Iran on the prevalence of *Shigella spp.* in different types of food. As illustrated in Figure 13, contamination with *Shigella spp*. is most commonly reported in RTE foods (1.72%) and vegetables (1.05%), followed by red meat (0.4%). In contrast to the data from Iran, a high prevalence of *Shigella spp*. contamination has been reported in vegetables (25.25%) in India [65], and in beef, chicken, and dairy products in Egypt [66]. According to our review, poultry meat should be considered a high-risk food with the potential to spread foodborne zoonoses in Iran. In general, poultry meat

Table 12.
Summary of the studies reporting the prevalence of *Shigella spp*. in Iran.

	Year	Sample type	Sample size	Positive samples (N)	Prevalence (%)	Type of *Shigella spp.*	References	Area
1	2022	raw milk, ground meat, and raw vegetable	580	13	2.24	*Shigella sonnei*	[364]	Tehran and Qazvin
2	2021	Vegetable salad, ground meat, and raw cow's milk	*405*	*18*	*4.44*	*Shigella spp.*	[365]	Qazvin
3	2021	meat, vegetable salad and raw milk	165	8	4.84	*Shigella spp.*	[366]	Qazvin
4	2019	Ready to eat food	250	2	0.8	*S. sonnei*	[64]	Isfahan, Fars, Hormozgan, Kohkiloyeh va Boyer Ahmad
				0	0	*S. flexneri*		
				0	0	*S. dysenteriae*		
				0	0	*S. boydii*		
		Fresh meat	150	1	0.7	*S. sonnei*		
				2	1.3	*S. flexneri*		
				0	0	*S. dysenteriae*		
				0	0	*S. boydii*		
		Frozen meat	150	0	0	*Shigella spp.*		
		Cow milk	100	0	0	*Shigella spp.*		
		Domestic cheese	100	0	0	*Shigella spp.*		
		Vegetables	650	8	1.2	*S. sonnei*		
				6	0.9	*S. flexneri*		
				0	0	*S. dysenteriae*		
				0	0	*S. boydii*		
		1400		19	1.4	*Shigella spp.*		
5	2018	Ready-to-Eat Salad	90	7	7.8	*Shigella spp.*	[367]	Tehran
6	2018	Food (vegetables, chicken, minced meat, fish)	100	6	6	*Shigella spp.*	[368]	Shiraz
7	2014	Camel milk	18	0	0	*Shigella spp.*	[369]	Golestan

is more susceptible to contamination during processing and handling due to its higher water content and pH levels, which provide an optimal environment for the proliferation of bacteria [67]. Moreover, poultry meat is frequently sold and consumed in its raw state, thereby increasing the probability of contamination if the requisite hygiene standards are not observed during slaughter, processing, and storage. In contrast, red meat and seafood have lower contamination rates compared to poultry meat, likely due to differences in processing and handling practices [68]. These findings underscore the necessity of developing strategies to reduce the contamination levels of poultry meat to effectively control and prevent foodborne illnesses in Iran.

The risk of food contamination, particularly in meat products, is significant. However, to effectively underscore the importance of foodborne diseases, it is imperative to document the consequences of infection with these pathogens and generalize this information to the population in Iran. Currently, foodborne diseases in Iran are not generally reported, leading to a likely gross underestimation of their burden. This underestimation is attributable to the fact that many foodborne illnesses do not exhibit sufficient severity, duration, or specific diagnostic criteria for accurate identification and intervention. Similar circumstances exist in developed countries, such as the United States. For instance, the CDC estimates that foodborne pathogens cause approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually in the US [70].

Therefore, it is crucial to emphasize the necessity of establishing robust monitoring systems in Iran.

Such a surveillance network would require the collaboration of multidisciplinary teams comprising medical doctors, veterinarians, microbiologists, public health specialists, and other relevant experts, in alignment with the One Health concept. By adopting a methodology similar to that employed by the CDC's Foodborne Diseases Active Surveillance Network (FoodNet), which monitors the incidence of nine foodborne pathogens in ten US cities, representing approximately 15% of the American population [71], Iran can enhance the awareness of foodborne disease events and trends. These practices enable the implementation of effective intervention and prevention strategies.

Authors' Contributions

MH suggested the topic and supervised the conduction of the systematic review. SA wrote the first draft of the manuscript. FA and SA performed the literature review. GS was the major contributor in writing the manuscript. AA gave advice for conducting and writing the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors declare that there is no conflict of interest.

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References

1. Touranlou FA, Noori SMA, Salari A, Afshari A, Hashemi M. Application of kefir for reduction of contaminants in the food industry: A systematic review. International Dairy Journal. 2023:105748. Doi:10.1016/j.idairyj.2023.105748.

2. Law JW-F, Ab Mutalib N-S, Chan K-G, Lee L-H. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Frontiers in microbiology. 2015;5:122692. Doi:10.3389/fmicb.2014.00770.

3. Imre K, Herman V, Morar A. Scientific achievements in the study of the occurrence and antimicrobial susceptibility profile of major foodborne pathogenic bacteria in foods and food processing environments in Romania: Review of the last decade. BioMed Research International. 2020;2020. Doi:10.1108/NFS-01-2023-0023.

4. Shakeri G, Jamshidi A, Ghazvini K. Isolation of broad-host-range bacteriophages against food-and patient-derived Shiga toxin-producing Escherichia coli. Iranian Journal of Veterinary Science and Technology. 2020;12(1):11-20. Doi:10.22067/veterinary.v12i1.85298.

5. Moghimani M, Noori SMA, Afshari A, Hashemi M. D-tryptophan, an eco-friendly natural, safe, and healthy compound with antimicrobial activity against food-borne pathogens: A systematic review. Food Science & Nutrition. 2024;12(5):3068-79. Doi:10.1002/fsn3.3987.

6. Gourama H. Foodborne pathogens. Food safety engineering: Springer; 2020. p. 25-49. Doi:10.1007/978-3-030-42660-6_2.

7. Tîrziu E, Bărbălan G, Morar A, Herman V, Cristina RT, Imre K. Occurrence and antimicrobial susceptibility profile of Salmonella spp. in raw and ready-to-eat foods and Campylobacter spp. in retail raw chicken meat in Transylvania, Romania. Foodborne Pathogens and Disease. 2020;17(8):479-84. Doi:10.1089/fpd.2019.2738.

8. Hashemi M, Erfani A, Dadgar Moghadam M, Dousti Nouri M, Asadi Touranlou F, Azadi N, et al. Assessment of knowledge, Attitudes, and Practices of Food Service Staff Regarding Food Safety at Mashhad University Hospitals. Journal of Nutrition, Fasting and Health. 2024. Doi: 10.22038/JNFH.2024.78388.1505.

9. WHO. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015: World Health Organization; 2015.

10. Tack DM, Marder EP, Griffin PM, Cieslak PR, Dunn J, Hurd S, et al. Preliminary incidence and trends of infections with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US Sites, 2015–2018. Morbidity and Mortality Weekly Report. 2019;68(16):369. Doi:10.15585/mmwr.mm6816a2.

11. Pires SM, Evers EG, Van Pelt W, Ayers T, Scallan E, Angulo FJ, et al. Attributing the human disease burden of foodborne infections to specific sources. Foodborne pathogens and disease. 2009;6(4):417-24. Doi:10.1089/fpd.2008.0208.

12. Mukhopadhyay S, Ramaswamy R. Application of emerging technologies to control Salmonella in foods: A review. Food Research International. 2012;45(2):666-77. Doi:10.1016/j.foodres.2011.05.016.

13. WHO. Estimating the burden of foodborne diseases: a practical handbook for countries: a guide for planning, implementing and reporting country-level burden of foodborne disease. 2021.

14. Yang J, Zhang Z, Zhou X, Cui Y, Shi C, Shi X. Prevalence and characterization of antimicrobial resistance in Salmonella enterica isolates from retail foods in Shanghai, China. Foodborne Pathogens and Disease. 2020;17(1):35-43. Doi:10.1089/fpd.2019.2671.

15. Nair DV, Kollanoor Johny A. Salmonella in poultry meat production. Food safety in poultry meat production. 2019:1-24. Doi:10.1007/978-3-030-05011-5_1.

16. Shafini A, Son R, Mahyudin N, Rukayadi Y, Zainazor TT. Prevalence of Salmonella spp. in chicken and beef from retail outlets in Malaysia. International Food Research Journal. 2017;24(1):437.

17. Carrasco E, Morales-Rueda A, García-Gimeno RM. Cross-contamination and recontamination by Salmonella in foods: A review. Food Research International. 2012;45(2):545-56. Doi:10.1016/j.foodres.2011.11.004.

18. Ehuwa O, Jaiswal AK, Jaiswal S. Salmonella, food safety and food handling practices. Foods. 2021;10(5):907. Doi:10.3390/foods10050907.

19. Ehuwa O, Jaiswal A, Jaiswal S. Salmonella, Food Safety and Food Handling Practices. Foods 2021, 10, 907. Foodborne Pathogens and Food Safety. 2021:175. Doi:10.3390/foods10050907.

20. Kowalska B. Fresh vegetables and fruit as a source of Salmonella bacteria. Annals of Agricultural and Environmental Medicine. 2023;30(1). Doi: 10.26444/aaem/156765.

21. Kadariya J, Smith TC, Thapaliya D. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed research international. 2014;2014. Doi:10.1155/2014/827965.

22. Naas HT, Edarhoby RA, Garbaj AM, Azwai SM, Abolghait SK, Gammoudi FT, et al. Occurrence, characterization, and antibiogram of Staphylococcus aureus in meat, meat products, and some seafood from Libyan retail markets. Veterinary World. 2019;12(6):925. Doi: 10.14202/vetworld.2019.925-931.

23. Dallal MMS, Foroushani AR, Sharifi–Yazdi S, Sharifi-Yazdi MK, Arfatahery N. Prevalence of Staphylococcus aureus in Shrimps in Tehran during 2013. Journal of Medical Bacteriology. 2015;4(5-6):42-6.

24. Pourbabaee M, Hadadi MR, Hooshyar H, Pourbabaee P, Nazari-Alam A. Prevalence of Staphylococcus aureus in Raw Hamburgers from Kashan in 2017. International Archives of Health Sciences. 2020;7(1):47-50. Doi: 10.4103/iahs.iahs_39_19.

25. Hamidiyan N, Salehi-Abargouei A, Rezaei Z, Dehghani-Tafti R, Akrami-Mohajeri F. The prevalence of Listeria spp. food contamination in Iran: A systematic review and meta-analysis. Food Research International. 2018;107:437-50. Doi:10.1016/j.foodres.2018.02.038.

26. Authority EFS, Prevention ECfD, Control. The European Union one health 2020 zoonoses report. EFSA Journal. 2021;19(12):e06971. Doi:10.2903/j.efsa.2021.6971.

27. Khademi F, Sahebkar A. A systematic review and meta-analysis on the prevalence of antibiotic-resistant Listeria species in food, animal and human specimens in Iran. Journal of food science and technology. 2019:1-17. Doi:10.1007/s13197-019-04040-w.

28. Dalton HR, Dreier J, Rink G, Hecker A, Janetzko K, Juhl D, et al. Coxiella burnetii-pathogenic agent of Q (query) fever. Transfusion medicine and hemotherapy. 2014;41(1):60-72. Doi:10.1159/000357107.

29. Abdali F, Hosseinzadeh S, Berizi E, Shams S. Prevalence of Coxiella burnetii in unpasteurized dairy products using nested PCR assay. Iranian Journal of Microbiology. 2018;10(4):220.

30. EFSA. The European Union one health 2018 zoonoses report. EFSa Journal. 2019;17(12):e05926. Doi:10.2903/j.efsa.2019.5926.

31. Rafiee S, Khoshnevisan B, Mohammadi I, Aghbashlo M, Clark S. Sustainability evaluation of pasteurized milk production with a life cycle assessment approach: an Iranian case study. Science of the total environment. 2016;562:614-27. Doi:10.1016/j.scitotenv.2016.04.070.

32. Moradnejad P, Esmaeili S, Maleki M, Sadeghpour A, Kamali M, Rohani M, et al. Q fever endocarditis in Iran. Scientific reports. 2019;9(1):15276. Doi:10.1038/s41598-019-51600-3.

33. Van den Brom R, Van Engelen E, Roest H, Van der Hoek W, Vellema P. Coxiella burnetii infections in sheep or goats: an opinionated review. Veterinary microbiology. 2015;181(1-2):119-29. Doi:10.1016/j.vetmic.2015.07.011.

34. Guidi F, Petruzzelli A, Ciarrocchi F, Duranti A, Valiani A, Amagliani G, et al. Prevalence of Coxiella burnetii in cows' and ewes' bulk tank milk samples from selected dairy farms of Central Italy. Italian Journal of Animal Science. 2017;16(4):673-6. Doi:10.1080/1828051X.2017.1321474.

35. Rozental T, Faria LSD, Forneas D, Guterres A, Ribeiro JB, Araújo FR, et al. First molecular detection of Coxiella burnetii in Brazilian artisanal cheese: a neglected food safety hazard in ready-to-eat raw-milk product. Brazilian Journal of Infectious Diseases. 2020;24(3):208-12. Doi:10.1016/j.bjid.2020.05.003.

36. Kim SG, Kim EH, Lafferty CJ, Dubovi E. Coxiella burnetii in bulk tank milk samples, United States. Emerging infectious diseases. 2005;11(4):619. Doi: 10.3201/eid1104.041036.

37. Berthold-Pluta A, Pluta A, Garbowska M, Stefańska I. Prevalence and toxicity characterization of Bacillus cereus in food products from Poland. Foods. 2019;8(7):269. Doi:10.3390/foods8070269.

38. Tewari A, Abdullah S. Bacillus cereus food poisoning: international and Indian perspective. Journal of food science and technology. 2015;52(5):2500-11. Doi:10.1007/s13197-014-1344-4.

39. Webb MD, Barker GC, Goodburn KE, Peck MW. Risk presented to minimally processed chilled foods by psychrotrophic Bacillus cereus. Trends in food science & technology. 2019;93:94-105. Doi:10.1016/j.tifs.2019.08.024.

40. Eglezos S, Huang B, Dykes GA, Fegan N. The prevalence and concentration of Bacillus cereus in retail food products in Brisbane, Australia. Foodborne pathogens and disease. 2010;7(7):867-70. Doi:10.1089/fpd.2009.0469.

41. Yu S, Yu P, Wang J, Li C, Guo H, Liu C, et al. A study on prevalence and characterization of Bacillus cereus in ready-to-eat foods in China. Frontiers in microbiology. 2020;10:3043. Doi:10.3389/fmicb.2019.03043.

42. Gupta V, Gulati P, Bhagat N, Dhar M, Virdi J. Detection of Yersinia enterocolitica in food: an overview. European Journal of Clinical Microbiology & Infectious Diseases. 2015;34(4):641-50. Doi:10.1007/s10096-014-2276-7.

43. Lucero-Estrada C, Favier GI, Escudero ME. An overview of Yersinia enterocolitica and related species in samples of different origin from San Luis, Argentina. Food microbiology. 2020;86:103345. Doi:10.1016/j.fm.2019.103345.

44. Mahdavi S, Farshchian MR, Amini K, Abbasi M, Rad MG, Ebadi AR. Survey of Yersinia enterocolitica contamination in distributed broiler meats in Tabriz City, Iran. African J Microbiol Res. 2012;6(12):3019-23. Doi: 10.5897/AJMR12.094.

45. Zadernowska A, Chajęcka-Wierzchowska W, Łaniewska-Trokenheim Ł. Yersinia enterocolitica: a dangerous, but often ignored, foodborne pathogen. Food Reviews International. 2014;30(1):53-70. Doi:10.1080/87559129.2013.853775.

46. Chlebicz A, Śliżewska K. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. International journal of environmental research and public health. 2018;15(5):863. Doi:10.3390/ijerph15050863

47. Soro AB, Whyte P, Bolton DJ, Tiwari BK. Strategies and novel technologies to control Campylobacter in the poultry chain: A review. Comprehensive Reviews in Food Science and Food Safety. 2020. Doi:10.1111/1541-4337.12544.

48. Rahimi E, Ameri M, Alimoradi M, Chakeri A, Bahrami AR. Prevalence and antimicrobial resistance of Campylobacter jejuni and Campylobacter coli isolated from raw camel, beef, and water buffalo meat in Iran. Comparative Clinical Pathology. 2013;22(3):467-73. Doi:10.1007/s00580-012-1434-5.

49. Thames H, Sukumaran A. A Review of Salmonella and Campylobacter in Broiler Meat: Emerging Challenges and Food Safety Measures. Foods. 2020;9(6):776. Doi:10.3390/foods9060776.

50. Quaglia NC, Dambrosio A. Helicobacter pylori: A foodborne pathogen? World journal of gastroenterology. 2018;24(31):3472. Doi: 10.3748/wjg.v24.i31.3472.

51. Bahrami AR, Rahimi E, Ghasemian Safaei H. Detection of Helicobacter pylori in city water, dental units' water, and bottled mineral water in Isfahan, Iran. The Scientific World Journal. 2013;2013. Doi:10.1155/2013/280510.

52. Souod N, Kargar M, Doosti A, Ranjbar R, Sarshar M. Genetic analysis of cagA and vacA genes in Helicobacter pylori isolates and their relationship with gastroduodenal diseases in the west of Iran. Iranian Red Crescent Medical Journal. 2013;15(5):371. Doi: 10.5812/ircmj.3732.

53. Fujimura S, Kawamura T, Kato S, Tateno H, Watanabe A. Detection of Helicobacter pylori in cow's milk. Letters in Applied Microbiology. 2002;35(6):504-7. Doi:10.1046/j.1472-765X.2002.01229.x.

54. Quaglia N, Dambrosio A, Normanno G, Parisi A, Patrono R, Ranieri G, et al. High occurrence of Helicobacter pylori in raw goat, sheep and cow milk inferred by glmM gene: a risk of food-borne infection? International journal of food microbiology. 2008;124(1):43-7. Doi:10.1016/j.ijfoodmicro.2008.02.011.

55. Meng X, Zhang H, Law J, Tsang R, Tsang T. Detection of Helicobacter pylori from food sources by a novel multiplex PCR assay. Journal of food safety. 2008;28(4):609-19. Doi:10.1111/j.1745-4565.2008.00135.x.

56. Mirnejad R, Jazi FM, Mostafaei S, Sedighi M. Epidemiology of brucellosis in Iran: A comprehensive systematic review and meta-analysis study. Microbial pathogenesis. 2017;109:239-47. Doi:10.1016/j.micpath.2017.06.005.

57. Dadar M, Fakhri Y, Shahali Y, Khaneghah AM. Contamination of milk and dairy products by Brucella species: A global systematic review and meta-analysis. Food Research International. 2020;128:108775. Doi:10.1016/j.foodres.2019.108775.

58. Moslemi E, Soltandalal MM, Beheshtizadeh MR, Taghavi A, Kheiri Manjili H, Mahmoudi Lamouki R, et al. Detection of Brucella spp. in dairy products by real-time PCR. Archives of Clinical Infectious Diseases. 2018;13(1). Doi:10.5812/archcid.12673.

59. Esmaeili S, Naddaf SR, Pourhossein B, Hashemi Shahraki A, Bagheri Amiri F, Gouya MM, et al. Seroprevalence of brucellosis, leptospirosis, and Q fever among butchers and slaughterhouse workers in south-eastern Iran. PloS one. 2016;11(1):e0144953. Doi:10.1371/journal.pone.0144953.

60. Islam MS, Islam MA, Rahman MM, Islam K, Islam MM, Kamal MM, et al. Presence of Brucella spp. in milk and dairy products: a comprehensive review and its perspectives. Journal of Food Quality. 2023;2023(1):2932883. Doi:10.1371/journal.pone.0144953.

61. Letchumanan V, Chan K-G, Lee L-H. Vibrio parahaemolyticus: a review on the pathogenesis, prevalence, and advance

molecular identification techniques. Frontiers in microbiology. 2014;5:705. Doi:10.3389/fmicb.2014.00705.

62. Odeyemi OA. Incidence and prevalence of Vibrio parahaemolyticus in seafood: a systematic review and meta-analysis. Springerplus. 2016;5(1):464. Doi:10.1186/s40064-016-2115-7.

63. Li Y, Xie T, Pang R, Wu Q, Zhang J, Lei T, et al. Food-Borne Vibrio parahaemolyticus in China: Prevalence, Antibiotic Susceptibility, and Genetic Characterization. Frontiers in microbiology. 2020;11:1670. Doi:10.3389/fmicb.2020.01670.

64. Shahin K, Bouzari M, Wang R, Yazdi M. Prevalence and molecular characterization of multidrug-resistant Shigella species of food origins and their inactivation by specific lytic bacteriophages. International journal of food microbiology. 2019;305:108252. Doi:10.1016/j.ijfoodmicro.2019.108252.

65. Gundappa M, Gaddad SM. Prevalence of Salmonella, Shigella and E. coli in Vegetables of Various Markets in Kalaburagi (India). Indian J Nat Sci. 2016;6(1):1-5. Doi:10.1007/s13197-017-2964-2.

66. Shahin K, Bouzari M. Bacteriophage application for biocontrolling Shigella flexneri in contaminated foods. Journal of food science and technology. 2018;55(2):550-9.

67. Rahman S, Park J, Song KB, Al-Harbi NA, Oh DH. Effects of slightly acidic low concentration electrolyzed water on microbiological, physicochemical, and sensory quality of fresh chicken breast meat. Journal of food science. 2012;77(1):M35-M41. Doi:10.1111/j.1750-3841.2011.02454.x.

68. Sofos JN. Meat and meat products. Food safety management: Elsevier; 2014. p. 119-62. Doi:10.1016/B978-0-12-381504-0.00006-8.

69. Pesavento G, Ducci B, Nieri D, Comodo N, Nostro AL. Prevalence and antibiotic susceptibility of Listeria spp. isolated from raw meat and retail foods. Food control. 2010;21(5):708-13. Doi:10.1016/j.foodcont.2009.10.012.

70. Centers for Disease Control and Prevention [Internet]. 2011. Available from: https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

71. Tack DM. Preliminary incidence and trends of infections with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US sites, 2015–2018. MMWR Morbidity and mortality weekly report. 2019;68.

72. Rahimi Z, Mahmoudi R, Ghajarbeyg P, Mosavi S, Mehrabi A. The Prevalence of Salmonella Enteritidis in Packaged and Tray Eggs Samples, Qazvin, Iran. Journal of Chemical Health Risks. 2021. Doi:10.22034/jchr.2021.1922123.1250.

73. Nazari Moghadam M, Rahimi E, Shakerian A, Momtaz H. Prevalence of Salmonella Typhimurium and Salmonella Enteritidis isolated from poultry meat: virulence and antimicrobial-resistant genes. BMC microbiology. 2023;23(1):1-8. Doi:10.1186/s12866-023-02908-8.

74. Saram M, Nosrati AC, Modiri L, Issazadeh K. Investigation of Genetic-molecular Sources of Multidrug Resistance of Salmonella Isolated from Eggs. Social Determinants of Health.8:1-11. Doi:10.22037/sdh.v8i1.39325.

75. Hassanzadeh P, Mohammadzadeh S. Antimicrobial Effects of Different Synbiotic Compounds against Pathogenic Bacteria Isolated from Beef, Mutton, and Chicken. Archives of Razi Institute. 2022;77(6):2105. Doi: 10.22092/ARI.2022.357834.2107.

76. Shayegh J, Shahbazi G, Ghazaei S, Movassagh Ghazani MH, Hanifian S. Prevalence, virulence factor and antibiotics susceptibility patterns of Salmonella spp. from poultry products in Ardabil province. 2022.

77. Rahimi Z, Ghajarbeygi P, Mahmoudi R, Mosavi S, Mehrabi A. PREVALENCE OF SALMONELLA STRAINS ISOLATED FROM INDUSTRIAL QUAIL EGGS AND LOCAL DUCK EGGS, IRAN. Carpathian Journal of Food Science & Technology. 2022;14(2). Doi:10.34302/crpjfst/2022.14.2.14.

78. Mir R, Salari S, Najimi M, Rashki A. Determination of frequency, multiple antibiotic resistance index and resistotype of Salmonella spp. in chicken meat collected from southeast of Iran. Veterinary Medicine and Science. 2022;8(1):229-36. Doi:10.1002/vms3.647.

79. Kazemeini H. Molecular detection of Campylobacter species and Salmonella spp. In cattle raw milk specimens in Mazandaran province. Journal of food science and technology (Iran). 2022;19(125):101-8. Doi:10.22034/FSCT.19.125.101.

80. Norbakhsh F, Behshood P. Determination of molecular properties of Salmonella isolates isolated from red meat in Shahrekord, Iran. Journal of Zoonosis. 2022;2(1):18-25.

81. Azizpour A. Prevalence and antibiotic resistance of Salmonella serotypes in chicken meat of Ardabil, Northwestern Iran. Iranian Journal of Medical Microbiology. 2021;15(2):232-46. Doi: 10.30699/ijmm.15.2.232.

82. Bahramianfard H, Derakhshandeh A, Naziri Z, Khaltabadi Farahani R. Prevalence, virulence factor and antimicrobial resistance analysis of Salmonella Enteritidis from poultry and egg samples in Iran. BMC veterinary research. 2021;17(1):196. Doi:10.1186/s12917-021-02900-2.

83. Sabzali S, Bouzari M. Isolation, identification and some characteristics of two lytic bacteriophages against Salmonella enterica serovar Paratyphi B and S. enterica serovar Typhimurium from various food sources. FEMS Microbiology Letters. 2021;368(7):fnab037. Doi:10.1093/femsle/fnab037.

84. Afshari A, Tavassoli M, Ram M, Ranjbar G. Assessment of Microbial and Chemical Quality of a Ready to Eat Food, Olivier Salad, in Mashhad City. Journal of Nutrition, Fasting and Health. 2019;7(4 (Spe):175-81.

85. Forouhar M, Harzandi N. Molecular Survey of Quinolone Resistance in Salmonella spp. Isolated From Poultry Products in Karaj, Iran. Int J Enteric Pathog. 2019;7(2):55-9. Doi: 10.15171/ijep.2019.13.

86. Mahdavi M, Jalali M, Safaei HG, Shamloo E. Microbial quality and prevalence of Salmonella and Listeria in eggs. International Journal of Environmental Health Engineering. 2012;1(1):48.

87. Hamzeh Pour S, Vaziri S, Molaee Aghaee E. Survey on the contamination rate and determination of antibiotic resistance of Staphylococcus aureus, Escherichia coli and Salmonella strains isolated from traditional cheeses distributed in Mahabad, Iran. Iranian Journal of Health and Environment. 2019;11(4):465-76.

88. Mahdavi S, Azizi Dehbokri M, Isazadeh A. Contamination of chicken meat with salmonella spp distributed in mahabad city, iran. Int J Enteric Pathog. 2018;6(3):65-8. Doi: 10.15171/ijep.2018.18

89. Hashemi M, Baygan A, Balouchzehi Z, Dousti Nouri M, Afshari A. Evaluation of microbial quality of traditional sweets, Zoolbia and Bamieh, during Ramadan in Mashhad, Iran. Journal of Nutrition, Fasting and Health. 2018;6(3):145-9. Doi:10.22038/JNFH.2018.34900.1147.

90. Karimiazar F, Soltanpour MS, Aminzare M, Hassanzadazar H. Prevalence, genotyping, serotyping, and antibiotic resistance of isolated Salmonella strains from industrial and local eggs in Iran. Journal of Food Safety. 2019;39(1):e12585. Doi:10.1111/jfs.12585.

91. Rezaee R, Mohammadi S, Faizee S. A study of salmonella spp. contamination of eggs in Sanandaj in 2017. Zanko Journal of Medical Sciences. 2018;19(61):43-9.

92. Khedmati Morasa H, Mahmoudi R, Ghajarbeygi P, Abasi N, Mousavy S. Effects of cold-water egg shell washing on Salmonella contamination in the shell and its contents. The Journal of Qazvin University of Medical Sciences. 2018;22(2):83-9.

93. Sana M, Hosseini Siahi Z. The determination of total microbial count and prevalence of Salmonella in the shrimp supply in Khuzestan province. Iranian Journal of Health and Environment. 2018;11(2):149-56.

94. Eslami A, Gholami Z, Nargesi S, Rostami B, Avazpour M. Evaluation of microbial contamination of ready-to-eat foods (pizza, frankfurters, sausages) in the city of Ilam. Environmental Health Engineering and Management Journal. 2017;4(2):117-22. Doi:10.15171/EHEM.2017.16.

95. Rahimi E, Shakeriyan A. Salmonella contamination of camel meat in various stages of destruction in Isfahan and Chaharmahal va Bakhtiari. Journal of Food Microbiology. 2017;4(2):29-36.

96. Sharafati Chaleshtori R, Mazroii Arani N, Taghizadeh M, Sharafati Chaleshtori F. Antibiotic resistance pattern of salmonella isolated from hamburgers and detection of their sensitivity to some essential oils. Journal of Mazandaran University of Medical Sciences. 2017;27(148):136-42.

97. Mojaddar Langroodi A, Ehsani A, Ebadi A. Comparison of bacteria in beef meat and poultry in terms of packaging and without packaging in Gilan province. Journal of Food Microbiology. 2016;3(1):33-42.

98. Dousandeh S, Asaadi Tehrani G, Amini B, Maziri P. Simultaneous detection of invA, STM4497, and fliC183 genes in Salmonella typhimurium by multiplex PCR method in poultry meat samples in Zanjan, Iran. Infection, Epidemiology and Microbiology. 2016;2(4):20-3.

99. Vahabi AN, Abbasvali M. Determination of microbial contamination of olovie salads consumed in Isfahan. 2016.

100. Khammar F, Alipour Eskandari M, Saadati D. Study of Salmonella contamination of traditional ice creams in Zabol City, Iran. Iranian Journal of Medical Microbiology. 2017;11(1):83-9.

101. Asadi S, Maram ZR, Kooshk F. Evaluation of microbial contamination of pastry cream in Arak city of Iran. Journal of Food Safety and Hygiene. 2015;1(1):26-9.

102. Tajbakhsh F, Tajbakhsh E, Momeni M. Detection of Staphylococcus aureus and Salmonella typimurium in traditional and industrial Olivier salads in shahrekord city. 2015.

103. Khodadadipour T, Amini K, Mahmoudi R. Evaluation of virulence and enterotoxin genes in Salmonella enteritidis strains isolated from Meat and Egg samples by Multiplex-PCR. Journal of Food Microbiology. 2016;3(2):25-33.

104. Ghorbani Ranjbary A. Study of Drug Resistance in Salmonella spp. Isolated from Native Eggs of Iran's Southern Region. Journal of pure and applied microbiology. 2015;9(2):175-9.

105. Khoramrooz S, Sarikhani M, Khosravani S, Farhang Falah M, Mahmoudi Y, Sharifi A. Microbial contamination determination of cream suit, traditional ice cream and Olovia in Yasuj City. Armaghane danesh. 2015;20(6):526-37.

106. Doosti A, Doosti E, Rahimi E, Ghasemi-Dehkordi P. Frequency of antimicrobial-resistant genes in Salmonella enteritidis isolated from traditional and industrial iranian white cheeses. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2017;87(1):73-80. Doi:10.1007/s40011-015-0572-3.

107. Moosavy M-H, Esmaeili S, Amiri FB, Mostafavi E, Salehi TZ. Detection of Salmonella spp in commercial eggs in Iran. Iranian journal of microbiology. 2015;7(1):50.

108. Kheyri A, Fakhernia M, Haghighat-Afshar N, Hassanzadazar H, Kazemi-Ghoshchi B, Zeynali F, et al. Microbial contamination of meat products produced in the factories of West Azerbaijan Province, North West of Iran. Global Veterinaria. 2014;12(6):796-802. Doi: 10.5829/idosi.gv.2014.12.06.83145.

109. Soltan Dallal MM, Emadi Koochak H, Sharifi Yazdi MK, Taheri Mirghaed A, Choobineh H. Determination of yersinia Spp. and Salmonella paratyphi B isolated from possibly contaminated cream samples in the city of Tehran. Journal of Payavard Salamat. 2014;8(1):34-43.

110. Dallal MMS. Prevalence of Salmonella spp. in packed and unpacked red meat and chicken in south of Tehran. Jundishapur Journal of Microbiology. 2014;7(4).

111. Sodagari HR, Mashak Z, Ghadimianazar A. Prevalence and antimicrobial resistance of Salmonella serotypes isolated from retail chicken meat and giblets in Iran. The Journal of Infection in Developing Countries. 2015;9(05):463-9. Doi: 10.3855/jidc.5945.

112. Zahedi M, Rahimi E, Zahedi M, Momtaz H. Prevalence of Salmonella enteritidis and S. typhimurium in marketed meat in Shahrekord in 2014. Journal of Shahrekord Uuniversity of Medical Sciences. 2017;19.

113. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A. Detection of Escherichia coli, Salmonella species, and Vibrio cholerae in tap water and bottled drinking water in Isfahan, Iran. BMC public health. 2013;13(1):556. Doi:10.1186/1471-2458-13-556.

114. Bidaki MZ, Pour AT, Dadpour S, Gholizadeh H. Prevalence of Salmonella in poultry carcasses serotypes in Birjand industrial slaughterhouses. Journal of Birjand University of Medical Sciences. 2013;20(02).

115. Monadi M, Kargar M, Naghiha A, Mohammadi R. Salmonella contamination of eggs of native Kohgiluyeh va Boyerahmad using PCR1 techniques and the evaluation of drug resistance. Armaghane danesh. 2014;19(2):179-87.

116. Amirmozaffari N, Rahmani Z, Iesazadeh K. Evaluation of the level of contamination with Salmonella spp. in red meat, chicken, and domestic and industrial eggs produced in Talesh city and assessment of their antibiotic resistance pattern, Iran. Qom University of Medical Sciences Journal. 2013;7(5):60-5.

117. Kaseb F, Shiranian M, Abdar M, Aminalroayaei H, Fallahzadeh H. The prevalence of salmonella and Staphylococcus aureus in industrial Olivier salad in Yazd in 2013. Tolooebehdasht. 2015;14(3):51-9.

118. Rahimi E, Shakerian A, Falavarjani AG. Prevalence and antimicrobial resistance of Salmonella isolated from fish, shrimp, lobster, and crab in Iran. Comparative Clinical Pathology. 2013;22(1):59-62. Doi:10.1007/s00580-011-1368-3.

119. Rahimi E. Prevalence and antimicrobial resistance of Salmonella spp isolated from retail chicken, turkey and ostrich by-products in Iran. Revue de MédecineVétérinaire. 2012;163(52):271-5. Doi:10.1007/s00580-011-1368-3.

120. Karimi Darehabi H, Esmailneshad F, Ebrahimi Mohammadi K. Contamination of Fresh Beef to Salmonella typhimurium and Salmonella enteritidis in Sanandaj during 2012. Food Hygiene. 2012;2(3 (7)):41-8.

121. Vaskas AT, Dahesht AE, Seifi S, Rahmani M, Motaghifar A, Safanavaee R. Study and comparison of the bacterial contamination outbreak of chicken meat consumed in some cities of Mazandaran Province (Iran). African Journal of Microbiology Research. 2012;6(33):6286-90. Doi: 10.5897/AJMR12.984.

122. Zarei M, Maktabi S, Ghorbanpour M. Prevalence of Listeria monocytogenes, Vibrio parahaemolyticus, Staphylococcus aureus, and Salmonella spp. in seafood products using multiplex polymerase chain reaction. Foodborne Pathogens and Disease. 2012;9(2):108-12. Doi:10.1089/fpd.2011.0989.

123. Tajbakhsh F, Tajbakhsh E, Momeni M, Rahimi E, Sohrabi R. Occurrence and Antibiotic Resistance of Salmonella spp isolated from raw Cow's Milk from Shahahrekord. Iran Inter J Microbiol Res. 2012;3:242-5. Doi: 10.5829/idosi.ijmr.2012.3.3.61302.

124. Safaei HG, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheimi E. The prevalence of bacterial contamination of table eggs from retails markets by Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. Jundishapur Journal of Microbiology. 2011;4(4).

125. Dallal MMS, Doyle MP, Rezadehbashi M, Dabiri H, Sanaei M, Modarresi S, et al. Prevalence and antimicrobial resistance profiles of Salmonella serotypes, Campylobacter and Yersinia spp. isolated from retail chicken and beef, Tehran, Iran. Food Control. 2010;21(4):388-92. Doi:10.1016/j.foodcont.2009.06.001.

126. Rahimi E, Ameri M, Kazemeini H, Elbagi M. PREVALENCE AND ANTIMICROBIAL RESISTANCE OF SALMONELLA ISOLATED FROM RETAIL RAW TURKEY, OSTRICH, AND PARTRIDGE MEAT IN IRAN. Bulgarian Journal of Veterinary Medicine. 2010;13(1).

127. Jamshidi A, Kalidari GA, Hedayati M. Isolation and identification of Salmonella Enteritidis and Salmonella Typhimurium from the eggs of retail stores in Mashhad, Iran using conventional culture method and multiplex PCR assay. Journal of food safety. 2010;30(3):558-68. Doi:10.1111/j.1745-4565.2010.00225.x.

128. Jamshidi Ae, basami m, afshari ns. Identification of Salmo-

nella spp. and Salmonella typhimurium by a multiplex PCR-based assay from poultry carcasses in Mashhad-Iran. 2009.

129. Shapouri R, Rahnema M, Eghbalzadeh S. PREVALENCE OF SALMONELLA SEROTYPES IN POULTRY MEAT AND EGG AND DETERMINE THEIR ANTIBIOTIC SENSIVITY IN ZANJAN CITY. 2009.

130. Soltan Dmm, Taremi M, Gachkar L, MODARESI S, Sanaei M, Bakhtiari R, et al. Characterization of antibiotic resistant patterns of Salmonella serotypes isolated from beef and chicken samples in Tehran. 2009.

131. Namaei M, Ziaee M, Kafi MG. Prevalence of salmonella contamination in locally (non-industrially) produced eggs in Birjand (2006). Journal of Birjand University of medical sciences. 2009;16(02).

132. Jalali M, Abedi D, Pourbakhsh SA, Ghoukasin K. Prevalence of salmonella spp. in raw and cooked foods in Isfahan-Iran. Journal of food safety. 2008;28(3):442-52. Doi:10.1111/j.1745-4565.2008.00122.x.

133. AR B. Qualitative and quantitative assessment of poultry carcasses contaminated with salmonella in Tehran industrial slaughter houses. 2008.

134. Daneshmand A, Rasooli M, Kargar M, Kyani S. A survey on bacterial contamination of fresh traditional cheeses with Salmonella spp. and Staphylococcus aureus in Jahrom, Fars Province. ISMJ. 2007;10(1):19-26.

135. Bonyadian M, Ale AS, MOTAHARI FA. Isolation and identification of Salmonellae from chicken carcasses in processing plants in Yazd province, central Iran. 2007.

136. Namaei M, ZIAEI M, GHANAD KM. Prevalence of salmonella contamination in locally (non-industrially) produced eggs in Birjand (2006). 2009.

137. Nourbakhsh SA, Rahimi E. The occurrence of some foodborne pathogens recovered from poultry meat in Shahrekord, Iran. Journal of Advanced Veterinary and Animal Research. 2023;10(2):205. Doi: 10.5455/javar.2023.j670.

138. Azad A, Shakerian A. Evaluation of Staphylococcus Aureus, Escherichia Coli, and Salmonella Contamination in Sausages and Bologna Sold in Tehran City, Iran. Journal of Alternative Veterinary Medicine| Spring. 2023;6(16).

139. Azimirad M, Nadalian B, Alavifard H, Panirani SN, Bonab SMV, Azimirad F, et al. Microbiological survey and occurrence of bacterial foodborne pathogens in raw and ready-to-eat green leafy vegetables marketed in Tehran, Iran. International Journal of Hygiene and Environmental Health. 2021;237:113824. Doi:10.1016/j.ijheh.2021.113824.

140. Doudaran HO, Mahsk Z, Kohdar V. Distribution Of Antibiotic Resistance Genes Amongst The Staphylococcus Aureus Strains Isolated From Raw Milk And Traditional Dairy Products. Journal of Pharmaceutical Negative Results. 2022:9735-41.

141. Feizolahnezhad A, Mashak Z. Study The Prevalence And Antibiotic Resistance Of Staphylococcus Aureus Bacteria Isolated From Street Ready To Eat Food Samples. Journal of Pharmaceutical Negative Results. 2022:9714-20. Doi:10.47750/pnr.2022.13.S09.1135.

142. Kabiri E, Mashak Z. Antimicrobial resistance properties and enterotoxigenic gene profile of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates from raw milk. Journal of Positive School Psychology. 2022;6(8):1244-52.

143. Pajohesh R, Tajbakhsh E, Momtaz H, Rahimi E. Relationship between Biofilm Formation and Antibiotic Resistance and Adherence Genes in Staphylococcus aureus Strains Isolated from Raw Cow Milk in Shahrekord, Iran. International Journal of Microbiology. 2022;2022. Doi:10.1155/2022/6435774.

144. Hassani S, Pakbin B, Allahyari S, Mahmoudi R, Mousavi S, Ghajarbeygi P. PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF FOODBORNE BACTERIAL PATHOGENS ISOLATED FROM BAGHLAVA AN IRANIAN EXPORTING PASTRY SWEET. Carpathian Journal of Food Science & Technology. 2022;14(3). Doi:10.34302/crpjfst/2022.14.3.5.

145. Shariatifar M, Salavati-Hamedani M, Rezaeian-Doloei R, Rezaeigolestani M, Mohsenzadeh M. Antibiotic resistance and enterotoxin gene profiles of Staphylococcus aureus isolated from raw milk in Iran. Journal of the Hellenic Veterinary Medical Society. 2023;74(1):5433-40. Doi:10.12681/jhvms.29671.

146. Bastam MM, Jalili M, Pakzad I, Maleki A, Ghafourian S. Pathogenic bacteria in cheese, raw and pasteurised milk. Veterinary Medicine and Science. 2021;7(6):2445-9. Doi:10.1002/vms3.604.

147. Abbasi K, Tajbakhsh E, Momtaz H. Antimicrobial resistance and biofilm encoding genes amongst the Staphylococcus aureus bacteria isolated from meat and meat products. Egyptian Journal of Veterinary Sciences. 2021;52(1):55-62. Doi:10.21608/EJVS.2020.39385.1186.

148. Maktabi S, Gharibi D, Hormozi T. Detection of Enterotoxin A Gene (sea) and Determination of Different Biotypes of Staphylococcus Aureus Isolates From Milk and Cheese in Khuzestan Province, Iran. Int J Enteric Pathog. 2021;9(3):117-23.

149. Haghi F, Zeighami H, Hajiloo Z, Torabi N, Derakhshan S. High frequency of enterotoxin encoding genes of Staphylococcus aureus isolated from food and clinical samples. Journal of Health, Population and Nutrition. 2021;40(1):1-6. Doi:10.1186/s41043-021-00246-x.

150. Mesbah A, Mashak Z, Abdolmaleki Z. A survey of prevalence and phenotypic and genotypic assessment of antibiotic resistance in Staphylococcus aureus bacteria isolated from ready-to-eat food samples collected from Tehran Province, Iran. Tropical Medicine and Health. 2021;49:1-12. Doi:10.1186/s41182-021-00366-4.

151. Dadmehr A, Sadighara P, Zeinali T. A study on microbial and chemical characterization of mechanically deboned chicken in Tehran, Iran. International Journal of Environmental Health Research. 2022;32(11):2396-405. Doi.org/10.1080/09603123.2021.1967889.

152. Saadati A, Mashak Z, Yarmand MS. Prevalence of Staphylococcal Cassette Chromosome mec and Panton-Valentine Leukocidin Gene Amongst the Methicillin-resistant Staphylococcus aureus Strains Isolated From Fowl Meat. Int J Enteric Pathog. 2019;7(3):93-8. Doi:10.15171/ijep.2019.20.

153. Mahdavi S, Isazadeh A. Investigation of contamination rate and determination of pattern of antibiotic resistance in coagulase positive staphylococcus aureus isolated from domestic cheeses in Maragheh, Iran. Pathobiology Research. 2019;22(2):85-9.

154. Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017;6(1):104. Doi:10.1186/s13756-017-0257-1.

155. Goudarzi B, Alipour V, Rezaei L, Dindarlu K, Heidari M, Rahmaniyan O. Bacteriological quality of ready to use salads at restaurants in Bandar Abbas Abstract. Journal of Preventive Medicine. 2016;3(3):38-1.

156. Varmazyar-najafi M, Pajohi-alamoti M, Mohammadzadeh A, Mahmoodi P. Detection of methicillin-resistance gene in Staphylococcus aureus isolated from traditional white cheese in Iran. Archives of Hygiene Sciences. 2016;5(4):302-9.

157. Arfatahery N, Davoodabadi A, Abedimohtasab T. Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus isolates in fishery products in Iran. Scientific reports. 2016;6(1):1-7. Doi:10.1038/srep34216.

158. Arabestani MR, Kazemian H, Tabar ZK, Hosseini SM. Prevalence of virulence genes, agr and antimicrobial resistance of Staphylococcus aureus isolated from food and dairy products in Hamadan, Iran. Der Pharmacia Lettre. 2016;8(8):62-7.

159. Dehkordi AA, Tajbakhsh E, Tajbakhsh F, Khamesipour F, Shahraki MM, Momeni H. Molecular typing of staphylococcus aureus strains from Iranian raw milk and dairy products by coagulase gene polymorphisms. Adv Stud Biol. 2015;7(4):169-77. Doi:10.12988/asb.2015.516.

160. Arfatahery N, Mirshafiey A, Abedimohtasab T, Zeinolabedinizamani M. Study of the prevalence of Staphylococcus aureus in marine and farmed shrimps in Iran aiming the future development of a prophylactic vaccine. Procedia in Vaccinology. 2015;9:44-9. Doi:10.1016/j.provac.2015.05.008.

161. Jamali H, Paydar M, Radmehr B, Ismail S, Dadrasnia A. Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. Food Control. 2015;54:383-8. Doi:10.1016/j.foodcont.2015.02.013.

162. Sadeghi M. The prevalence of Enterotoxigenic Isolates of Staphylococcus aureus in Bovine and Sheep Bulk Tank Milk Samples of Maku. 2015. Doi:10.1016/j.foodcont.2015.02.013.

163. Eslami M, Koohi M, Zadehashem E, Khadiri B, Keshavarz H. Survey the presence of coagulase positive staphylococcus aureus in cottage cheeses produced from sheep milk and sold in Marand county. Journal of Food Science & Technology (2008-8787). 2014;12(46).

164. Madahi H, Rostami F, Rahimi E, Dehkordi FS. Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. Jundishapur journal of microbiology. 2014;7(8). Doi: 10.5812/jjm.10237.

165. Khoshkholgh MR, Isazadeh K. Evaluation of Microbial quality of food samples collected in the East region of Gilan. Journal of Food Microbiology. 2015;2(1):27-37.

166. Shabani S, Sadeghi Mahoonak A, Jalali H. Microbial contamination of pastry cream supplied in Gorgan. Medical Laboratory Journal. 2014;8(2):62-6.

167. Saadat YR, Fooladi AAI, Shapouri R, Hosseini MM, Khiabani ZD. Prevalence of enterotoxigenic Staphylococcus aureus in organic milk and cheese in Tabriz, Iran. Iranian journal of microbiology. 2014;6(5):345.

168. Fadaei A. Bacteriological quality of raw cow milk in Shahrekord, Iran. Veterinary World. 2014;7(4):240-3.

169. Al-musawi MHJ, Al-Rubaye MTS, Hosseini M. Culture and Molecular Detection of Staphylococcus aureus in Dairy Products of Ahwaz. Al-Mustansiriyah Journal for Pharmaceutical Sciences. 2014;14(2):103-7.

170. Farajvand N, Alimohammadi M. Prevalence of Staphylococcus aureus in Four Famous Brand ofDoogh Produced in Iran. Iranian Journal of Health and Environment. 2014;7(1):85-94.

171. Shojaei M, KARIMI DH, Javadi A. Distribution of genes encoding biofilm production in S. aureus isolated from raw milk in Kurdistan. 2014.

172. Sepidarkish M, Ghane M. Isolation, identification and the presence of enterotoxin A gene in Staphylococcus aureus from meat products. 2014.

173. Shanehbandi D, Baradaran B, Sadigh-Eteghad S, Zarredar H. Occurrence of methicillin resistant and enterotoxigenic Staphylococcus aureus in traditional cheeses in the North West of Iran. International Scholarly Research Notices. 2014;2014. Doi:10.1155/2014/129580

174. Rahimi E. Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran. Brazilian Journal of Microbiology. 2013;44(2):393-9.

175. Rahimi E, Alian F. Presence of enterotoxigenic Staphylococcus aureus in cow, camel, sheep, goat, and buffalo bulk tank milk. Veterinarski arhiv. 2013;83(1):23-30.

176. Mirzaei H, Farhoudi H, Tavassoli H, Farajli M, Monadi A. Presence and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus in raw and pasteurized milk and ice cream in Tabriz by culture and PCR techniques. African Journal of Microbiology Research. 2012;6(32):6224-9. Doi: 10.5897/AJMR12.1701

177. Tavakoli H, Farhang K, AA KZ HE. Bacteriological quality of ready to eat food in four military restaurants. Journal Mil Med. 2012;13(4):207-12.

178. Shahraz F, Dadkhah H, Khaksar R, Mahmoudzadeh M, Hosseini H, Kamran M, et al. Analysis of antibiotic resistance patterns and detection of mecA gene in Staphylococcus aureus isolated from packaged hamburger. Meat science. 2012;90(3):759-63. Doi:10.1016/j.meatsci.2011.11.009

179. Alian F, Rahimi E, Shakerian A, Momtaz H, Riahi M, Momeni M. Antimicrobial resistance of Staphylococcus aureus isolated from bovine, sheep and goat raw milk. Global Veterinaria. 2012;8(2):111-4.

180. Sharafati Chaleshtori R, Sharafati Chaleshtori F, Karimi A. Antibiotic resistance pattern of staphylococcus strains isolated from orange and apple juices in Shahre-kord, Iran. Pakistan Journal of Medical Sciences. 2010;26(3):615-8.

181. Tavakoli HR, Riazipour M. Microbial quality of cooked meat foods in Tehran University's Restaurants. Pakistan Journal of Medical Sciences. 2008;24(4):595-9.

182. Basti AA, Misaghi A, Salehi TZ, Kamkar A. Bacterial pathogens in fresh, smoked and salted Iranian fish. Food control. 2006;17(3):183-8. Doi:10.1016/j.foodcont.2004.10.001

183. Raeisi M, Afshari A, Shirzad-Aski H, Seifi S, Hashemi M, Khoshbakht R, et al. The Occurrence of Serotypes and Virulence Genes of Listeria monocytogenes in Various Food Products. Journal of food quality and hazards control. 2023. Doi:10.18502/jfqhc.10.2.12673

184. Kiyanpour Berjoee R, Momtaz H, Lotfollahi L, Bamzaheh Z. Detection and antibiotic resistance pattern of Listeria monocytogenes strains isolated from curd and cheese. Journal of Food Microbiology. 2022;9(4):18-29. Doi:10.30495/jfm.2022.1943942.1741

185. Farahbakhsh S, Kariminik A. Detection of Listeria Monocytogenes in non-Pasteurized Milk in Kerman City by Phenotypic and Molecular Techniques. Journal of Quality and Durability of Agricultural Products and Food Stuffs. 2021;1(1):30-9. Doi:10.30495/qafj.2021.685560

186. Dehnavi M, Khanjari A, Rezaei E. Prevalence of Listeria monocytogenes in traditional cheeses obtained from food sale centers of Tehran, Iran. Journal of Food Safety and Hygiene. 2021;7(3):135-40. Doi:10.18502/jfsh.v7i3.9133

187. Faridi Z, Dallal MMS, Koohy-Kamaly P, Javadi NHS, Hadian Z, Fard RMN. Prevalence and Antimicrobial Resistance of Listeria monocytogenes in Raw Milk in Tehran, Iran. Doi:10.22037/afb.v8i3.34379

188. Mashak Z, Banisharif F, Banisharif G, Reza Pourian M, Eskandari S, Seif A, et al. Prevalence of listeria species and serotyping of Listeria monocytogenes bacteria isolated from seafood samples. Egyptian Journal of Veterinary Sciences. 2021;52(1):1-9. Doi:10.21608/EJVS.2020.17893.1105

189. Farhoumand P, Hassanzadazar H, Soltanpour MS, Aminzare M, Abbasi Z. Prevalence, genotyping and antibiotic resistance of Listeria monocytogenes and Escherichia coli in fresh beef and chicken meats marketed in Zanjan, Iran. Iranian Journal of Microbiology. 2020;12(6):537. Doi: 10.18502/ijm.v12i6.5028

190. Soleimani M, Khalili Sadrabad E, Hamidian N, Heydari A, Akrami Mohajeri F. Prevalence and Antibiotic Resistance of Listeria Monocytogenes in chicken meat retailers in Yazd, Iran. Journal of Environmental Health and Sustainable Development. 2019;4(4):895-902. Doi:10.18502/jehsd.v4i4.2022

191. Akrami-Mohajeri F, Derakhshan Z, Ferrante M, Hamidiyan N, Soleymani M, Conti GO, et al. The prevalence and antimicrobial resistance of Listeria spp in raw milk and traditional dairy products delivered in Yazd, central Iran (2016). Food and Chemical Toxicology. 2018;114:141-4. Doi:10.1016/j.fct.2018.02.006

192. Lotfollahi L, Chaharbalesh A, Rezaee MA, Hasani A. Prevalence, antimicrobial susceptibility and multiplex PCR-serotyping of Listeria monocytogenes isolated from humans, foods and livestock in Iran. Microbial pathogenesis. 2017;107:425-9. Doi:10.1016/j.micpath.2017.04.029.

193. Zeinali T, Jamshidi A, Bassami M, Rad M. Isolation and identification of Listeria spp. in chicken carcasses marketed in northeast of Iran. International Food Research Journal. 2017;24(2):881.

194. Pournajaf A, Rajabnia R, Sedighi M, Kassani A, Moqarabzadeh V, Lotfollahi L, et al. Prevalence, and virulence determination of Listeria monocytogenes strains isolated from clinical and non-clinical samples by multiplex polymerase chain reaction. Revista da Sociedade Brasileira de Medicina Tropi-

cal. 2016;49(5):624-7.

195. Abdollahzadeh E, Ojagh SM, Hosseini H, Ghaemi EA, Irajian G, Heidarlo MN. Antimicrobial resistance of Listeria monocytogenes isolated from seafood and humans in Iran. Microbial pathogenesis. 2016;100:70-4. Doi:10.1016/j.micpath.2016.09.012.

196. Rahimi E, Jahanmard M, SAFARI S, Ansari M, TORKI Z. PREVALENCE AND ANTIMICROBIAL RESISTANCE OF LISTERIA SPECIES ISOLATED FROM FILLETED ARGYROSOMUS HOLOLEPIDOTUS, SCOMBEROMORUS COMMERSON AND ALBURNUS SPP. 2016.

197. Abbasinejad B, Neyriz-Nagadehi M, Taher Talatappeh N. Prevalence and antimicrobial susceptibility of Listeria monocytogenes in Koozeh cheeses of Urmia retails. Food Hygiene. 2015;5(1 (17)):27-34.

198. Maktabi S, Pourmehdi M, Zarei M, Moalemian R. Occurrence and antibiotic resistance of Listeria monocytogenes in retail minced beef distributed in Ahvaz, South-West of Iran. Journal of food quality and hazards control. 2015;2(3):101-6.

199. Jamali H, Paydar M, Ismail S, Looi CY, Wong WF, Radmehr B, et al. Prevalence, antimicrobial susceptibility and virulotyping of Listeria species and Listeria monocytogenes isolated from open-air fish markets. BMC microbiology. 2015;15(1):144. Doi:10.1186/s12866-015-0476-7.

200. Haghi F, Zeighami H, Naderi G, Samei A, Roudashti S, Bahari S, et al. Detection of major food-borne pathogens in raw milk samples from dairy bovine and ovine herds in Iran. Small Ruminant Research. 2015;131:136-40. Doi:10.1016/j.smallrumres.2015.08.005.

201. Abdimoghadam Z, Shamloo E, Atefi M. Frequency of Listeria species in raw milk and traditional dairy products in Isfahan, Iran. Iranian Journal of Nutrition Sciences & Food Technology. 2015;10(3):101-7.

202. Mansouri-Najand L, Kianpour M, Sami M, Jajarmi M, editors. Prevalence of Listeria monocytogenes in raw milk in Kerman, Iran. Veterinary Research Forum; 2015: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

203. Rahimi E, Shakerian A. Prevalence of Listeria Species in Ready-to-Eat Food in Shahrekord Restaurants. Medical Laboratory Journal. 2014;8(2):83-7.

204. Haj Hosseini A, Sharifan A, Tabatabaee A. Isolation of Listeria monocytogenes from meat and dairy products. Journal of Medical Microbiology and Infectious Diseases. 2014;2(4):159-62.

205. Rahimi E, Momtaz H, Behzadnia A, Baghbadorani ZT. Incidence of Listeria species in bovine, ovine, caprine, camel and water buffalo milk using cultural method and the PCR assay. Asian Pacific Journal of Tropical Disease. 2014;4(1):50-3. Doi:10.1016/S2222-1808(14)60313-3.

206. Rahimi E. Prevalence and antimicrobial resistance of Listeria species isolated from smoked and salted fish. Iran J Med Microbiol: Volume. 2014;8(3).

207. Jamileh N, SIASIE TE, Baharvand R. Isolation of Listeria monocytogenes from meat products and study on prfA gene in bacteria isolated and comparing with clinical and standard samples. 2013.

208. Khamesipour F, Khodadoustan Shahraki A, Moumeni M, Khadivi Boroujeni R, Yadegari M. Prevalence of Listeria monocytogenes in the crayfish (Astacus leptodactylus) by polymerase chain reaction in Iran. International Journal of Biosciences. 2013;3(10):160-9. Doi:10.12692/ijb/3.10.160-169.

209. Sohrabi R, Rashedi M, Dorcheh MP, Shahbazi AM. Prevalence of Listeria species in raw milk in Esfahan Province, Iran. African Journal of Microbiology Research. 2013;7(19):2057-60. Doi: 10.5897/AJMR12.1463.

210. Jamali H, Paydar M, Looi CY, Wong WF. Prevalence of Listeria species and Listeria monocytogenes serotypes in ready mayonnaise salads and salad vegetables in Iran. African Journal of Microbiology Research. 2013;7(19):1903-6. Doi: 10.5897/AJMR2013.5658.

211. Fallah AA, Saei-Dehkordi SS, Mahzounieh M. Occurrence and antibiotic resistance profiles of Listeria monocytogenes isolated from seafood products and market and processing environments in Iran. Food control. 2013;34(2):630-6. Doi:10.1016/j.foodcont.2013.06.015.

212. Jamali H, Radmehr B, Thong KL. Prevalence, characterisation, and antimicrobial resistance of Listeria species and Listeria monocytogenes isolates from raw milk in farm bulk tanks. Food Control. 2013;34(1):121-5. Doi:10.1016/j.foodcont.2013.04.023.

213. Akya A, Najafi F, Moradi J, Mohebi Z, Adabagher S. Prevalence of food contamination with Listeria spp. in Kermanshah, Islamic Republic of Iran. EMHJ-Eastern Mediterranean Health Journal, 19 (5), 474-477, 2013. 2013.

214. Momtaz H, Yadollahi S. Molecular characterization of Listeria monocytogenes isolated from fresh seafood samples in Iran. Diagnostic Pathology. 2013;8(1):1-6. Doi:10.1186/1746-1596-8-149

215. Dorcheh MP, Sohrabi R, Salajegheh M. Prevalence of Listeria species in retail quail products from Isfahan, Iran. Journal of Veterinary Medicine and Animal Health. 2013;5(1):16-9. Doi: 10.5897/JVMAH12.019

216. Khalili Borujeni F, Moshtaghi H, Bonyadian M. Study on contamination of sheep meat in Shahrekord area with Listeria ivanovii and determination its antibiotic resistance pattern.

Iranian Journal of Medical Microbiology. 2013;7(1):15-21.

217. Rahimi E, Yazdi F, Farzinezhadizadeh H. Prevalence and antimicrobial resistance of Listeria species isolated from different types of raw meat in Iran. Journal of food protection. 2012;75(12):2223-7. Doi:10.4315/0362-028X.JFP-11-565

218. Fallah AA, Saei-Dehkordi SS, Rahnama M, Tahmasby H, Mahzounieh M. Prevalence and antimicrobial resistance patterns of Listeria species isolated from poultry products marketed in Iran. Food Control. 2012;28(2):327-32. Doi:10.1016/j.foodcont.2012.05.014

219. Rahimi E, Shakerian A, Raissy M. Prevalence of Listeria species in fresh and frozen fish and shrimp in Iran. Annals of Microbiology. 2012;62(1):37-40. Doi:10.1007/s13213-011-0222-9

220. Modaresi R, Mardani K, Tukmechi A, Ownagh A. Prevalence of Listeria spp. in fish obtained from Urmia fish markets. African Journal of Microbiology Research. 2011;5(30):5398-401. Doi: 10.5897/AJMR11.888

221. Rahimi E, Ameri M, Momtaz H. Prevalence and antimicrobial resistance of Listeria species isolated from milk and dairy products in Iran. Food Control. 2010;21(11):1448-52. Doi:10.1016/j.foodcont.2010.03.014

222. Jami S, Jamshidi A, Khanzadi S. The presence of Listeria monocytogenesin raw milk samples in Mashhad, Iran. Iranian Journal of Veterinary Research. 2010;11(4):363-7.

223. Mahmoodi MM. Occurrence of Listeria monocytogenes in raw milk and dairy products in Noorabad, Iran. Journal of Animal and Veterinary Advances. 2010;9(1):16-9.

224. Jalali M, Abedi D. Prevalence of Listeria species in food products in Isfahan, Iran. International journal of food microbiology. 2008;122(3):336-40. Doi:10.1016/j.ijfoodmicro.2007.11.082

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225. Rahimi E, Momtaz H, Hemmatzadeh F. The prevalence of Escherichia coli O157: H7, Listeria monocytogenes and Campylobacter spp. on bovine carcasses in Isfahan, Iran. Iranian Journal of Veterinary Research. 2008;9(4):365-70. Doi: 10.22099/IJVR.2008.2620

226. Shahbazi N, Javadi B, Afshari A, Jamshidi A, Ghavidel M. Identification of Coxiella Burnetti and Mycobacterium SPP through Touch-down PCR Examination in Unpasteurized Camel Milk in North-East of Iran. Journal of Nutrition, Fasting & Health. 2022;10(4). Doi: 10.22038/JNFH.2022.64095.1382.

227. Mokarizadeh K, Ownagh A, Tajik H, editors. Molecular detection of Coxiella burnetii in Kope cheese and cattle milk in West Azerbaijan, Iran. Veterinary Research Forum; 2023: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Doi:10.30466/vrf.2022.544750.3323

228. Mobarez AM, Mostafavi E, Khalili M, Esmaeili S. Identification of Coxiella burnetii in raw milk of livestock animal in Iran. International Journal of Microbiology. 2021;2021. Doi:10.1155/2021/6632036

229. Kazemeini H, Partovi R, Nazaktabar A, Shokri H. Detection of Coxiella burnetii in raw milk samples collected from dairy farms in Mazandaran province, north of Iran. Journal of Food Safety and Hygiene. 2022. Doi:10.18502/jfsh.v7i3.9137

230. Nourozhaghi NA, RAHIMI E, SHAKERIAN A. Prevalence of Coxiella burnetii in bovine bulk milk by Nested-PCR in Gilan province. 2020.

231. Khademi P, Ownagh A, Ataei B, Kazemnia A, Enferadi A, Khalili M, et al. Prevalence of C. burnetii DNA in sheep and goats milk in the northwest of Iran. International Journal of Food Microbiology. 2020;331:108716. Doi:10.1016/j.ijfoodmicro.2020.108716

232. Esmaeili S, Mobarez AM, Khalili M, Mostafavi E. High prevalence and risk factors of Coxiella burnetii in milk of dairy animals with a history of abortion in Iran. Comparative immunology, microbiology and infectious diseases. 2019;63:127-30. Doi:10.1016/j.cimid.2019.01.015

233. Khademi P, Ownagh A, Mardani K, Khalili M. Prevalence of Coxiella burnetii in milk collected from buffalo (water buffalo) and cattle dairy farms in Northwest of Iran. Comparative immunology, microbiology and infectious diseases. 2019;67:101368. Doi:10.1016/j.cimid.2019.101368

234. Reisi M, Rahimi E, Momeni M. Prevalence of Coxiella burnetii in Traditional and Industrial Butter and Cream Using Nested Polymerase Chain Reaction in Shahrekord, Iran. Avicenna Journal of Clinical Microbiology and Infection. 2019;6(2):61-5. Doi: 10.34172/ajcmi.2019.12

235. Norouzian H, Diali HG, Azadpour M, Afrough P, Shakib P, Mosavi SM, et al. PCR detection of Coxiella burnetii in milk samples of ruminants, Iran. Journal of Medical Bacteriology. 2018;7(1-2):31-5.

236. Rahmdel S, Sadat Moezzi M, Azimzadeh N, Hosseinzadeh S. PCR Detection of Coxiella Burnetii in bovine bulk tank milk samples in Shiraz, southern Iran. International Journal of Nutrition Sciences. 2018;3(4):198-201. Doi: 10.30476/IJNS.2019.82644.1022

237. Mosleh N, Moslehishad M, Moosakhani F. Detection of Coxiella burnetii by Real-Time PCR in Raw Milk and Traditional Cheese Distributed in Tehran Province. Journal of Pharmaceutical & Health Sciences. 2018;6(2):149-55.

238. Lorestani S, Jaydari A, Maleki S, Khademi P. Genomic detection of Coxiella burnetii in sheep milk samples by Nest-

ed-PCR method in Khorramabad, Iran. 2016.

239. Kargar M, Rashidi A, Doosti A, Najafi A, Ghorbani-Dalini S. The sensitivity of the PCR method for detection of Coxiella burnetii in the milk samples. Zahedan Journal of Research in Medical Sciences. 2015;17(6). Doi:10.17795/zjrms988.

240. Ahmadizadeh C, Moosakhani F, Jamshidian M. Detection and Identification of Coxiella burnetii in Milk Cattles of Tehran Province. Advances in Bioresearch. 2015;6(4).

241. Khalili M, Diali HG, Mirza HN, Mosavi SM. Detection of Coxiella burnetii by PCR in bulk tank milk samples from dairy caprine herds in southeast of Iran. Asian Pacific Journal of Tropical Disease. 2015;5(2):119-22. Doi:10.1016/S2222-1808(14)60638-1.

242. Khademi MP. Genomic detection of Coxiella burnetii in cattle milk samples by Nested-PCR method, Iran. Iran J Med Microbiol: Volume. 2015;9(2).

243. Khanzadi S, Jamshidi A, Razmyar J, Borji S. Identification of Coxiella burnetii by touch-down PCR assay in unpasteurized milk and dairy products in North-East of Iran. Iranian Journal of Veterinary Medicine. 2014;8(1):15-9.

244. Borji S, Jamshidi A, Khanzadi S, Razmyar J. Detection of Coxiella burnetii and sequencing the IS1111 gene fragment in bulk tank milk of dairy herds. Iranian Journal of Veterinary Science and Technology. 2014;6(2):21-8. Doi:10.22067/VETERINARY.V6I2.26330.

245. Amin J, Peyman K, Samira L, Shahram M. DEVELOPMENT OF NESTED-PCR METHOD FOR DETECTING COXIELLA BURNETII IN GOAT MILK SAMPLES. Iranian Journal of Public Health. 2014;43(2):171.

246. Ghalyanchi Langeroudi A, Babkhani N, Zolfaghari MR, Majidzadeh Arbadili K, Morovvati A, Soleimani M. Detection of Coxeilla brunetii in bulk tank milk samples from dairy bovine farms using nested-PCR in Qom, Iran, 2011. Iranian Journal of Veterinary Medicine. 2013;7(3):207-11.

247. Kargar M, Rashidi A, Doosti A, Ghorbani-Dalini S, Najafi A. Prevalence of Coxiella burnetii in bovine bulk milk samples in southern Iran. Comparative clinical pathology. 2013;22(3):331-4. Doi:10.1007/s00580-012-1406-9.

248. Rahimi E, Ameri M, Karim G, Doosti A. Prevalence of Coxiella burnetii in bulk milk samples from dairy bovine, ovine, caprine, and camel herds in Iran as determined by polymerase chain reaction. Foodborne pathogens and disease. 2011;8(2):307-10. Doi:10.1089/fpd.2010.0684.

249. Rahimi E. Coxiella burnetii in goat bulk milk samples in Iran. African Journal of Microbiology Research. 2010;4(21):2324-6.

250. Rahimi E, Doosti A, Ameri M, Kabiri E, Sharifian B. Detection of Coxiella burnetii by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Zoonoses and public health. 2010;57(7-8):e38-e41. Doi:10.1111/j.1863-2378.2009.01289.x.

251. Torki Baghbadorani S, Rahimi E, Shakerian A. Investigation of Virulence and Antibiotic-Resistance of Bacillus cereus Isolated from Various Spices. Canadian Journal of Infectious Diseases and Medical Microbiology. 2023;2023. Doi:10.1155/2023/8390778.

252. Zeighami H, Nejad-dost G, Parsadanians A, Daneshamouz S, Haghi F. Frequency of hemolysin BL and non-hemolytic enterotoxin complex genes of Bacillus cereus in raw and cooked meat samples in Zanjan, Iran. Toxicology reports. 2020;7:89-92. Doi:10.1016/j.toxrep.2019.12.006.

253. Heydarzadeh M, Javadi A, Rad MG. Detection of emetic toxin (ces) gene and antimicrobial susceptibility of Bacillus cereus isolates from Iranian traditional dairy products. 2020. Doi:10.21203/rs.3.rs-61769/v1.

254. Deilami Khiabani Z, Noori E, Rahnama M, Shapouri R, Bigdelo Y, Ghamari D, et al. Detection of NHE Complex Genes in Bacillus cereus Isolated from Rice Samples from Zanjan, Iran by Multiplex PCR. Pathobiology Research. 2012;14(4):89-97.

255. Heydarzadeh M, Javadi A. Isolation and enumeration of Bacillus cereus in raw milk distributed in Tabriz, Iran and detection of ces gene among the isolates. Food Hygiene. 2018;8(2 (30)):35-42.

256. Ghourchian S, Douraghi M, Baghani A, Soltan Dallal M. Bacillus cereus Assessment in Dried Vegetables Distributed in Tehran, Iran. Journal of food quality and hazards control. 2018;5(1):29-32. Doi: 10.29252/jfqhc.5.1.29.

257. Hassanzadazar H, Taami B, Abbasi Z, Aminzare M. Microbial Contamination of Cream filled Pastries supplied in Confectioneries of Zanjan, Iran. Journal of Nutrition, Fasting and Health. 2018;6(Issue):30-4. Doi:10.22038/jnfh.2018.33470.1119.

258. Soleimani M, Hosseini H, Pilevar Z, Mehdizadeh M, Carlin F. Prevalence, molecular identification and characterization of Bacillus cereus isolated from beef burgers. Journal of Food Safety. 2018;38(1):e12414. Doi:10.1111/jfs.12414.

259. Ranjbar R, Shahreza MHS. Prevalence, antibiotic-resistance properties and enterotoxin gene profile of Bacillus cereus strains isolated from milk-based baby foods. Tropical Journal of Pharmaceutical Research. 2017;16(8):1931-7. Doi:10.4314/tjpr.v16i8.25.

260. Soltan Dallal MM, Nezamabadi S, Mardaneh J, Rajabi Z, Sirdani A. Detection of toxigenic bacillus cereus strains in powdered infant formula (PIF) milk by PCR assay. Tehran University Medical Journal TUMS Publications. 2017;75(3):179-86.

261. Rasoulpour T, Mahdavi S. Study of frequency of NHE complex genes in Bacillus cereus isolated from milk in Tabriz city in spring, 2017. 2020.

262. Molayi Ks, deilami Kz, Ghasemian A, SHAPOURI R, Taghinejad J, Eslami M, et al. Detection of hbla and bal genes in Bacillus cereus isolates from cheese samples using the polymerase chain reaction. 2016.

263. Keshtkar M, Momtaz H, Ghavamizadeh M, Rahimi E. Detection of Enterotoxin Genes Profile of Bacillus cereus in Pasteurized and Sterile Milk, Baby Food and Dairy Products. International Medical Journal. 2016;23(2).

264. Mashak Z, Langroodi AM, Ehsani A, Ilkhanipoor A, Fathabad AE. Microbiological quality of ready-to-eat foods of Tehran province. African Journal of Food Science. 2015;9(5):257-61. Doi: 10.5897/AJFS2015.1260.

265. Teymori R, Ghazanfarirad N, Dehghan K, Asadzadeh J, Hajigholizadeh G, Bahmani M. A survey of bacterial and mold contamination of imported rice into West Azerbaijan Province, northwest of Iran. Asian Pacific Journal of Tropical Disease. 2014;4:S833-S5. Doi:10.1016/S2222-1808(14)60737-4.

266. Rahimi E, Abdos F, Momtaz H, Torki Baghbadorani Z, Jalali M. Bacillus cereus in infant foods: prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. The Scientific World Journal. 2013;2013. Doi:10.1155/2013/292571.

267. Hosseini H, Hippe B, Denner E, Kollegger E, Haslberger A. Isolation, identification and monitoring of contaminant bacteria in Iranian Kefir type drink by 16S rDNA sequencing. Food Control. 2012;25(2):784-8. Doi:10.1016/j.foodcont.2011.12.017.

268. Rahimifard N, Fatholahzadeh B, Noory Z, Saadati S, Zavar M, Pirouz B, et al. Bacillus cereus contamination in infant formula: a study in food and drug control laboratory. Tehran University Medical Journal TUMS Publications. 2007;65(8):64-8.

269. Yazdi SS, Mobasseri B, Bonab PT, Yazdi SS, Mirbagheri Z, Dallal MMS. Prevalence and Characteristics of Yersinia Enterocolitica and Yersinia Pseudotuberculosis from Raw Milk Supplied in Tehran. Journal of Nutrition and Food Security. 2022. Doi:10.18502/jnfs.v8i1.11776.

270. Najjar Asiabani F, Bazaei M, Rais Sadat SA. Study of Yersinia enterocolitica contamination in red meat supplied In Shiraz, Iran. Journal of Zoonotic Diseases. 2021;5(3):28-33. Doi:10.22034/jzd.2021.13491.

271. Movafagh F, Zeinali T, Jamshidi A. Contamination Rate of Bovine Raw Milk with Yersinia enterocolitica Biotypes in Mashhad, Iran. Journal of Human Environment and Health Promotion. 2021;7(1):30-4. Doi: 10.29252/jhehp.7.1.5.

272. Tavassoli M, Jamshidi A, Ranjbar G, Torabi MR, Moghddam AA. Prevalence, Biotyping, and Antimicrobial Resistance of Yersinia enterocolitica Isolated from Traditional Iranian Cheeses-Evaluation of Yersinia enterocolitica in Traditional Cheeses. City. 2021;1(1B):5.

273. Tavassoli M, Jamshidi A, Movafagh F, Afshari A. Virulence Characteristics of Yersinia enterocolitica Isolated from Dairy Products in the Northeast of Iran. Journal of Human, Environment and Health Promotion. 2019;5(2):72-8. Doi:10.29252/jhehp.5.2.5.

274. Sirghani K, Zeinali T, Jamshidi A. Detection of Yersinia enterocolitica in retail chicken meat, Mashhad, Iran. Journal of pathogens. 2018;2018. Doi:10.1155/2018/1286216.

275. Alavi S, Rahimi E, Tajbakhsh E, Branch S. Prevalence of Yersinia enterocolitica in raw small ruminant milk in Shahrekord, Iran. Bulgarian J Vet Med. 2018;21(3):364-70. Doi: 10.15547/bjvm.1088.

276. Hadad A, Tajbakhsh E, Reiysi M. Identification of virulence genes in serotype O: 3 Yersinia enterocolitica isolated from turkey meat by PCR method in Shahrekord. Journal of Food Microbiology. 2018;5(2):1-10.

277. Dabiri H, Aghamohammad S, Goudarzi H, Noori M, Hedayati MA, Ghoreyshiamiri SM. Prevalence and antibiotic susceptibility of Campylobacter species isolated from chicken and beef meat. International Journal of Enteric Pathogens. 2016;2(2):6-17087. Doi:10.3168/jds.2014-8853.

278. Jamali H, Paydar M, Radmehr B, Ismail S. Prevalence, characterization, and antimicrobial resistance of Yersinia species and Yersinia enterocolitica isolated from raw milk in farm bulk tanks. Journal of dairy science. 2015;98(2):798-803. Doi:10.3168/jds.2014-8853.

279. Rahimi E, Sepehri S, Dehkordi FS, Shaygan S, Momtaz H. Prevalence of Yersinia species in traditional and commercial dairy products in Isfahan Province, Iran. Jundishapur Journal of Microbiology. 2014;7(4). Doi: 10.5812/jjm.9249

280. Saberianpour S, Tajbakhsh E, Khamesipour F, Branch S. Prevalence of virulence genes and biotyping of Yersinia enterocolitica isolated from chicken meat in Shahrekord, Iran. Vidyabharati International Interdisciplinary Research Journal. 2014;3:71-6.

281. Mehdi SDM, Hamid EK, Kazem SYM, Ali TM, Hamid C. Determination Of Yersinia Spp. And Salmonella Paratyphi B Isolated From Possibly Contaminated Cream Samples In The City Of Tehran. Payavard Salamat. 2014;8(1).

282. Momtaz H, Rahimian MD, Dehkordi FS. Identification and characterization of Yersinia enterocolitica isolated from raw chicken meat based on molecular and biological techniques. Journal of Applied Poultry Research. 2013;22(1):137-45. Doi:10.3382/japr.2012-00549.

283. Hanifian S, Khani S. Prevalence of virulent Yersinia enterocolitica in bulk raw milk and retail cheese in northern-west of Iran. International Journal of Food Microbiology. 2012;155(1-2):89-92. Doi:10.1016/j.ijfoodmicro.2012.01.012.

284. Mahdavi S, Farshchian MR, Amini K, Rad MAG, Ebadi AR. Survey of Yersinia enterocolitica contamination in distributed broiler meats in Tabriz City, Iran. African Journal of Microbiology Research. 2012;6(12):3019-23. Doi: 10.5897/AJMR12.094.

285. MK SY, Soltan-Dallal M, Bakhtiari R. Incidence and antibiotic susceptibilities of Yersinia enterocolitica and other Yersinia species recovered from meat and chicken in Tehran, Iran. African Journal of Microbiology Research. 2011;5(18):2649-53. Doi: 10.5897/AJMR11.248.

286. Mirmoeini SS, Sari AA, Goudarztalejerdi A, Alamoti MP, Staji H. Prevalence of Campylobacter spp. Among Broiler Carcasses at Industrial Slaughterhouses in Hamedan, Iran. Archives of Hygiene Sciences. 2023;12(2). Doi: 10.34172/AHS.12.2.1.393.

287. Mousavinafchi SB, Rahimi E, Shakerian A. Campylobacter spp. isolated from poultry in Iran: Antibiotic resistance profiles, virulence genes, and molecular mechanisms. Food Science & Nutrition. 2023;11(2):1142-53. Doi:10.1002/fsn3.3152.

288. Emami MS, Shakerian A, Chaleshtori RS, Rahimi E. Distribution of Virulence Genes in Campylobacter spp. Isolated from Agaricus Mushrooms in Iran. BioMed research international. 2023;2023. Doi:10.1155/2023/1872655.

289. Hadiyan M, Momtaz H, Shakerian A. Prevalence, antimicrobial resistance, virulence gene profile and molecular typing of Campylobacter species isolated from poultry meat samples. Veterinary Medicine and Science. 2022;8(6):2482-93. Doi:10.1002/vms3.944.

290. Sadeghi A, Ganji L, Fani F, Pouladfar G, Eslami P, Doregiraee F, et al. Prevalence, species diversity, and antimicrobial susceptibility of Campylobacter strains in patients with diarrhea and poultry meat samples: one-year prospective study. Iranian Journal of Microbiology. 2022;14(3):362. Doi: 10.18502/ijm.v14i3.9775.

291. Shahreza MS, Dehkordi NG, Nassar MF, Al-Saedi R. Genotyping of Campylobacter jejuni isolates from raw meat of animal species. Academic Journal of Health Sciences: Medicina balear. 2022;47(4):52-7. Doi: 10.3306/AJHS.2022.37.04.52.

292. Jahromi RR, Moradi F, Erfanian S, Pourahmadi M. Evaluation of the Contamination of Poultry Carcasses with Campylobacter jejuni and Campylobacter coli in Southern Iran: A Molecular Study. Jundishapur Journal of Health Sciences. 2021;13(3). Doi:10.5812/jjhs.116991.

293. Shafiei A, Rahimi E, Shakerian A. Prevalence, Virulence, and Antimicrobial Resistance of Campylobacter Species Isolated From Carcasses of Camels Slaughtered in Slaughterhouses of Chaharmahal and Bakhtiari Province, 2018-2019. Epidemiology and Health System Journal. 2021;8(3):115-21. Doi:10.34172/ijer.2021.22.

294. Far hang Zargar M. Identification of Campylobacter species in Jahrom slaughterhouse chickens in 2016-17. Journal of Jahrom University of Medical Sciences. 2019;17(3):1-6. Doi:10.52547/JMJ.17.3.1.

295. Maktabi S, Ghorbanpoor M, Hossaini M, Motavalibashi A, editors. Detection of multi-antibiotic resistant Campylobacter coli and Campylobacter jejuni in beef, mutton, chicken and water buffalo meat in Ahvaz, Iran. Veterinary Research Forum; 2019: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Doi: 10.30466/vrf.2019.34310.

296. Fani F, Aminshahidi M, Firoozian N, Rafaatpour N. Prevalence, antimicrobial resistance, and virulence-associated genes of Campylobacter isolates from raw chicken meat in Shiraz, Iran. Iranian journal of veterinary research. 2019;20(4):283.

297. Modirrousta S, Shapouri R, Rezasoltani S, Molaabaszadeh H. Prevalence of Campylobacter spp. and their Common Serotypes in 330 Cases of Red-meat, Chicken-meat and Egg-shell in Zanjan City, Iran. 2016. Doi: 10.7508/iem.2016.01.003.

298. Hosseinzadeh S, Mardani K, Aliakbarlu J, Ghorbanzadehghan M. Occurrence of Campylobacter in chicken wings marketed in the northwest of Iran. International Food Research Journal. 2015;22(1).

299. Zendehbad B, Khayatzadeh J, Alipour A. Prevalence, seasonality and antibiotic susceptibility of Campylobacter spp. isolates of retail broiler meat in Iran. Food Control. 2015;53:41-5. Doi:10.1016/j.foodcont.2015.01.008.

300. Dabiri H, Aghamohammad S, Goudarzi H, Noori M, Ahmadi Hedayati M, Ghoreyshiamiri SM. Prevalence and antibiotic susceptibility of Campylobacter species isolated from chicken and beef meat. Int J Enteric Pathog. 2014;2(2):1-4.

301. Rahimi E, Sepehri S, Momtaz H. Prevalence of Campylobacter species in milk and dairy products in Iran. Revue de Médecine Vétérinaire. 2013;164(5):283-8.

302. Kazemeini H, Valizade Y, Parsaei P, Nozarpour N, Rahimi E. Prevalence of Campylobacter species in raw bovine milk in Isfahan, Iran. Middle-East J Sci Res. 2011;5:664-6.

303. Rahimi E, Ameri M. Antimicrobial resistance patterns of Campylobacter spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran. Food Control. 2011;22(8):1165-70. Doi:10.1016/j.foodcont.2011.01.010.

304. Rahimi E, Kazemeini HR, Safaei S, Allahbakhshi K, Momeni M, Riahi M. Detection and identification of Campylobacter spp. from retail raw chicken, turkey, sheep and goat meat

in Ahvaz, Iran. African journal of microbiology research. 2010;4(15):1620-3.

305. Rahimi E, Ameri M, Kazemeini HR. Prevalence and antimicrobial resistance of Campylobacter species isolated from raw camel, beef, lamb, and goat meat in Iran. Foodborne pathogens and disease. 2010;7(4):443-7. Doi:10.1089/fpd.2009.0421.

306. Rahimi E, Tajbakhsh E. Prevalence of Campylobacter species in poultry meat in the Esfahan city, Iran. Bulg J Vet Med. 2008;11(4):257-62.

307. Asadi S, Rahimi E, Shakerian A. Helicobacter pylori Strains Isolated from Raw Poultry Meat in the Shahrekord Region, Iran: Frequency and Molecular Characteristics. Genes. 2023;14(5):1006. Doi:10.3390/genes14051006.

308. Mashak Z, Jafariaskari S, Alavi I, Shahreza MS, Dehkordi FS. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020;13:257. Doi:10.2147/IDR.S233612.

309. Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019;39(2):e12594. Doi:10.1111/jfs.12594.

310. Gilani A, Razavilar V, Rokni N, Rahimi E, editors. VacA and cagA genotypes of Helicobacter pylori isolated from raw meat in Isfahan province, Iran. Veterinary Research Forum; 2017: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

311. Talimkhani A, Mashak Z. Prevalence and genotyping of helicobacter pylori isolated from meat, Milk and vegetable in Iran. Jundishapur Journal of Microbiology. 2017;10(11). Doi: 10.5812/jjm.14240.

312. Hemmatinezhad B, Momtaz H, Rahimi E. VacA, cagA, iceA and oipA genotypes status and antimicrobial resistance properties of Helicobacter pylori isolated from various types of ready to eat foods. Annals of clinical microbiology and antimicrobials. 2016;15(1):1-9. Doi:10.1186/s12941-015-0115-z.

313. Saeidi E, Sheikhshahrokh A. VacA genotype status of Helicobacter pylori isolated from foods with animal origin. BioMed research international. 2016;2016. Doi:10.1155/2016/8701067.

314. Gilani A, Razavilar V, Rokni N, Rahimi E. VacA and cagA genotypes status and antimicrobial resistance properties of Helicobacter pylori strains isolated from meat products in Isfahan province, Iran. Iranian journal of veterinary research. 2017;18(2):97.

315. Ranjbar R, Khamesipour F, Jonaidi-Jafari N, Rahimi E. Helicobacter pylori isolated from Iranian drinking water: vacA, cagA, iceA, oipA and babA2 genotype status and antimicrobial resistance properties. FEBS Open Bio. 2016;6(5):433-41. Doi:10.1002/2211-5463.12054.

316. Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. Tropical Journal of Pharmaceutical Research. 2016;15(8):1631-6. Doi:10.4314/tjpr.v15i8.5.

317. Talaei R, Souod N, Momtaz H, Dabiri H. Milk of livestock as a possible transmission route of Helicobacter pylori infection. Gastroenterology and hepatology from bed to bench. 2015;8(Suppl1):S30.

318. Esmaeiligoudarzi D, Tameshkel FS, Ajdarkosh H, Arsalani M, Sohani MH, Behnod V. Prevalence of Helicobacter pyloriinIranian milk and dairy products using culture and ureC based-PCR techniques. Biomedical and Pharmacology Journal. 2015;8(1):179-83. Doi:10.13005/bpj/597.

319. Atapoor S, Dehkordi FS, Rahimi E. Detection of Helicobacter pylori in various types of vegetables and salads. Jundishapur Journal of Microbiology. 2014;7(5). Doi: 10.5812/jjm.10013.

320. Yahaghi E, Khamesipour F, Mashayekhi F, Safarpoor Dehkordi F, Sakhaei MH, Masoudimanesh M, et al. Helicobacter pylori in vegetables and salads: genotyping and antimicrobial resistance properties. BioMed Research International. 2014;2014. Doi:10.1155/2014/757941.

321. Rahimi E, Kheirabadi EK. Detection of Helicobacter pylori in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne Pathogens and Disease. 2012;9(5):453-6. Doi:10.1089/fpd.2011.1060.

322. Ansarian Barezi A, Shakerian A, Rahimi E, Esfandiari Z. Examining the Extent of Contamination, Antibiotic Resistance, and Genetic Diversity of Clostridioides (Clostridium) difficile Strains in Meat and Feces of Some Native Birds of Iran. BioMed Research International. 2023;2023. Doi:10.1155/2023/3524091.

323. Ghorbani Filabadi P, Rahimi E, Shakerian A, Esfandiari Z. Prevalence, antibiotic resistance, and genetic diversities of Clostridium difficile in meat nuggets from various sources in Isfahan, Iran. Journal of Food Quality. 2022;2022. Doi:10.1155/2022/9919464.

324. Hassani S, Mahmoudi R, Kabudari A, Ghajarbeygi P, Peymani A, Moosavi S, et al. The Prevalence of Clostridium difficile and Clostridium perfringens in Minced and Ground Beef in Iran. Journal of Chemical Health Risks. 2022;12(4):575-80.

325. Bacheno H, Ahmadi M, Fazeli F, Ariaii P. Antibiotic Resistance Of Clostridioides Difficile Isolated From Bovine, Vone And Caprine Raw Meat. Journal of Pharmaceutical Negative Results. 2022:9721-6. Doi: 10.47750/pnr.2022.13.S09.1136.

326. Hassani S, Pakbin B, Brück WM, Mahmoudi R, Mousavi S. Prevalence, antibiotic resistance, toxin-typing and genotyping of Clostridium perfringens in raw beef meats obtained from Qazvin city, Iran.Antibiotics.2022;11(3):340.Doi:10.3390/antibiotics11030340.

327. Ram M, Tavassoli M, Ranjbar G, Afshari A. The Microbial and Chemical Quality of Ready-to-Eat Olivier Salad in Mashhad, Iran. Journal of Nutrition, Fasting and Health. 2019;7(4):175-81. Doi: 10.22038/jnfh.2019.40572.1199.

328. Hosseinzadeh S, Bahadori M, Poormontaseri M, Dehghani M, Fazeli M, Nazifi S. Molecular characterization of Clostridium perfringens isolated from cattle and sheep carcasses and its antibiotic resistance patterns in Shiraz slaughterhouse, southern Iran. Veterinarski arhiv. 2018;88(5):581-91. Doi:10.24099/vet.arhiv.0009.

329. Shahdadnejad N, Mohammadabadi M, Shamsadini M. Typing of clostridium perfringens isolated from broiler chickens using multiplex PCR. Genetics in the 3rd millennium. 2016;14(4):4368-74.

330. Doosti A, Pasand M, Mokhtari-Farsani A, Ahmadi R, Chehelgerdi M. Prevalence of Clostridium perfringens type a isolates in different tissues of broiler chickens. Bulgarian Journal of Veterinary Medicine. 2017;20(1). Doi: 10.15547/bjvm.919.

331. Hajimohammadi B, Dehghani A, Javadzadeh M, Zandi H, Eslami G. Clostridium botulinum spores and fungal contamination in honeys of Iran. Tolooebehdasht. 2017;15(6):1-9.

332. Shakerian A, Rahimi E, Mesbah J, Mousavi M. Molecular Detection of Clostridium Perfringens in Some Raw Animal Food Origin Products in Shahrekord, 2014. Journal of Payavard Salamat. 2017;11(4):391-9.

333. Afshari A, Jamshidi A, Razmyar J, Rad M, editors. Genotyping of Clostridium perfringens isolated from broiler meat in northeastern of Iran. Veterinary Research Forum; 2015: Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

334. Afshari A, Jamshidi A, Razmyar J, Rad M. Molecular typing of Clostridium perfringens isolated from minced meat. Iranian Journal of Veterinary Science and Technology. 2015;7(1):32-9. Doi:10.22067/VETERINARY.V7I1.39040.

335. Poormontaseri M, Hosseinzadeh S, Shekarforoush S. Characterization of Clostridium botulinum spores and its toxin in honey. Iranian Journal of Veterinary Research. 2014;15(1):36-9.

336. Sadeghi Sarvestani MV, Hosseinzadeh S, Poormontaseri M, Fazeli M. Monitoring the Various Types of Clostridium botulinumin in Four Kinds of Food Stuffs Using Multiplex PCR. Journal of Fasa University of Medical Sciences. 2014;3(4):380-6.

337. Shahcheraghi F, Nobari S, Asl HM, Aslani MM. Identification of botulinum toxin type in clinical samples and foods in Iran. Archives of Iranian medicine. 2013;16(11):0-.

338. Hosseini H, Tavakoli HR, Meshgi MA, Khaksar R, Hosseini M, Khakpour M. Survey of Clostridium botulinum toxins in Iranian traditional food products. Comparative clinical pathology. 2010;19(3):247-50. Doi:10.1007/s00580-009-0865-0.

339. Majzobi MM, Karami P, Khodavirdipour A, Alikhani MY. Brucellosis in humans with the approach of brucella species contamination in unpasteurized milk and dairy products from Hamadan, Iran. Iranian Journal of Medical Microbiology. 2022;16(4):282-7.

340. Asgari MH, Ahmadi E. Contamination of bovine milk with Brucella spp.: a current public health menace in Kurdistan province of Iran. Avicenna Journal of Clinical Microbiology and Infection. 2021;8(1):17-22. Doi: 10.34172/ajcmi.2021.04.

341. Majzoobi MM, Karami P, Khodavirdipour A, yousef Alikhani M. The contamination rate of Brucella spp. in unpasteurized dairy products used in urban and rural areas of Hamadan County, west of Iran. 2020. Doi:10.21203/rs.2.22703/v1.

342. Alamian S, Dadar M. Brucella abortus contamination of camel milk in two Iranian regions. Preventive veterinary medicine. 2019;169:104708. Doi:10.1016/j.prevetmed.2019.104708.

343. Mirnejad R, Jazi FM, Mostafaei S, Sedighi M. Molecular investigation of virulence factors of Brucella melitensis and Brucella abortus strains isolated from clinical and non-clinical samples. Microbial pathogenesis. 2017;109:8-14. Doi:10.1016/j.micpath.2017.05.019.

344. Shirazi Z, Khalili M, Sadeghi B, Sharifi H, Ashrafganjooyi S. Detection of Brucella spp. in the sheep and goats milks from southeastern Iran using culture and PCR. Journal of Medical Microbiology and Infectious Diseases. 2017;5(3):40-2.

345. Ashrafganjooyi S, Saedadeli N, Alamian S, Khalili M, Shirazi Z. Isolation and biotyping of Brucella spp. from sheep and goats raw milk in southeastern Iran. Trop Biomed. 2017;34(3):507-11.

346. Majid Y, Somayeh N, Parisa S, Behrooz A, Reza FN, Javad R, et al. Prevalence of Brucella melitensis and Brucella abortus in raw milk and dairy product by real time PCR technique. The Ulutas Medical Journal. 2016;2(1):7-11. Doi:10.5455/umj.20151004054235.

347. Khalili M, Aflatoonian MR, Aliabadi FS, Abshenas J. Brucella contamination in raw milk by polymerase chain reaction. Tehran University Medical Journal TUMS Publications. 2016;74(7):517-21.

348. Shakerian A, Deo P, Rahimi E, Shahjavan A-R, Khamesipour F. Molecular detection of Brucella melitensis in sheep and goat milk in Iran. Tropical Journal of Pharmaceutical Research. 2016;15(5):913-8. Doi:10.4314/tjpr.v15i5.3.

349. Izadi A, Moslemi E, Tabatabaei Panah A, Kheiri Manjili H. Brucella spp. detection in dairy products using nested and hemi nested PCR techniques. Ann Biol Res. 2014;5(1):124-31.

350. Gholizadeh SS, Zali M, Hashempour A, AHMADI EM. Investigation of brucellosis in cattle and sheep in Urmia-Iran. Yüzüncü yıl Üniversitesi Veteriner Fakültesi Dergisi. 2013;24(3):133-4.

351. Karimi Alavigeh A, Sharifzadeh A. Study on the Frequency of Vibrio species in fish from Isfahan. Journal of Food Microbiology. 2021;8(4):47-55.

352. Shohreh P, Azizkhani M, Tooryan F. Food Poisoning Bacteria from Commercially Important Fish Species Collected from Markets in Mazandaran Province. Fisheries Science and Technology. 2019;8(1):1-6.

353. Asgarpoor D, Haghi F, Zeighami H. Detection and molecular characterization of Vibrio parahaemolyticus in shrimp samples. The Open Biotechnology Journal. 2018;12(1).

354. Mashak Z, Khalajzadeh A, Koohdar V. Study the bacterial and fungal quality and physicochemical properties of cold smoked salted fishes of Caspian sea, İran. Biosciences Biotechnology Research Asia. 2016;13(3):1811-20. Doi: 10.13005/bbra/2334.

355. Raissy M, Rahimi E, Azargun R, Moumeni M, Sohrabi H. Molecular detection of Vibrio spp. in fish and shrimp from the persian gulf. Journal of Food Biosciences and Technology. 2015;5(2):49-52.

356. Hosseini S, Safarpoor Dehkordi F, Rahimi E, Shakerian A. Prevalence study of Vibrio species and frequency of the virulence genes of Vibrio parahaemolyticus isolated from fresh and salted shrimps in Genaveh seaport. Food Hygiene. 2014;4(2 (14)):17-26.

357. Khamesipour F, Noshadi E, Moradi M, Raissy M. Detection of Vibrio spp. in shrimp from aquaculture sites in Iran using polymerase chain reaction (PCR). Aquaculture, Aquarium, Conservation & Legislation. 2014;7(1):1-7.

358. Dehkordi FS, Hosseini S, Rahimi E, Momeni M, Yahaghi E, Darian EK. Study the distribution of virulence genes of Vibrio parahaemolyticus isolated from fish, lobster and crab caught from Persian Gulf. Iran J Med Microbiol: Volume. 2014;8(2).

359. Raissy M, Khamesipour F, Rahimi E, Khodadoostan A. Occurrence of Vibrio spp., Aeromonas hydrophila, Escherichia coli and Campylobacter spp. in crayfish (Astacus leptodactylus) from Iran. Iranian Journal of Fisheries Sciences. 2014;13(4):944-54.

360. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A. Detection of Escherichia coli, Salmonella species, and Vibrio cholerae in tap water and bottled drinking water in Isfahan, Iran. BMC public health. 2013;13(1):1-7. Doi:10.1186/1471-2458-13-556.

361. Raissy M, Moumeni M, Ansari M, Rahimi E. Occurrence of Vibrio spp. in lobster and crab from the Persian Gulf. Journal of Food Safety. 2012;32(2):198-203. Doi:10.1111/j.1745-4565.2012.00368.x.

362. Rahimi E, Ameri M, Doosti A, Gholampour AR. Occurrence of toxigenic Vibrio parahaemolyticus strains in shrimp in Iran. Foodborne pathogens and disease. 2010;7(9):1107-11. Doi:10.1089/fpd.2010.0554.

363. Hosseini H, Cheraghali AM, Yalfani R, Razavilar V. Incidence of Vibrio spp. in shrimp caught off the south coast of Iran. Food control. 2004;15(3):187-90. Doi:10.1016/S0956-7135(03)00045-8.

364. Pakbin B, Didban A, Brück WM, Alizadeh M. Phylogenetic analysis and antibiotic resistance of Shigella sonnei isolates. FEMS microbiology letters. 2022;369(1):fnac042. Doi:10.1093/femsle/fnac042.

365. Pakbin B, Amani Z, Allahyari S, Mousavi S, Mahmoudi R, Brück WM, et al. Genetic diversity and antibiotic resistance of Shigella spp. isolates from food products. Food Science & Nutrition. 2021;9(11):6362-71. Doi:10.1002/fsn3.2603.

366. Pakbin B, Didban A, Monfared YK, Mahmoudi R, Peymani A, Modabber MR. Antibiotic susceptibility and genetic relatedness of Shigella species isolated from food and human stool samples in Qazvin, Iran. BMC research notes. 2021;14(1):1-6. Doi:10.1186/s13104-021-05554-3.

367. Tehrani ST, Harzandi N, Jabalameli L. Molecular detection of Shigella spp. contamination in ready-to-eat salad samples in west of Tehran. International Journal of Enteric Pathogens. 2018;6(2):41-4. Doi: 10.15171/ijep.2018.11.

368. Bahador N, Ajampour I. Phenotypic and genotypic characterization of isolated Shigella spp. from food based on IpaH gene and evaluation of their antibiotic patterns. Journal of Food Microbiology. 2019;5(4):55-65.

369. Yam BAZ, Khomeiri M, Mahounak AS, Jafari SM. Hygienic quality of camel milk and fermented camel milk (chal) in Golestan Province, Iran. Journal of Microbiology Research. 2014;4(2):98-103. Doi: 10.5923/j.microbiology.20140402.09.

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Cytotoxic Effects of Titanium Dioxide Nanoparticles on MCF-7 Cancer Cell Line

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ABSTRACT

Cancer is a widespread disease of various types worldwide that affects many people. Today, titanium dioxide nanoparticles have substantial therapeutic applications. We investigated how harmful titanium dioxide is to breast cancer cells. MCF-7 cancer cells and HFF cell lines were cultured. The survival of cells exposed to different amounts of titanium dioxide nanoparticles was tested. The examined concentrations were 25, 50, 100, and 200 µg/ml. The survival rate was measured after 48 and 72 hours and IC50 was determined. We found that the highest toxicity occured while MCF-7 and HFF cells were exposed to 200 µg/ml of titanium dioxide. Apoptosis in MCF-7 and HFF cells emerged as shown with Annexin V-PI staining and flow cytometry. Under a microscope, it was found that titanium dioxide nanoparticles could be harmful in specific amounts. At a dose of 200 µg/ml, after 48 and 72 hours of treatment, MCF-7 and HFF cells were affected. The mitochondrial membrane broke when breast cells were exposed to titanium dioxide nanoparticles. The matrix leaked into the cytoplasm, and the rough endoplasmic reticulum swelled. These observations occurred after 72 hours of treatment with a concentration of 200 µg/ml. Considering the acquired effects, titanium dioxide nanoparticles may be advocated as potential medicinal candidates for pharmaceutical purposes even though further research is required.

Keywords

TiO_2 Nanoparticles, Flow Cytometry, Micronucleus Assay, MTT Assay, TEM

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Abbreviations

NP: Nanoparticle
TiO_2: Titanium dioxide
FBS: Fetal bovine serum
TEM: Transmission electron microscope
DMSO: Dimethyl Sulfoxide

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Introduction

Nanotechnology is a broad field of applied physical sciences, chemical engineering, and biological engineering. NPs are tiny particles with dimensions less than 100 nm. Nanotechnology is used to supply cosmetics and medications. NPs have ancient records and were utilized by artisans in the 9th century to polish the surface of pottery. TiO_2, known as titanium IV, was first commercially extracted from ore in 1923. Titania exists in three forms: rutile, anatase, and brookite. Due to its luster, TiO_2 is used as a light-reflecting coating in papers and tablets as a white pigment, and also as an ultraviolet inhibitor in sunscreen.

Oxidative stress caused by environmental pollution and harmful gases, such as greenhouse gases, plays an essential role in aging. Moreover, the accumulation of reactive oxygen species damages the nucleic acid of the cells and can lead to aging. NPs build up rapidly in the environment, and the unique behavior of NPs, especially their high surface-to-volume ratio, is the reason for their high reactivity and ability to pass through cell membranes. NPs cause cell damage and inflammation due to the production of free radicals in cells [1].

The autosomal cell cycle has four phases, the first of which is called the S phase, where DNA synthesis and replication occur. The second phase is the M phase, where cell separation happens. In addition to the S and M phases, the cell cycle has three other phases: G1, G2, and G0. Cyclin kinases are responsible for controlling these phases. There are two types of tumors: benign and malignant. Malignant tumors are cancerous and grow fast. They can spread to other tissues and invade the body [2]. Cancerous cells can enter the bloodstream or lymphatic system and spread to distinct elements of the frame. All forms of most cancers are a consequence of troubles inside the cells. Normal cells usually divide to make new cells at the same time as they are needed, preserving stability amongst cell increase and cellular loss of life. While the managing system that regulates cellular boom is disrupted, cells divide uncontrollably and form tumors. Mutations that increase cell branches and prevent the loss of cell existence can cause cancers . In the modern day, scientists have discovered mutated genes in human cancer cells. These genes are divided into three groups: proto-oncogenes, tumor suppressors, and DNA restore genes. Mutations in these genes can set off proto-oncogenes or inhibit tumor suppressor genes, leading to out-of-control cell growth and immortality. Mutations in DNA repair genes can also cause the accumulation of more significant mutations. Cancers are now the second leading cause of death worldwide. Breast cancer is the most cancer for women. It causes more deaths than lung and stomach cancers. A survey reported 40,430 deaths from breast cancers (40,000 women and 430 men) in 2014 [3]. Cancer is the leading cause of death in Iran, after coronary heart disease and accidents [4]. Reducing death rates from breast cancer is difficult in developing countries, including Iran [5]. Treating breast cancer is difficult because chemotherapy has limited effectiveness and side effects. Therefore, today the focus is on controlled and targeted drug delivery systems. NPs can target and deliver anticancer agents to maximize their effectiveness and minimize the side effects [6]. There is an urgent need to find new substances for treatment. NPs are an alternative for treating diseases due to their biological effects and small and unique size. TiO_2 is used in many industries, such as paper, plastic, cosmetics, and paint. It is also used as a disinfectant and biological sensor to kill tumor cells [7]. The anticancer influences of TiO_2 have been investigated on several cancers, and the experiments showed that the viability of most cancers depends on the particle doses and counseled that TiO_2 NPs can be used for cancer treatment. TiO_2 NPs affect MCF-7 and HFF cell traces in distinctive concentrations. This cell line has been considerably used as a human breast cancer cell line for the development of recent antitumor treatments. The purpose of this was to assess the cytotoxic activity of TiO_2 NPs.

Result

Cell Growth Inhibition by TiO_2 NPs

TEM Technique

TEM image represents the morphology and organelles of MCF-7 and HFF cells via the NPs. TEM analysis shows the structural adjustments and damages occurring following treatment with TiO_2. Intracellular uptake of TiO_2 NPs was established by TEM. The MCF-7 and HFF cells were incubated for 72 h with 0 and 200 µg/ml of TiO_2 NPs.

Figure 1 (A and B) demonstrates the fibroblast cells without treatment, where the organelles are seen as healthy and intact. After 72 h, the results showed that the mitochondrial organelle was elongated and the rough endoplasmic reticulum was inflamed. Figure 2 (A and B) shows breast cancer cells without treatment. Compared to normal cells, these cells have many mitochondrial organelles due to multiple and misplaced mitotic divisions. Figure 2 (C and D) indicates the breast cancer cells treated with a concentration of 200 µg/ml for a period of 72 h. Electron microscope images show the presence of TiO_2 NPs inside the cell, as well as mitochondrial membrane rupture

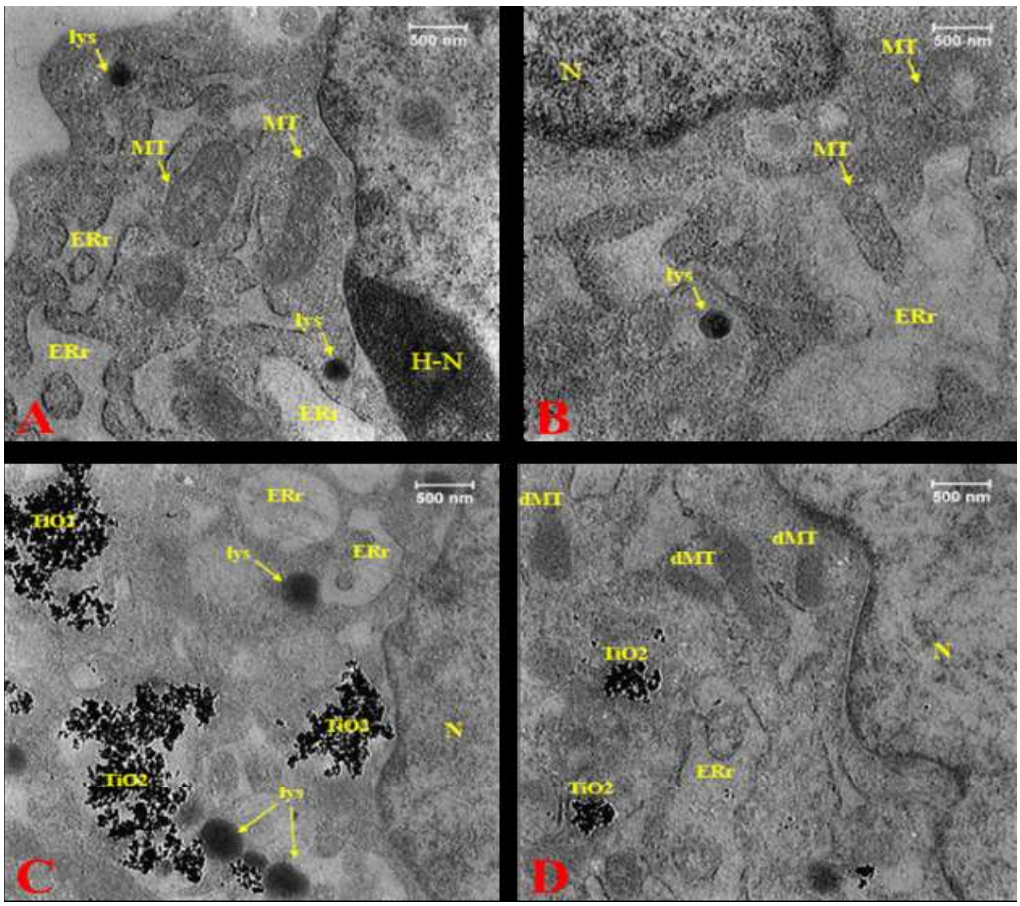

Figure 1.
Effect of 200 µg/ml concentrations of TiO_2 NPs on HFF cell line. A, B: Control group-HFF cell, ×4000; C, D: Dose 200 µg/ml-HFF cell, ×4000. Lys, lysosome; MT, mitochondria; ERr, rough endoplasmic reticulum; H-N, heterochromatin nucleus; N, nucleus; TiO_2, TiO_2 nanoparticle; dMT, damage mitochondria; N, nucleus.

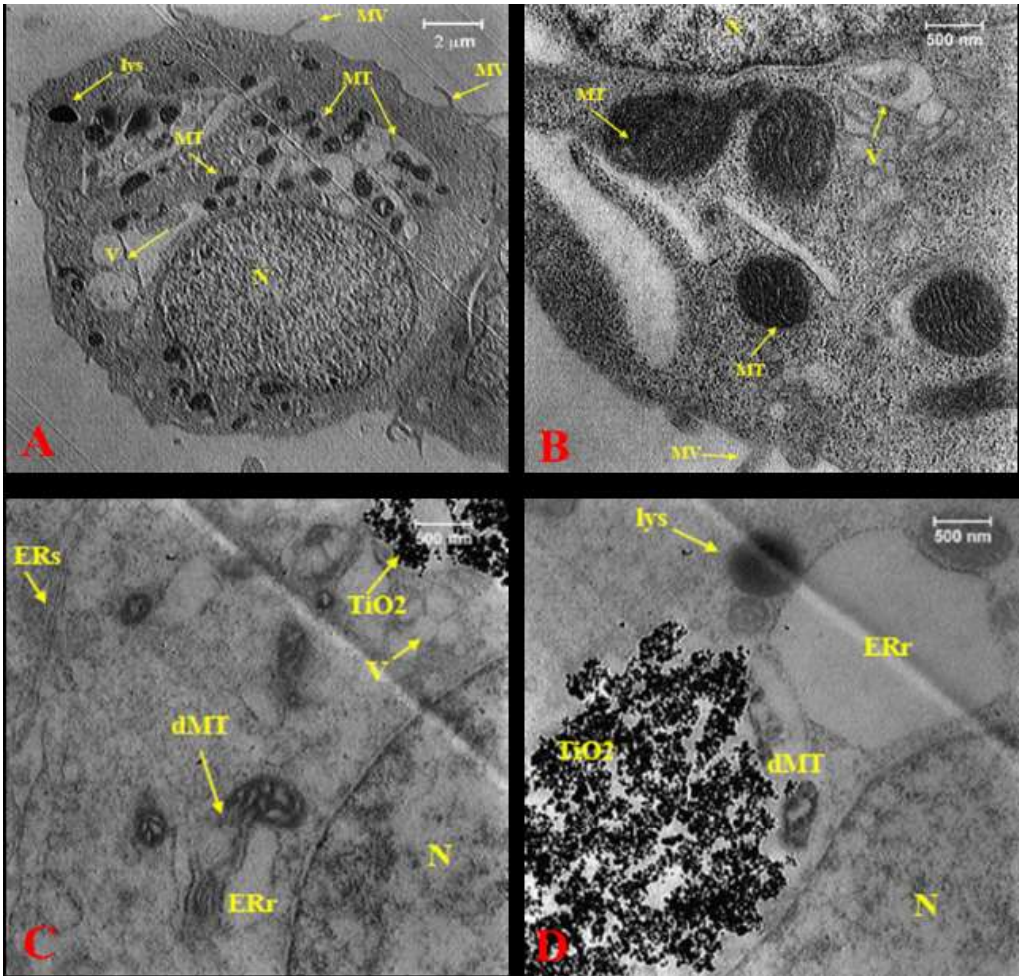

Figure 2.
Effect of 200 µg/ml concentrations of TiO2 NPs on MCF-7 cell line.
A, B: Control group-MCF-7 ×4000; C, D: Dose 200 µg/ml-MCF-7. Lys, lysosome; MT, mitochondria; MV microvilli; V, vacuole cytoplasm; N, nucleus; ERs, smooth endoplasmic reticulum; dMT, damage mitochondria; ERr, rough endoplasmic reticulum; TiO_2, TiO_2 nanoparticle.

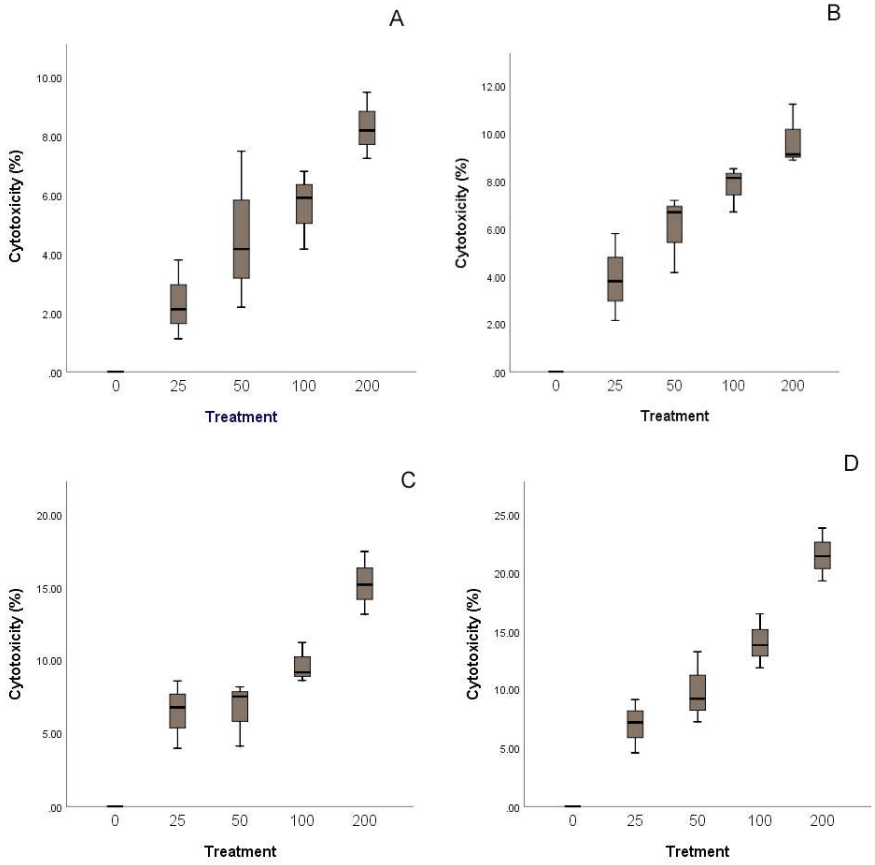

Figure 3.
Effect of different concentrations of TiO2 NPs (µg/ml) on HFF and MCF-7 cells line. A: 48h, HFF cell line; B: 72h, HFF cell line; C: 48h, MCF-7 cell line; D: 72h, MCF-7 cell line.

and leakage. Moreover, they present the swelling of the rough endoplasmic reticulum.

MTT Assay

In our study, we used TiO_2 NPs to treat MCF-7 and HFF cell lines. The used concentrations were in the range of 25-200 µg/ml. We used the MTT assay to measure cell growth inhibition. As the concentration of TiO_2 NPs increased, we found that cell growth was increasingly inhibited. This was observed after 48 and 72 h of exposure to the NPs. Cell viability decreased in a dose-dependent manner. Figure 3 shows that the amount of inhibition of cells depends on the concentration. At the concentration of 200 µg/ml, cell death percentages were significantly lower after 48 and 72 h of treatment ($p = 0.01$, $p < 0.05$). We calculated the IC50 values for TiO_2 NPs. The values represent the concentrations that cause 50% toxicity or death in the MCF-7 cancer cells and normal HFF cells. The IC50 was 420 and 1000 µg/ml for MCF-7 and HFF, respectively.

Micronucleus Assay

The micronucleus technique is a very convenient and fast method for examining the structural abnormalities of chromosomes. This technique is extensively used to study the cell morphology. These groups included the absence of or presence of NPs. The results of light microscopy showed that TiO_2 NPs can cause concentration-dependent toxicity at a dose of 200 µg/ml in the treatment periods of 48 and 72 h in the MCF-7 and HFF cell lines. As a result, at the concentration of 200 µg/ml, the percentage of cell death in the treatment periods of 48 and 72 h ($p = 0.01$) is significantly lower than other groups ($p < 0.05$) (Figure 4).

Flow Cytometry Assay

To assess the rate of cell death due to TiO_2 NPs, the MCF-7 and HFF cells were handled with IC50 attention. The cells were then stained with FITC Annexin V and PI and were analyzed by flow cytometry. The flow cytometry results are presented in Figure 5. While exposed to 420 µg/ml of TiO_2 NPs, the MCF-7 and HFF cells experienced a substantial decrease in viable cells. Moreover, the proportion of apoptotic cells extended. In the meantime, the percentage of necrotic cells was tiny and negligible.

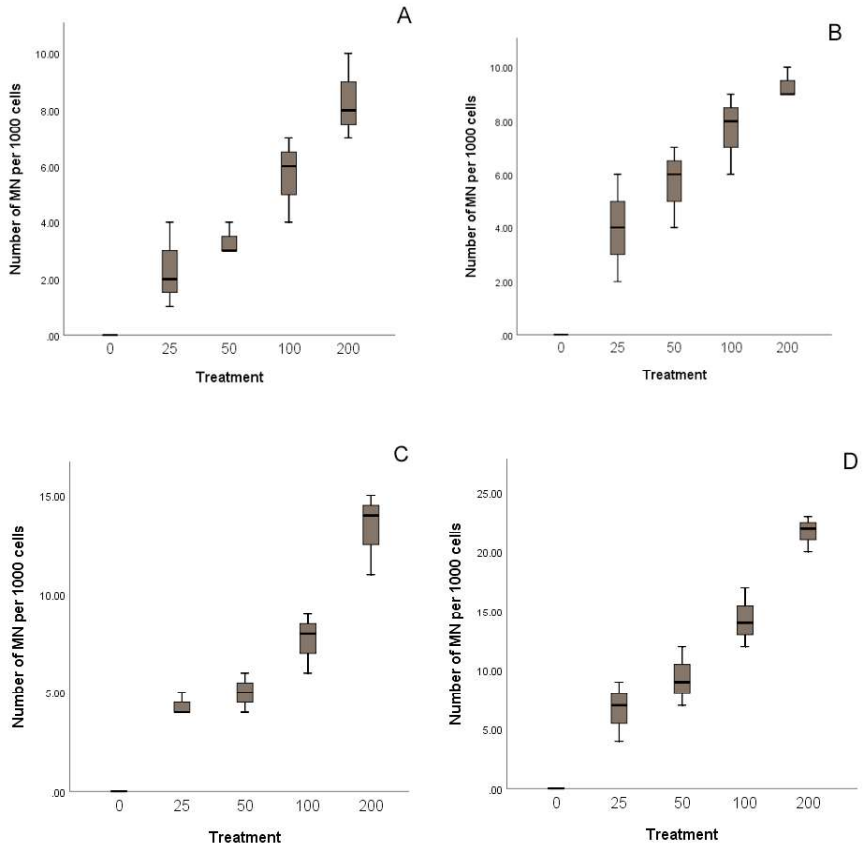

Figure 4.
Effects of different concentrations of TiO2 NPs (µg/ml) on HFF and MCF-7 cells line. A: 48h, HFF cell line; B: 72h, HFF cell line; C: 48h, MCF-7 cell line; D: 72h, MCF-7 cell line.

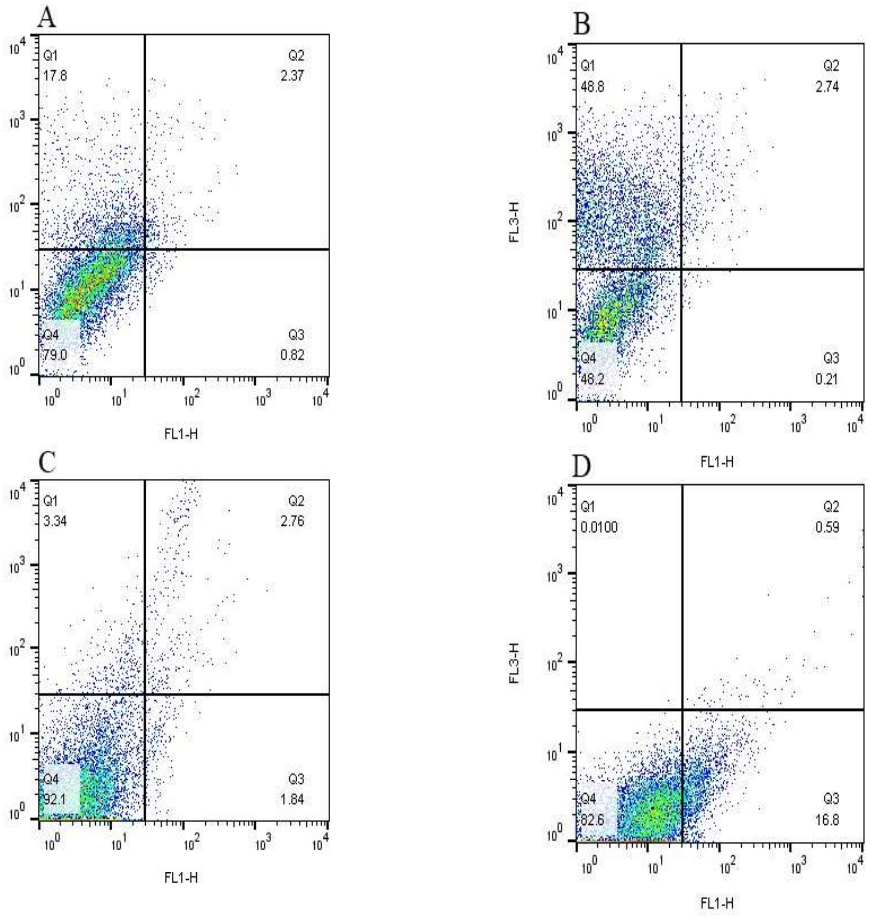

Figure 5.
Effect of TiO_2 NPs 200 (µg/ml) on HFF and MCF-7 cells line. A: control, HFF cell line; B: 72h, HFF cell line; C: control, MCF-7 cell line; D: 72h, MCF-7 cell line.

Discussion

NPs are a necessity in physics and dentistry because they can contest bacteria, fungi, and viruses (8-10). However, researchers have limited studies on the antineoplastic effects of TiO_2 NPs. The TiO_2 NPs did not harm glioma C6, RG2, mouse, or human glioma U373 cells. Copper-TiO_2 NP complex turned out to be much less poisonous than copper alone, indicating some protection from the harm of TiO_2 NPs. However, the complex became somewhat more venomous than cisplatin. The copper-TiO_2 complex can be a part of mitochondria and ATP composition. It may also lower the shaping of nitrogenic bases. Moreover, it may reach the cell nucleus and hook up with DNA base pairs. This occurs through interplay or groove binding. In the long run, it can trigger apoptotic cellular death [11].

Reports indicated that TiO_2 NPs, with added Au and Pt, effectively destroyed the K562 tumor cells [12]. The way TiO_2 NPs are taken up is not well understood. Some studies have suggested that TiO_2 NPs are taken up and stored in different cell parts, such as vacuoles, endosomes, and lysosomes. They may also be found in the cytoplasm because the lysosomal membrane breaks. Recently, it was found that TiO_2 NPs can enter human cells through a specific receptor. Human bronchial epithelial BEAS 2B cells were treated with nm uncoated anatase TiO_2-NPs smaller than 25 and SiO_2-lined rutile TiO_2-NPs of 10-40 nm.

Regardless of the truth that the uncoated TiO_2-NPs increased the micronucleus, the SiO_2-protected NPs no longer [13]. Guichard et al. [14] determined that none of the TiO_2-NPs or TiO_2 bulk behavior momentous starting of micronuclei shape after 24 h of the exposure of these particles to SHE cells. Determind that none of the TiO_2 NPs or TiO_2 bulk resulted in significant formation of micronulei shape after 24 hours exposure to SHE cells.The genotoxic potential of 20 n.m TiO_2 was assessed in SHE cells.The cells were treated with 0.1 mg/cm2 of the particles for 12,24,48,66 and 72 hours.The micronuleuse frequenies were increased by the treatment in a time dependet manner. [15] . The genotoxic capability of 20 nm TiO_2-NPs became charged in the SHE cells. The cells were treated with 1 mg/ml TiO_2-NPs for 12, 24, 48, 66, and 72 h. The micronucleus frequencies were extended by treatment in an age-setting method [15]. The HepG2 cells were treated with a low dose of 30 nm TiO_2-NPs and a vital increase inside the micronucleus commonness was placed in the treated cells [16].

Human epidermal cells (A431) were treated with 50 nm anatase TiO_2-NPs at a dose of 80 mg/ml. The treatment caused tremendous chromosome change at a dose of 80 mg/ml [17]. Human lung cancer cells, A549, were treated with 10 and 50 mg/ml of TiO_2-NPs for 24 h, and a micronucleus assay was performed to decide the genotoxicity of the debris. There was a high-quality response within the micronucleus induction for each of the treatment concentrations [18, 19]. The toxicity of NPs for cancer cells was compared to normal cells. Investigations showed that the rate of the inhibition of cells depended on the concentration. However, with raising the concentration, the rate of toxicity increased, and on the other hand, the rate of cell survival diminished. Further investigations are required to explain the TiO_2 NPs and to clarify the toxicity of TiO_2 NPs on cells for finding modern methodologies for treating cancer and other diseases.

Conclusion

According to the observed effects, TiO_2 NPs may be endorsed as potential medicinal candidates for

Materials and Methods

We obtained chemicals and reagents from different companies. RPMI1640 and FBS were purchased from Biosera in France. TiO2 NPs (nanopowder) were obtained from Sigma-Aldrich (Germany). We purchased MTT, plastic dishes, 6-well and 96-well plates, doxorubicin, and DMSO from BETACELL in Belgium .

TiO_2 Np Topography and NPs Characterization

We purchased TiO_2 NPs from Sigma-Aldrich company (United Kingdom). The TiO2 NPs used in this study were titanium (IV) oxide, and anatase, with a purity of 99.7%. We weighed the TiO2 NPs and placed them in natural water. In order to reduce the length of NP aggregates, NPs were sonicated three times for 30 min. We analyzed the TiO_2 NPs using a particle length analyzer (D mean number 35.51 n.m) (NanoQ Report). The topographical surface was then studied with a TEM (LEO 912 AB) (Figure 1).

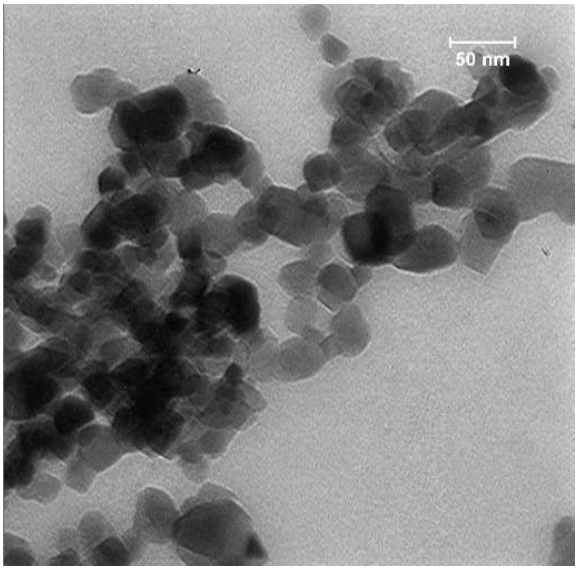

Figure 1. Detection of aggregation of TiO_2 nanoparticles (NPs) by transmission electron microscopy (TEM).

Cell Culture

The MCF-7 and HFF cells (Ferdowsi University) were cultured at 37˚C in RPMI 1640 and Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated FBS, 500 µg/ml penicillin-streptomycin, and 200 µg/ml amphotericin B in a humidified atmosphere with 5% CO_2. Cells were then harvested by treatment with 0.25% trypsin-EDTA.

Cytotoxic Activity

MTT test is used to check the share of living cells. A cell flask with 60% confluence was trypsinized, and after cell counting, it was transferred to a 6-well plate, so that about 150×103 cells were placed in 200 µl of way-of-life medium for every concentration of TiO_2 NPs, and were incubated at 37°C for 48 and 72 h. Cellular survival was assessed by adding 100 µl MTT (5 mg/ml in PBS containing 10% FBS) to each well, and the cells were incubated every other 4 hours. To dissolve the resultant formazan, 100 µl dimethyl sulfoxide was delivered, and absorbance was measured by a spectrophotometer at a wavelength of 540 nm. Moreover, cell death was calculated by the following formula. SPSS software and the Kruskal Wallis test were used for the statistical analysis of the data with a significance level of $p < 0.05$.

Survival Percentage = OD test/OD count ×100

Analysis of Apoptosis by Flow Cytometry

To measure cell death, we used a flow cytometry test called annexin V-FITC apoptosis detection kit. The test was performed following the instructions provided by the manufacturer. We treated the MCF-7 and HFF cells (5×105) with a solution of TiO2 NPs at different concentrations of 25, 50, 100, and 200 µg/ml. After 48 and 72 h, we gently removed the cells from the dishes, washed them once with a medium containing serum, and put them in 500 µl buffer. Next, we added 5 µl of annexin V-FITC and 5 µl of propidium iodide. The cells were incubated at room temperature for 15 min in the dark. Finally, cells were analyzed using a BD Falcon flow cytometer (USA).

Intracellular Uptake of TiO_2 NPs

We exposed cells to different amounts of TiO_2 NPs for 48 and 72 h. Afterwards, we washed the cells three times with PBS and stuck them for 1 h with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH: 7.4) at 4˚C. We collected the cells with a rubber scraper, dried them, and embedded them in Araldite M. Samples were stained with uranyl acetate and lead citrate. Finally, thin sections were observed under a LEO 912 AB TEM at a magnification of ×4,000.

Slide Preparation for Micronucleus Assay

We created two slides for each sample. The quantity of fixative varied depending on the pellet. We produced a total of four slides for each sample . On each slide, we introduced two 20 µl drops of cell suspension. Next, we allowed the slides to dry in the airand stained them with 10% Giemsa stain in phosphate buffer for 10 min. Afterwards, we allowed the slides to dry in the air for a single day. Subsequently, the slides were covered with a cover slip.

Statistical Analysis

The distribution of MTT and micronucleus values among the control and four test groups receiving different doses of TiO_2 was compared using the non-parametric Kruskal Wallis test. A pairwise comparison was performed using the Mann-Whitney U test with Bonferroni adjustment. MTT and micronucleus values were compared between MCF7 and HFF cell lines and also between two assessing times (48 and 72 h) using the Mann-Whitney U test. The data were analyzed using IBM SPSS Statistics version 26 software (IBM SPSS Statistics, Chicago, USA).

Authors' Contributions

Investigation, writing the original draft, and statistical analysis: RJ. Conceptualization, supervision, software, draft review, and editing: RJ, AR, HM, MA. All authors were involved in writing the article and accepted responsibility for its content.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. Kleinjans J, Janssen Y, Van Agen B, Hageman G, Schreurs J. Genotoxicity of coal fly ash, assessed in vitro in Salmonella typhimurium and human lymphocytes, and in vivo in an occupationally exposed population. Mutat Res Genet Toxicol. 1989; 224(1): 127-34. Doi: 10.1016/0165-1218(89)90011-6.

2. Hamel P A,Hanley-Hyde J. G1 cyclins and control of the cell division cycle in normal and transformed cells. Cancer Investig. 1997; 15(2): 143-52. Doi: 10.3109/07357909709115767.

3. American Cancer Society. Global Cancer Facts & Figures. 2 ed. Atlanta: American Cancer Society; 2014.

4. Goya M. Iranian Annual Cancer Registration Report 2005/2006. Tehran: Center for Disease Control and Prevention, Iranian Ministry of Health and Medical Education; 2007.

5. Sadjadi A, Nouraie M, Ghorbani A, Ali Mohammadian M,-Malekzadeh R. Epidemiology of breast cancer in the Islamic Republic of Iran: first results from a population-based cancer registry. East. Mediterr. Health J. 2009; 15:1426-31.

6. Qi L, Xu Z,Chen M. In vitro and in vivo suppression of hepatocellular carcinoma growth by chitosan nanoparticles. Eur J Cancer. 2007; 43(1): 184-93. Doi:10.1016/j.ejca.2006.08.029.

7. Wang J J, Sanderson B J,Wang H. Cyto-and genotoxicity of ultrafine TiO2 particles in cultured human lymphoblastoid cells. Mutat Res Genet Toxicol Environ Mutagen. 2007; 628(2): 99-106. Doi: 10.1016/j.mrgentox.2006.12.003.

8. Dhas S P, John S P, Mukherjee A,Chandrasekaran N. Autocatalytic growth of biofunctionalized antibacterial silver nanoparticles. Biotechnol. Appl. Biochem. 2014; 61(3): 322-332. Doi:/10.1002/bab.1161.

9. García-Contreras R, Argueta-Figueroa L, Mejía-Rubalcava C, Jiménez-Martínez R, Cuevas-Guajardo S, Sanchez-Reyna P A, et al. Perspectives for the use of silver nanoparticles in dental practice. Int. Dent. J. 2011; 61(6): 297-301. Doi:/10.1111/j.1875-595X.2011.00072.x.

10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1983; 65(1-2): 55-63. Doi: 10.1016/0022-1759(83)90303-4.

11. Lazau C, Mocanu L, Miron I, Sfirloaga P, Tanasiea G, Tatua C, et al. Consideration regarding the use of TiO2 doped nanoparticles in medicine. Dig. J. Nanomater. Biostructures 2007; 2(3): 257-63.

12. Lopez T, Ortiz-Islas E, Guevara P,Gomez E. Catalytic nanomedicine technology: copper complexes loaded on titania nanomaterials as cytotoxic agents of cancer cell. Int J Nanomedicine. 2013; 581-92. Doi:10.2147/IJN.S37118.

13. Saimon S M, Kanehira K,Taniguchi A. Comparison of cellular uptake and inflammatory response via Toll-like receptor 4 to lipopolysaccharide and titanium dioxide nanoparticles. Int. J. Mol. Sci. 2013; 14:13154-170. Doi: 10.3390/ijms140713154.

14. Guichard Y, Schmit J, Darne C, Gaté L, Goutet M, Rousset D, et al. Cytotoxicity and genotoxicity of nanosized and microsized titanium dioxide and iron oxide particles in Syrian hamster embryo cells. Ann Occup Hyg. 2012; 56:63144. Doi:10.1093/annhyg/mes006.

15. Prasad R Y, Wallace K, Daniel K M, Tennant A H, Zucker R M, Strickland J, et al. Effect of treatment media on the agglomeration of titanium dioxide nanoparticles: impact on genotoxicity, cellular interaction, and cell cycle. ACS nano. 2013; 7(3): 1929-42. Doi:10.1021/nn302280n.

16. Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss D G, et al. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ. Health Perspect. 2002; 110(8): 797-800. Doi: 10.1289/ehp.02110797.

17. Shukla R K, Kumar A, Gurbani D, Pandey A K, Singh S, Dhawan A, et al. TiO2 nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. Nanotoxicology. 2013; 7(48e60).DOI: 10.3109/17435390.2011.629747.

18. Shukla R K, Sharma V, Pandey A K, Singh S, Sultana S, Dhawan A, et al. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicol In Vitro. 2011; 25:231e241.Doi: 10.1016/j.tiv.2010.11.008.

19. Srivastava R K, Rahman Q, Kashyap M P, Lohani M,Pant A B. Ameliorative effects of dimetylthiourea and N-acetylcysteine on nanoparticles induced cyto-genotoxicity in human lung cancer cells-A549. PLoS One 2011; 6:e25767. DOI: 10.1371/journal.pone.0025767.

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Antidiabetic and Protective Effects of *Ferula assa-foetida* L. oleo Gum Resin Ethanolic Extract on the Testis of Streptozotocin-Induced Diabetic Rats: A Histopathological Study

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ABSTRACT

Diabetes is one of the most common metabolic diseases worldwide which affects all organs, including the reproductive system. Today, many researchers use medicinal plants instead of chemical agents to achieve fewer side effects. *Ferula assa-foetida* L. is one of the medicinal plants used to treat many diseases traditionally for years. The present study evaluated the antidiabetic and protective effects of *Ferula assa-foetida* L. on the testis of streptozotocin-induced diabetic male rats. The histomorphologic study of diabetic rats treated with Ferula assa-foetida L. extract showed a significant improvement in testes. Histological studies revealed that treatment with the Ferula assa-foetida L. extract significantly increased sperm count in the seminiferous tubules and reduced fibrosis. Our study confirmed the improving effects of *Ferula assa-foetida* L. on histomorphometric and biochemical parameters in diabetes and related testicular damage, which are partially attributed to the bioactive compounds and antioxidants in *Ferula assa-foetida* L.

Keywords

Ferula assa-foetida, Rat, Diabetes, Reproductive system, Spermatogenesis, Medicinal plants

Number of Figures: 5
Number of Tables: 1
Number of References:: 51
Number of Pages: 11

Abbreviations

FAE: Ferula assa-foetida L. oleo gum resin ethanolic extract
SOD: Superoxide Dismutase
Met: Metformin
STZ: Streptozotocin
FSH: Follicle-Stimulating Hormone
LH: Luteinizing Hormone

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Introduction

One of the common metabolic and endocrine diseases that is a serious threat to public health, especially in developing countries, is diabetes [1]. Diabetes is caused by a decrease in insulin secretion or sensitivity [2]. Disturbances in carbohydrate, lipid, and protein metabolism affect the secretion and function of insulin which is very worrying for the health of society [3]. Therefore, improper lifestyle and nutrition leading to obesity and overweight have an effective role in the prevalence and occurrence of diabetes [4].

An increase in blood glucose level is one of the clear symptoms of diabetes, which results in structural and functional changes in various tissues and organs, such as the reproductive system [5]. Abnormal feedback of sex steroids in the hypothalamus-pituitary axis, which is observed in diabetic rats, is the result of abnormal transfer of steroids or decreased sensitivity of the pituitary gland [6, 7]. Moreover, various studies have shown that hyperglycemia in diabetes negatively affects male and female fertility [8,9]. Testes are sensitive to hyperglycemia [10]. Weight loss[11], abnormal germinal epithelium [5, 12], and disruption of the testicular blood barrier are among the complications of diabetes [13]. When the blood glucose level rises, glucose autoxidation causes excessive production of free radicals and finally oxidative stress [14]. A medication with fewer side effects to treat diabetes is Met which is used to control hyperglycemia and inhibits gluconeogenesis in hepatocytes. Its mechanism of action is inhibiting mitochondrial respiration and reducing cellular energy levels, which decreases glucose production by hepatocytes [15]. Treatment of male diabetic mice with Met preserves the structure and function of the testis [16]. In order to reduce the negative effects of free radicals on the reproductive system and testes, many investigations have evaluated the impact of antioxidant compounds on this system. Among these antioxidant compounds, natural antioxidants found in medicinal plants have attracted the attention of scientists due to fewer side effects than chemical antioxidants on living organisms. Some medicinal plants, such as curcumin[17], Ficus Carica [18], Telferia Occidentalis [19], and Ginger [20], have been investigated. Another medicinal plant with antioxidant properties is Ferula assa-foetida L. [21,22, 23] which has been used to treat many diseases for centuries. This plant, which is native to Iran, is also called Anghuzeh, a member of the Umbelliferae (Apiaceae) family [24]. Components of Ferula assa-foetida L. oleogum resin are Ferulic acid, esters, coumarins, other terpenoids [24], umbelliferone [25], bisabolol, and quercetin[23]. It has been effective and available for the treatment of neurological disorders, stomachache, intestinal parasites, weak digestion, asthma, bronchitis, influenza, infertility, and diabetes for many years [24, 26, 27]. Research has shown that the use of Ferula assa-foetida L. is effective in the treatment of liver and kidney diseases, hyperglycemia, and hyperlipidemia [28, 29]. In addition, the anti-obesity impacts of Ferula assa-foetida L. were investigated and the results showed that leptin and blood glucose levels decreased after consuming Ferula assa-foetida L. [30]. Therefore, Ferula assa-foetida L. can be a good candidate for the treatment of diabetes because of its availability and natural antioxidant properties. As the number of people with diabetes is increasing rapidly [1], the age of diabetes is decreasing[31] which raises the number of people with diabetes in reproductive age. Therefore, identifying the mechanisms that destroy the testes in diabetes, discovering effective substances and medications, and preventing infertility and reproductive disorders are important issues. With this background, we decided to investigate the protective effects of FAE on the testis of STZ-induced diabetic rats.

Result

Morphometric Data

According to Figure 1 and Table 1, the thickness of the epithelium of seminiferous tubules in group 5 (diabetic rats treated with 250 mg/kg FAE) decreased significantly compared to other groups (Figure 1).

The examination of the size of the seminiferous tubules in different experimental groups showed that the size of the seminiferous tubules in groups 1, 3, and 4 increased significantly compared to groups 2 and 5, also in groups 3 and 4 compared to group 2 (Figure 1; Table. 1).

Johnson, s Score

According to Figure 1 and Table 1, Johnson's Score decreased in groups 2 and 5 compared to other groups.

Biochemical Evaluation

The comparison between groups 1 and 2 showed that diabetes caused a significant decrease in testosterone levels in rats. Furthermore, a significant increase in testosterone levels in group 4 compared to group 2 indicated the positive effect of FAE on testosterone levels in diabetes (Figure 2.d).

As shown in Figure 3, the level of blood glucose increased in diabetic control group rats compared to non-diabetic control group rats. Moreover, the lack of significant difference between groups 1 and 3 showed that the low dose of FAE in non-diabetic rats did not

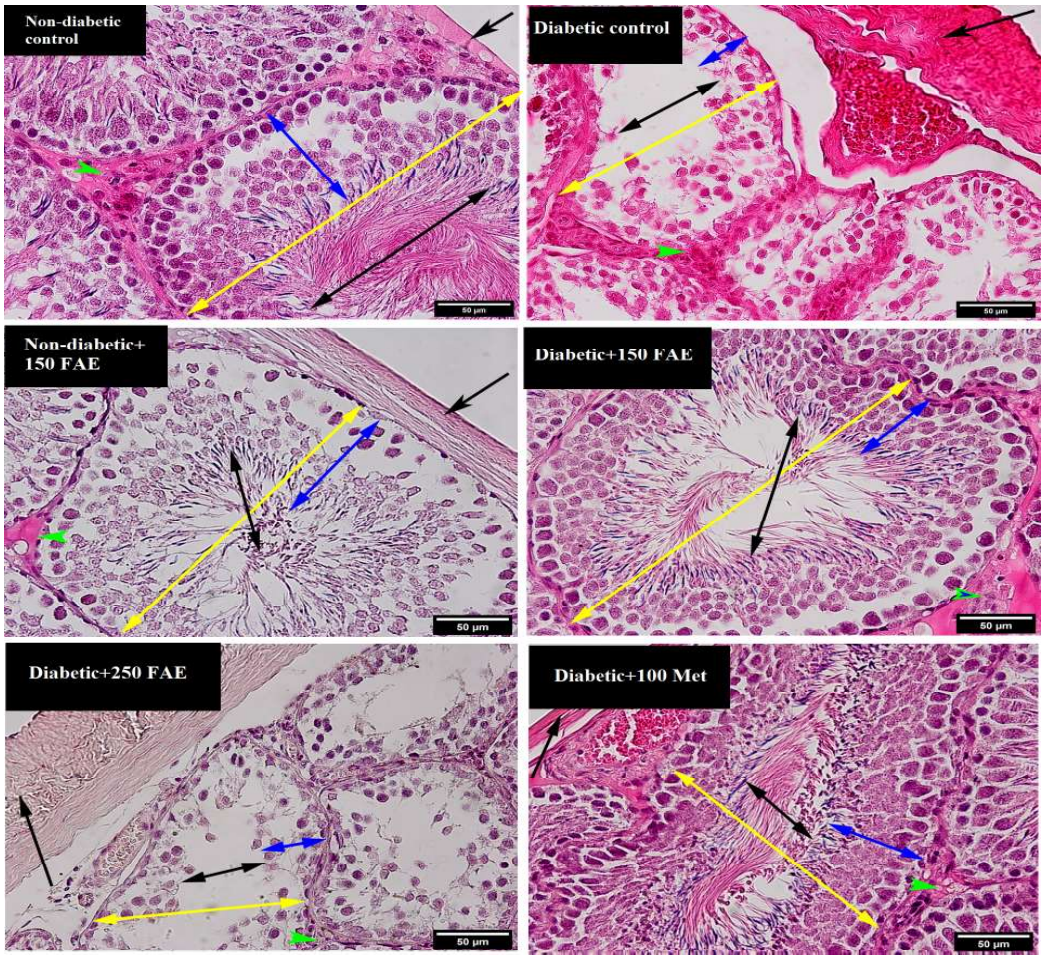

Figure 1.
Cross section of the testis from different groups showing Tubular Diameter (TD) and different parts of the testis. The yellow arrows showing tubular diameter (TD); the black double-sided arrow indicate lumen diameter (LD); green arrow heads showing interstitial tissue (IT); black single-side arrows show tunica albuginea (TA); the blue double-sided arrow indicate epithelium thickness (ET).

reduce blood sugar levels. A significant decline in blood glucose levels was observed in group 4 compared to groups 2 and 5, which shows that a lower dose of FAE reduces blood sugar levels in diabetes (Figure 2. b).

The results of evaluating blood insulin levels in different groups showed that insulin secretion in group 3 rose compared to groups 1, 2, 5, and 6. The levels of insulin in groups 3 and 4 did not differ significantly. The high levels of insulin in group 3 compared to other groups indicated the positive effect of a lower dose of FAE on insulin levels in non-diabetic subjects (Figure 2. c).

Table 1.
FAE improves the morphometric features of the testis tubules in diabetes-induced rats.

	Johnson, s score	Tubule Diameter	Epithelium thickness
Group1	$9.58 \pm 0.54^{\ddagger\#}$	$168.83 \pm 9.06^{\#\ddagger}$	46.16 ± 1.19
Group2	$5.57 \pm 0.49^{\dagger\pm\S}$	$114.66 \pm 84^{\dagger\pm\S}$	$33.83 \pm 1.30^{*\dagger\pm}$
Group3	9.89 ± 0.32	$174.40 \pm 6.72^{\ddagger}$	$47.60 \pm 1.72^{\ddagger}$
Group4	$9.51 \pm 0.17^{\ddagger}$	$176.80 \pm 3.18^{\ddagger}$	47.40 ± 1.07
Group5	$5.34 \pm 1.22^{\S\dagger}$	88.50 ± 12.40	$21.66 \pm 4.79^{*\#\pm\S}$
Group6	9.41 ± 0.40	$163.00 \pm 8.61^{\ddagger}$	40.80 ± 2.78

Data showing the tubular diameter, epithelium thickness and Johnson's Score in different experimental groups.
Statistics: Data are mean values ± SEM; One-way ANOVA with Tukey test; $P < 0.05$ was considered as significant; *, significant in comparisons with non-diabetic control; #, significant in comparison with diabetic control; †, significant in comparison with non-diabetic+150 mg/kg b.w FAE ; ±, significant in comparison with diabetic rats treated with 150 mg/kg b.w FAE; ‡,significant in comparison with diabetic rats treated with 250 mg/kg b.w FA; ¤, significant in comparison with diabetic rats treated with 100 mg/kg b.w Met.

Enzyme Activity

As shown in Figure 3, SOD enzyme activity decreased in diabetes and low dose of FAE increased the activity of this enzyme in diabetic and non-diabetic rats.

Weight Evaluation

A significant difference in body weight was observed between group 5 and other groups (Figure 4. c). As shown in Fig. 4, the study groups were not significantly different in terms of the relative weight of the testes neither in the right testis nor in the left testis (Figure 4.g&h). According to Figure 5, there was a significant difference between groups 1, 2, 5, and 6,

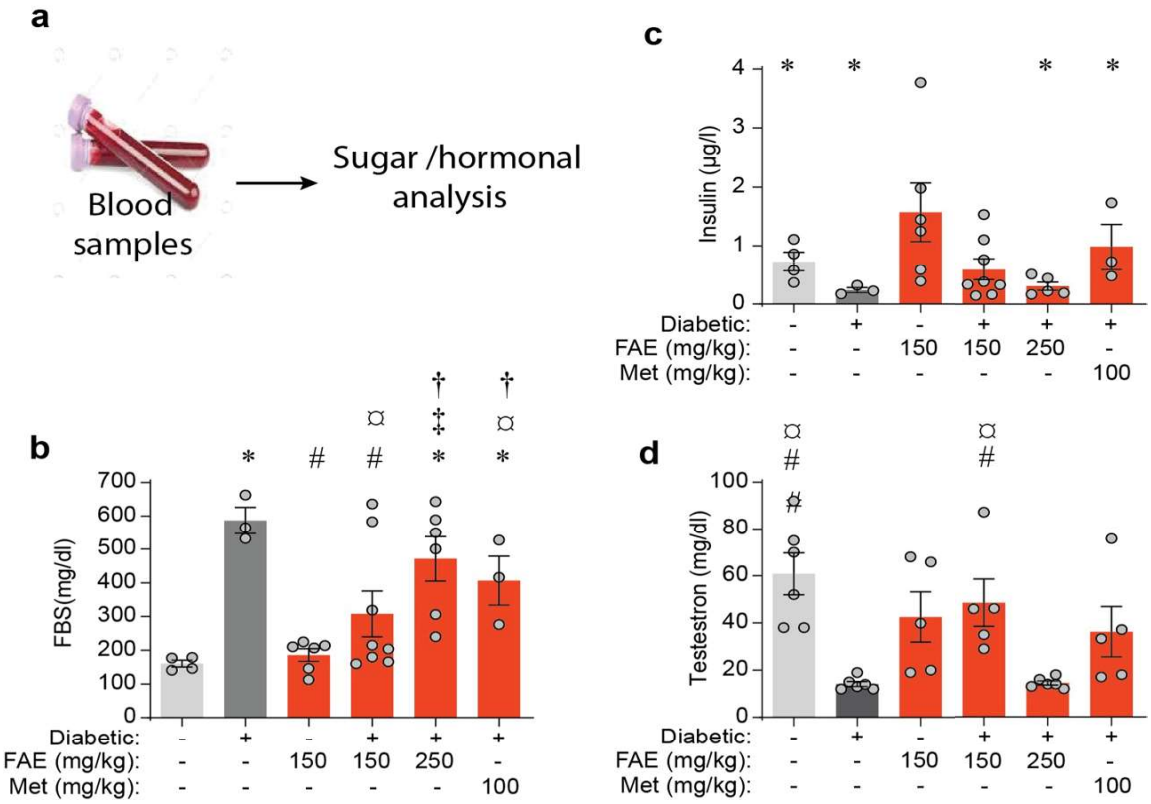

Figure 2.
FAE (150 mg/kg b.w) corrects sugar and hormonal level in the blood of treated rats and its positive effect reduces at higher doses.
a, collected blood samples from tail vein of the animals before organ collcetion. b, bar graph showing the FBS level in different experimental groups. c, bar graph showing the serum insulin level in different experimental groups. d, bar graph showing the testestrone level in different experimental groups.
Statistics: bar graphs are mean values ± SEM; One-way ANOVA with Tukey test; $P < 0.05$ was considered as significant; d & b, *, significant in comparisons with non-diabetic control; #, significant in comparison with diabetic control; †, significant in comparison with non-diabetic+150 mg/kg b.w FAE; ‡, significant in comparison with diabetic rats treated with 150 mg/kg b.w FAE; ¤, significant in comparison with diabetic rats treated with 250 mg/kg b.w FAE.C, *; significant in comparison with non-diabetic+150 mg/kg b.w FAE.

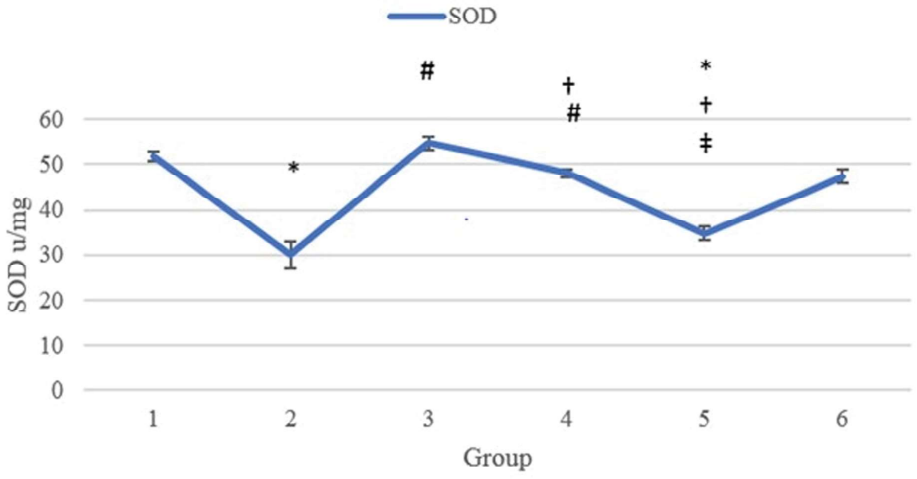

Figure 3.
FAE increases antioxidative enzyme (SOD): super oxide dismutase in diabetes-induced rats.
Statistics: bar graphs are mean values ± SD; One-way ANOVA with Tukey test; $P < 0.05$ was considered as significant; *, significant in comparisons with non-diabetic control (Group1); #, significant in comparison with diabetic control (group2); †, significant in comparison with non-diabetic+150 mg/kg b.w FAE (group3); ‡, significant in comparison with diabetic rats treated with 150 mg/kg b.w FAE (group4).

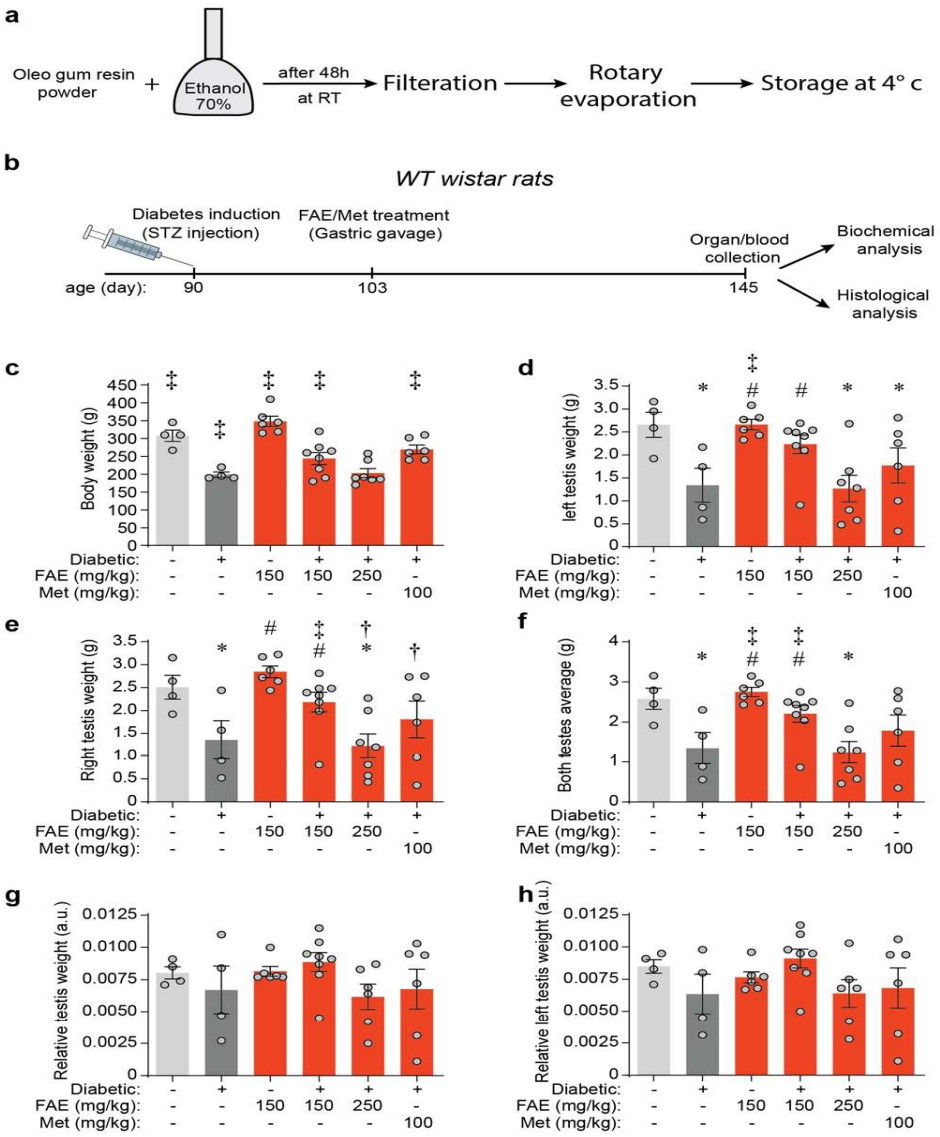

Figure 4.
FAE balances the body and testis weight in diabetic wistar rats.
a, Schematic drawing of the used protocol for FAE extraction. b, a drawing of the protocol used for induction of diabetes in rat and organ/blood collection from treated/untreated animals. c, representing the weight of the animals at the time of organ collection. d-f, showing the weight of the left, right and the average mass of both testes respectively. g & h, are the bar graphs showing the relative testis weight obtained by dividing the mass of each testis to the weight of the body. Statistics: bar graphs are mean values ± SEM; One-way ANOVA with Tukey test; $P < 0.05$ was considered as significant; *, significant in comparisons with non-diabetic control; #, significant in comparison with diabetic control; †, significant in comparison with non-diabetic+150 mg/kg b.w FAE; ±, significant in comparison with diabetic rats treated with 150 mg/kg b.w FAE; ‡, significant in comparison with diabetic rats treated with 250 mg/kg b.w FAE.

which shows that diabetes had a negative effect on testis weight. Moreover, the weight of testicles in group 2 was reduced compared to group 4, which confirmed the positive effect of FAE on increasing testis weight (Figure 4.d). The decrease in testis weight in groups 2 and 5 compared to group 1 indicated the negative effect of diabetes on the absolute weight of the testis. The weight of the testicle in group 4 increased compared to groups 2 and 5, which emphasizes the positive effect of FAE as a treatment for diabetes. Groups 4 and 5 showed that a low dose of FAE has an increasing effect on the weight of the testis compared to its high dose. In addition, the absence of a significant difference between the untreated diabetic group and the diabetic group treated with a high dose of FAE indicated the destructive effect of high amounts of FAE on the testis tissue (Figure 4. e).

Testis Tissue Changes

H&E Staining

Histological findings of testes (Figure 1) by H&E staining showed that in groups 1 and 3, the structure and shape of tubules and sperm cells were normal compared to group 2. In group 2, the structure and shape of the seminiferous tubules changed. The number of cell layers and spermatozoa reduced and secondary spermatids were not seen. Degenerated spermatic cells and apoptotic cells with pyknotic nuclei were visible.

As seen in images (Figure 1), histopathological changes in the diabetic groups treated with FAE (group 4) and Met (group 6) were less than in the diabetic control group (group 2) and the arrangement and quality of sperm cells and the structure of the tubules improved (Figure 1).

In groups 2 and 5, the number of sperm cells decreased and secondary spermatids were not seen in the seminiferous tubules. In addition, the number of cell layers decreased (Figure 1). In group 3 (Figure 1),

good and normal spermatogenesis was observed in most of the tubules. We found that in group 4, spermatogenesis was good and normal, and Ferula prevented the effect of diabetes on the tubules (Figure 1). Good and normal spermatogenesis was observed in group 6 (Figure 1).

Masson's Trichrome Staining

Masson's Trichrome staining was used in order to show the changes in the connective tissue. The results showed that the connective tissue was normal in groups 1 and 3 (Figure 5). In group 2, the seminiferous tubules in the testes were degenerated. The extension of connective tissue into interstitial tissue, called fibrosis, increased significantly (Figure 5). Fibrosis in the group receiving the lower dose of FAE was less than in other diabetic groups (Figure 5), in group 5 was similar to group 2, and it was less in group 6 than in group 2 (Figure 5).

Discussion

Many studies have shown that diabetes has complex effects on the male reproductive system and spermatogenesis [5, 6, 8-10, 12, 17, 38-40]. Some of these influences include decreasing testosterone and insulin levels and increasing blood glucose. These changes were observed in the current investigation (Figures 1, 2, and 5). These biochemical alterations lead to decreased protein synthesis and increased cell apoptosis [40]. In the current study, the effects of diabetes on testicular tissue included a reduction in the number of germinal epithelial cell layers and a change in the number of germinal cells. These alterations are a result of apoptosis. The increase in testosterone levels in group 4 compared to groups 2 and 5 shows the positive effect of a lower dose of FAE and the negative effect of a higher dose of FAE on the testosterone level in diabetic rats (Figure 2.d). Diabetes affects pituitary gonadotropins and causes ultrastructural changes in the Sertoli and Leydig cells, and these changes disrupt normal spermatogenesis [41]. Moreover, low and high doses of FAE increase and decrease testosterone levels in male rats, respectively [26]. We indicated that these hormonal alterations are along with tissue changes, including alterations in the number of cells and thickness of germinal epithelium in diabetic

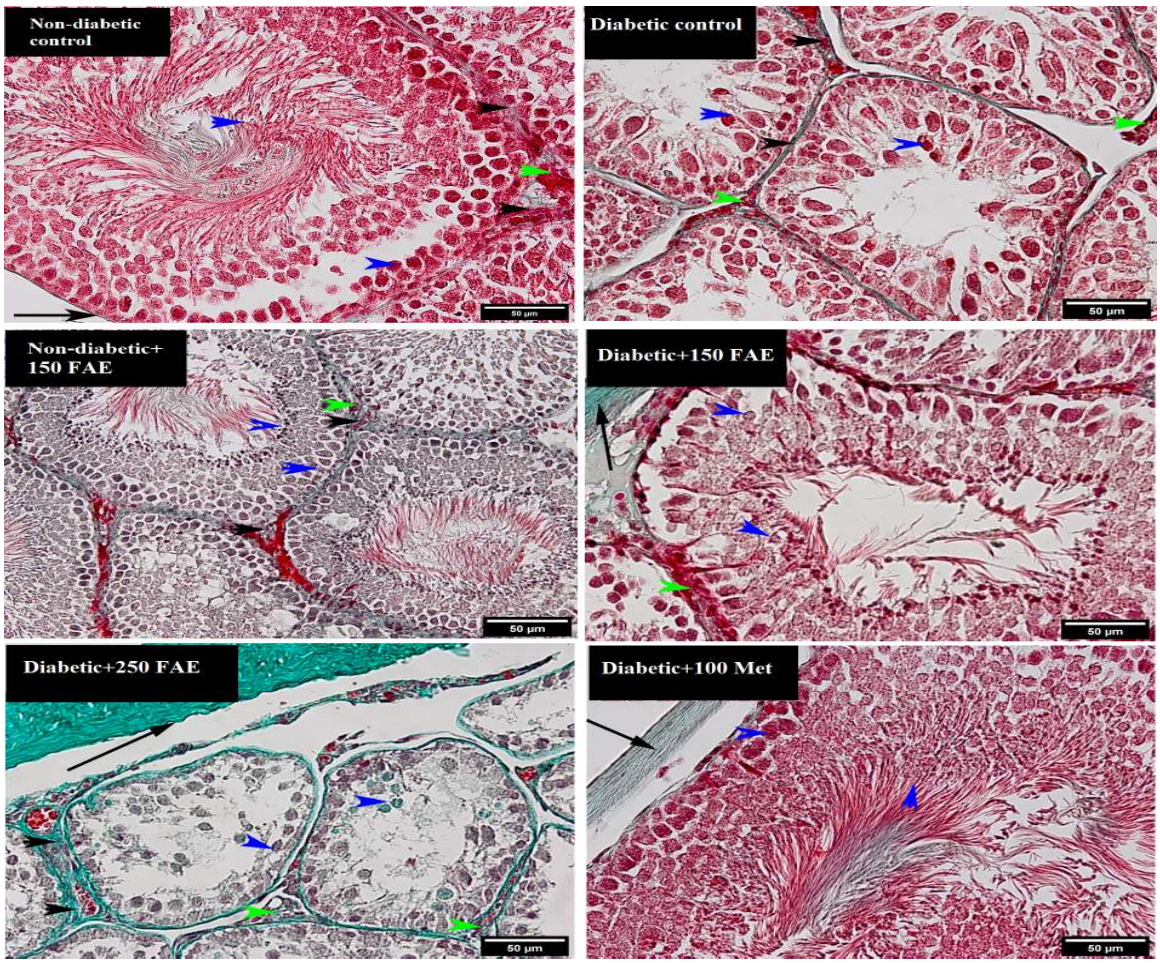

Figure 5.
Cross section of the testis from different groups showing the semineferous tubules with fibrosis. Arrows showing connective tissue and cells in different groups. The green arrow heads showing interstitial tissue (IT); the black single-side arrows show tunica albuginea (TA); the blue arrow head indicate germinal cell; the black arrow heads show connective tissue.

testis. A low dose of FAE improves the disruptive effects of diabetes on the testis, while a high dose does not have such effects. According to the previous and present research, seminiferous tubule diameter and germinal epithelium thickness declined in diabetic subjects [5]. These morphometric alterations are attributable to the apoptotic and oxidative effects of diabetes [5] and more interestingly, FAE improved the morphometric features of the testis tubules in diabetic rats. It seems that these restorative effects of FAE are due to the presence of antioxidant and anti-apoptotic compounds in FAE.

The effect of diabetes on Johnson's score was evaluated and the obtained data were consistent with the results of the previous study [41]. In addition, the positive effect of a low dose of FAE on Johnson's score and the negative effect of a high dose of FAE on Johnson's score in diabetic and non-diabetic animals were observed in this study (Table 1).

The effect of FAE, as a compound containing natural antioxidants, on spermatogenesis in diabetic rats was investigated. The results were consistent with previous studies. For example, one previous study showed that FAE has positive effects on spermatogenesis and by increasing the dose of FAE, spermatogenesis increases, although tissue damage such as vacuolation of Leydig cells were observed . [26]. There are many natural active compounds in FAE, which make it a good candidate for the treatment of diabetes and infertility. Ferulic acid, quercetin, and umbelliferon are the three important compounds found in FAE [23-25]. Ferulic acid and quercetin have antioxidant and anti-apoptotic properties [43].

Park et al. showed that the effects of ferulic acid on increasing testosterone levels result from inhibiting testosterone-reducing enzymes in the liver [44]. Furthermore, quercetin increases the level of testosterone, FSH, and LH, while diabetes negatively affects these parameters. These changes in diabetic conditions are attributed to the production of reactive oxygen species that reduce the secretion of LH and FSH, and these events lead to a decrease in the number of Leydig cells and testosterone levels [45]. In our study, a low dose of FAE, as a rich source of ferulic acid and quercetin, reduced blood glucose levels (Figure 2. b) and increased insulin (Figure 2. c) and testosterone (Figure 2.d) levels. In the current study, the treatment of diabetic and non-diabetic rats with a low dose of FAE corrected the weight loss of the testis (Figure 4.d and 4. e). This weight loss is due to insulin deduction which leads to decreasing structural protein synthesis [46, 47]. Ferulic acid and quercetin reverse these weight changes by hyperglycemia control and insulin levels increase [43]. SOD and glutathione peroxidase are two key enzymes that neutralize free radicals and clean the testis from reactive oxygen species, thus reducing oxidative stress [48]. These enzymes decline in diabetic subjects, as in our study, the amount of SOD decreased (Figure 3). The activity of the SOD enzyme (Figure 3) rose in group 4 compared to group 2. These results emphasize that the presence of antioxidant compounds in FAE prevents the destruction and apoptosis of the testis. Our study showed that the effects of FAE can be attributed to the antioxidant compounds, including ferulic acid and quercetin. Umbelliferon is another antioxidant component found in FAE with many useful properties. Reduction of insulin resistance, hyperglycemia, and hyperlipidemia in diabetic rats are the effects of umbelliferon. Moreover, the increase of FSH, LH, and testosterone, and the upregulation of FSH, LH, and Peroxisome Proliferator-Activated Receptor γ (PPAR-γ) in the testes of rats are other effects of umbelliferon. PPAR-γ increases insulin sensitivity [49]. Umbelliferon reduces oxidant factors, including reactive oxygen species, malondialdehyde, and nitric oxide, and augments antioxidant factors, namely SOD, glutathione, and catalase [50]. As can be seen in our study, in Figure 2, the increase in testosterone in group 4 compared to group 2 shows the beneficial effects of FAE and its natural antioxidant compounds, including umbelliferon. The mammalian testis is very sensitive to lipid peroxidation due to the presence of unsaturated fatty acids. Therefore, treatment with umbelliferon before testicular ischemia prevents the harmful effects of oxidative stress [50, 51].

As shown in previous studies, antioxidants have ameliorating effects on diabetes and reproductive system disorders. Furthermore, our study emphasizes the antioxidant and improving effects of FAE, as a compound rich in antioxidants, in the treatment of

Materials and Methods

Ethical Considerations

All the experimental procedures were performed in compliance with the policies of the Animal Care and Ethics Committee (ACEC) of Ferdowsi University of Mashhad (No. 41,391). According to ACEC recommendations, we tried our best to minimize research animal pain and suffering.

Animals

All the Wistar rats used in this study were wild-type. A total of 42 male Wistar rats at 3 months of age (weighting 270±20 grams) were obtained from the animal house. To adapt animals to the new environment they were kept in the laboratory for two weeks before the experiments. The rats were housed under the standard conditions at 23°C±1°C with a 12:12h light: dark cycle and had access to food and water ad libitum.

Medications and Materials

In this study, we used STZ (Sigma Aldrich, USA) for diabetes induction and Met (Merck, Germany) as a reference for the

treatment of diabetes. A rat insulin enzyme-linked immunosorbent assay kit (Merccodia, Sweden) was used for insulin measurement. Testosterone and glucose were also measured by standard kits (testosterone was measured by rat testosterone ELIZA kit (Cayman Chemical, USA) and glucose were measured by Pars Azmoon glucose kit (Pars Azmoon, Iran)).

Plant Collection, Specimen Voucher, and Ethanolic Extract of Ferula assa-Foetida Preparation

Ferula assa-Foetida L. was collected from Bastak desert in Hormozgan province at latitude 27° 16' 25" N and longitude 54°21' 51"E in the height of 1650 meters. The plant was identified by Ferdowsi University of Mashhad Herbarium with a voucher specimen (accession number: E-1165 FUMH) in 2020. The plant name was checked with http://www.theplantlist.org.

Ferula assa-Foetida L. ethanolic extract was prepared as previously reported [28]. Briefly, the dried oleo gum resin was collected and powdered by a grinder. A total of 100 grams of the powder was dissolved in 1 liter of ethanol 70° and after 48 h at room temperature, the solution was filtered four times using Whatman filter paper (grade 40). The filtered solution was dried using a rotary evaporator and the product was frozen on dry ice before storage at 4°C for further use.

Diabetes Induction and Experimental Groups

Diabetes was induced by injecting a single dose of STZ (55 mg/kg b.w, intraperitoneally) as reported [32]. All non-diabetic groups in this study (including the control) received the same volume of citrate buffer (0.01 M, pH: 4.5) as the vehicle. Fasting blood sugar was monitored after STZ injection for 10 consecutive days, and animals with a constant fasting blood sugar level upper than 250 mg/dl [33] were considered diabetic [34] and were used in our study. For studying the effect of Ferula assa-Foetida L. ethanolic extract on the reproductive organs of male rats, animals were divided into six groups including 1) non-diabetic control group that did not receive any treatment, 2) diabetic control group that was injected with only a single dose of STZ for diabetes induction, 3) non-diabetic treatment group treated with FAE (150 mg/kg b.w, gavage), 4 and 5) diabetic treatment groups treated with FAE (150 and 250 mg/kg b.w, respectively, gavage, and 6) diabetic positive control group that received Met (100 mg/kg b.w, intraperitoneally). Both FAE and Met were dissolved in distilled water and the final volume used for treating the animals via gastric gavage was 1 ml.

Organ Collection, Tissue Processing, and Microscopy

The animals were anesthetized with ether and then euthanized with CO_2 gas for organ collection 42 days after treatment (28). The testes were separated, weighed, and washed in normal saline before fixation in 10% neutral buffered formalin and Bouin-Hollande's. Subsequently, tissue sections of 5 µm thickness were prepared and stained routinely by Hematoxylin & Eosin (H&E) (Merck) and Masson's Trichrome (Merck) [35]. The stained tissue sections were studied by light microscope and the acquired images were used for further quantifications.

Histomorphometric Analysis

To study the histomorphology of animal testes, the obtained images were opened with Image J software (version 1.44 p), and some parameters, such as the diameter of the seminiferous tubules and germinal epithelium thickness, were measured. To measure the diameter of the seminiferous tubule, two opposite points were considered in the circumference of the tubules from the location of the connective tissue in the basement membrane. The basement membrane was defined based on the connective tissue and myoid cells. To measure the germinal epithelium thickness, the distance between the round spermatid and the basement membrane was reported as epithelium thickness (Figure 1) (Table 1). In total, 20 tubules were analyzed in each tissue section and their average was reported as a single data point.

Spermatogenesis Evaluation

Johnson's score is a measure for evaluating spermatogenesis in the seminiferous tubules [42]. For studying the FAE effect on spermatogenesis, Johnson's score values were calculated and analyzed. In this way, Johnson's score in each seminiferous tubule was determined based on a score of 1 to 10. At each tissue section, 50 tubules were studied and their average was considered a data point (Table 1).

Biochemical Evaluation

Insulin, testosterone, and glucose were evaluated by standard kits. SOD activity was determined by the Marklund method [37].

Data Analysis

For each experimental group, 5-7 rats were analyzed. The means of the calculated values for each rat were reported as single data points and were used for making the graphs. Graphs were drawn with GraphPad Prism, Adobe Illustrator, and Microsoft Excel. Data were statistically analyzed by the SPSS software (version 22). One-way analysis of variance and Tukey post hoc test were used and significance levels were considered at $p \leq 0.05$. The error bars on the graph present the mean ± SEM.

Authors' Contributions

Design and conception: ZA, AAM and EL. Methodology validation, Preparation and chemical constituent analysis: EL, AAM and HN. Diabetes induction and daily treatment: EL and AAM. Preparation of samples for biochemical analysis: ZA, EL and AAM. Data analysis and manuscript drafting: ZA, and EL. Data validation and manuscript revision: ZA, EL and HN.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. WHO, Global report on Diabetes—World Health Organization. 2016. https://www.who.int/publications-detail-redirect/9789241565257.

2. Vetere A, Choudhary A, Burns SM, Wagner BK. Targeting the pancreatic β-cell to treat diabetes. Nat Rev Drug Discov. 2014; 13(4): 278–289. Doi:10.1038/nrd4231"10.1038/nrd4231.

3. American diabetes assiciation, Diagnosis and classification of diabetes mellitus, Diabetes Care,2010; 32: S64–S69. Doi:10.2337%2Fdc10-S062"10.2337/dc10-S062.

4. Rosenbloom AL, Joe JR, Young RS, Winter WE. 1999. Emerging epidemic of type 2 diabetes in youth.Diabetes Care.1999;22: 345–354. Doi: 10.2337/diacare.22.2.345 .

5. Kianifard D, Sadrkhanlou RA., Hasanzadeh Sh.The Histological, Histomorphometrical and Histochemical Changes of Testicular Tissue in the Metformin Treated and Untreated StreptozotocinInduced Adult Diabetic Rats. Veterinary Research Forum .2011;2(1):13 – 24.

6. Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, et al. Insulin-dependent diabetes in men is associated with hypothalamo–pituitary derangement and with impairment in semen quality. Hum. Reprod.2002;17: 2673–2677. Doi:10.1093/humrep/17.10.2673"10.1093/humrep/17.10.2673 .

7. Dong Q, Lazarus RM, Wong LS, Vellios M, Handelsman DJ. Pulsatile LH secretion in streptozotocin-induced diabetes in the rat. J Endocrinol.1991;131: 49–55. Doi:10.1677/joe.0.1310049"10.1677/joe.0.1310049 .

8. Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Current Diabetes Reviews.2008; 4(1): 46–54. Doi:10.2174/157339908783502398"10.2174/157339908783502398 .

9. Agbaje IM, Rogers DA, McVicar CM, McClure N, Atkinson AB, Mallidis C, et al. Insulin dependent diabetes mellitus: implications for male reproductive function. Human Reproduction. 2007; 22:1871–1877. Doi:10.1093/humrep/dem077 .

10. Maresch CC, Stute DC, Alves MJ. Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. Human Reproduction Update;2018: 24(1): 86–105.

11. Khamis T, Abdelalim AF, Abdallah SH, Saeed AA, Edress NM, Arisha AH. Early intervention with breast milk mesenchymal stem cells attenuates the development of diabetic-induced testicular dysfunction via hypothalamic Kisspeptin/Kiss1r-GnRH/GnIH system in male rats. 2020. Biochimica et Biophysica Acta - Molecular Basis of Disease,1866. 1, 165577. Doi:10.1016/j.bbadis.2019.165577"10.1016/j.bbadis.2019.165577.

12. Sajadi E, DadrasS, Bayat M, Abdi S, Nazarian H, Ziaeipour S, et al. Impaired spermatogenesis associated with changes in spatial arrangement of Sertoli and spermatogonial cells following induced diabetes. Journal of Cellular Biochemistry.2020; 120(10): 17312– 17325. Doi:10.1002/jcb.28995"10.1002/jcb.28995 .

13. Jiang YP, Ye RJ, Yang JM, Liu N, Zhang W, Ma L, et al. Protective effects of salidroside on spermatogenesis in streptozotocin induced type-1 diabetic male mice by inhibiting oxidative stress mediated blood-testis barrier damage. Chem Biol Interact.2020; 315, 108869. Doi: 10.1016/j.cbi.2019.108869.

14. Kangralkar VA, Patil SD, Bandivadekar RM. Oxidative Stress and Diabetes: A Review. Int J Pharma Appl,2012; 1: 38-45. Doi:10.1002/jbt.10058"10.1002/jbt.10058 .

15. Foretz M, He´ brard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J Clin Invest.2010; 120: 2355–2369. Doi: 10.1172/JCI40671 .

16. Liu Y, Yang Z,Kong D, Zhang Y, Zha W. Metformin Ameliorates Testicular Damage in Male Mice with Streptozotocin-Induced Type 1 Diabetes through the PK2/PKR Pathway. Oxidative Medicine and Cellular Longevity. 2019; Article ID 5681701, 14 pages, Doi:10.1155/2019/5681701"10.1155/2019/5681701 .

17. Belhan SYS, Huyut Z, Özdek U, et al. Effects of curcumin on sperm quality, lipid profile, antioxidant activity and histopathological changes in streptozotocin induced diabetes in rats. Andrologia.2020. 52(6): e13584. https://pubmed.ncbi.nlm.nih.gov/32293755/.

18. Belhan S,Yildirim S, Huyut Z, Özdek U, Oto G, Algul S. Effects of curcumin on sperm quality, lipid profile, antioxidant activity and histopathological changes in streptozotocin induced diabetes in rats. Andrologia.2020. 52(6): e13584. Doi:10.1111/and.13584"10.1111/and.13584 .

19. Akang EN, Oremosu A A, Dosumu OO, et al. The effect of fluted pumpkin (Telferia occidentalis) seed oil (FPSO) on testis and semen parameters. Agriculture and Biology Journal of North America. 2010; 14:697-703.

20. Zahedi A, Fathiazad F, Khaki A, Ahmadnejad B. Protective Effect of Ginger on Gentamicin-Induced Apoptosis in Testis of Rats. Advanced Pharmaceutical Bulletin. 2012;2(2):197-200.Doi:10.5681%2Fapb.2012.030"10.5681/apb.2012.030.

21. Dehpour A A, Ebrahimzadeh M A, Nabavi S F, Nabavi S M. Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition,Grasas Y Aceites 2009:60(4): 405–412. Doi: 10.3989/gya.010109.

22. Karimian V, Sepehry A, Barani H, et al. Productivity, essential oil variability and antioxidant activity of Ferula assa-foetida L. oleogum- resin during the plant exploitation period.J essen oil res.2020;32(6):545-555. Doi: 10.1016/j.foodchem.2012.11.131.

24. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)-A review. J EthnoPharmacol. 2011; 134:1–10. Doi: 10.1016/j.jep.2010.11.067.

25. Hofer O, Widhalm M, Greger H. Circular dichroism of sesquiterpene-umbelliferone ethers and structure elucidation of a new derivative isolated from the gum resin "Asa Foetida. Monatshefte Fa*f*a.rChemie-Chem.Mon.1984;115:1207–1218.

26. Bagheri S M, Yadegari M, Porentezari M, Mirjalili A, Hasanpor A, Dashti M H, et al. Effect of Ferula assa-foetida oleo gum resin on spermatic parameters and testicular histopathology in male wistar rats.J Ayur & Integ Med.2015;6(3): 175-180. Doi: 10.4103/0975-9476.146552..

27. Nejatbakhsh F, Shirbeigi L, Rahimi R, Abolhasani H. Review of local herbal compounds found in the Iranian traditional medicine known to optimise male fertility. Andrologia.2016; 48:8: 850-859. Doi:10.1111/and.12675"10.1111/and.12675.

28. Latifi E, Mohammadpour A A, Fathi H B, Nourani H. Antidiabetic and antihyperlipidemic effects of ethanolic Ferula assa-foetida oleo-gum-resin extract in streptozotocin-induced diabetic wistar rats. Biomed pharmacother. 2019; 110:197-202.Doi:10.1016/j.biopha.2018.10.152"10.1016/j.biopha.2018.10.152.

29. Rafiee S, Shafi MM, Molavi M, Nazemi S. Effect of Ethyl Acetate Extract of Ferula asafoetida Oleo-Gum Resin on the Glucose Level and Lipid Profile in Streptozotocin-Induced Diabetic Rats. Jundishapur J Nat Pharm Prod.2020;15,2. e67042. Doi:10.5812/jjnpp.67042" 10.5812/jjnpp.67042.

30. Azizian H, Rezvani ME, Esmaeilidehaj M, Bagheri S M. Anti-Obesity, Fat Lowering and Liver Steatosis Protective Effects of Ferula asafoetida Gum in Type 2 Diabetic Rats: Possible Involvement of Leptin., Iranian Journal of Diabetes and Obesity. 2012; 4(3): 120-6.

31. Tuomilehto J. The emerging global epidemic of type 1 diabetes, Current Diabetes Reports.2013;(13)6:795–804. Doi:10.1007/s11892-013-0433-5"10.1007/s11892-013-0433-5 .

32. Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy R C. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. J Ethnopharmacol.2004;90:185–189. Doi:10.1016/j.jep.2003.09.024"10.1016/j.jep.2003.09.024 .

33. Kodikonda M, Naik PR. Ameliorative effect of borneol, a natural by cyclicmonoterpene against hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic Wistar rats. Biomed Pharmacother.2017;96:336–347. Doi:10.1016/j.biopha.2017.09.122"10.1016/j.biopha.2017.09.122.

34. Chandran R, George BP, Abrahamse H, Parimelazhagan T. Therapeutic effects of Syzygium mundagam bark methanol extract on type-2 diabetic complications in rats. Biomed Pharmacother.2017;95:167–174.Doi:10.1016/j.biopha.2017.08.061"10.1016/j.biopha.2017.08.061.

35. Luna LG. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3ed. NewYork: McGraw-Hill.1968.

36. Johnsen SG. Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1(1):2-25.Doi:10.1159/000178170"10.1159/000178170.

37. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry.1974; 47(3):469–474. Doi:10.1111/j.1432-1033.1974.tb03714.x.

38. Sexton W J, Jarow J P. Effect of diabetes mellitus upon male reproductive function. Urology. 1997;49: 508–513. Doi:10.1016/s0090-4295(96)00573-0"10.1016/s0090-4295(96)00573-0.

39. Singh A, Kukreti R, Luciano S, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. Molecules.2022;27(3):950. DOI:10.3390/molecules2703095040.

40. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunol Today.1994;15: 7–10. Doi:10.1016/0167-5699(94)90018-3.

41. Kianifard D, Sadrkhanlou RA, Hasanzadeh Sh. The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. Iran J Basic Med Sci.2012;15(1):623-35. PMID: 23493249.

42. Shokoohi M, Gholami Farashah MS, Khaki A, Khaki A A, Ouladsahebmadarek E, Aref Nezhad R. Protective Effect of Fumaria parviflora Extract on Oxidative Stress and Testis Tissue Damage in Diabetic Rats. Crescent Journal of Medical and Biological Sciences. 2019; 6:355-360. https://www.cjmb.org/uploads/pdf/pdf_CJMB_375.pdf.

43. Khaki A, Fathiazad F, Nouri M, Khaki A A, Maleki N A, Jabbari Khamnei H, et al. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytother Res.2010; 24:1285–1291. Doi:10.1002/ptr.3100"10.1002/ptr.3100.

44. Park JS, Han K. The spermatogenic effect of yacon extract and its constituents and their inhibition effect of testosterone metabolism. Biomol Ther (Seoul).2013;21: 153–160. Doi:10.4062%2Fbiomolther.2012.093"10.4062/biomolther.2012.093.

45. Adedara IA, Abolaji A O, Ladipo E O, Fatunmibi OJ, Farombi AO.4-Vinylcyclohexene diepoxide disrupts sperm characteristics, endocrine balance and redox status in testes and epididymis of rats. Redox Report.2016;22(6): 388–398. Doi:10.1080/13510002.2016.1259718"10.1080/13510002.2016.1259718.

46. Rajkumar L, Srinivasan N, Balasubramanian K, Govindarajulu P. Increased degradation of dermal collagen in diabetic rats. Indian J Exp Biol.1991; 29:1081–1083.

47. Proud CG. Regulation of protein synthesis by insulin. Biochemical Society Transactions.2006; 34: 213-216. Doi:10.1042/bst20060213"10.1042/BST20060213.

48. Fuji J, Iuchi Y, Matsuki S, Ishii T. Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. Asian J Androl.2003;5: 231–242.

49. Allam M, Khowailed A A, Elattar S, Mahmoud AM. Umbelliferone ameliorates oxidative stress and testicular injury, improves steroidogenesis and upregulates peroxisome proliferator-activated receptor gamma in type 2 diabetic rat. Journal of Pharmacy and Pharmacology.2022; 74: 573–584. Doi:10.1093/jpp/rgab083"10.1093/jpp/rgab083.

50. Alotaibi M F, Al-Joufi F, Abou Seif H S, Alzoghaibi MA, Djouhri L, Ahmeda AF et al. Umbelliferone Inhibits Spermatogenic Defects and Testicular Injury in Lead-Intoxicated Rats by Suppressing Oxidative Stress and inflammation, and Improving Nrf2/HO-1 Signaling. Drug Des Dev and Ther.2020;14: 4003–4019. Doi: 10.2147%2FDDDT.S416910"10.2147/DDDT.S416910.

51. Mahmoud AM, Hussein O E. Protective Effects of Umbelliferone in Experimental Testicular Ischaemia/Reperfusion Injury in Rats.Anat Physio: cur res.2015; 6:1. Doi:10.4172/2161-0940.1000189"10.4172/2161-0940.1000189.

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Preventive Effects of Silymarin on Diclofenac-induced Toxicity in the Domestic Pigeon (*Columba livia*)

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ABSTRACT

This study aimed to evaluate the effects of silymarin on diclofenac-induced acute liver and kidney poisoning in domestic pigeons (Columba livia). The use of NSAIDs leads to adverse drug effects, such as cardiovascular and gastrointestinal hemorrhage and renal side effects. The vast amount of pharmacological attributes possessed by silymarin describes the remarkable content of research aimed at understanding its effect in the remedy of diverse diseases. Fifteen pigeons were randomly assigned into three groups (1, 2, and 3). Group 1 pigeons served as the negative control group and only were given tap water. Groups 2 and 3 were administered diclofenac (15 mg/kg PO q12h) since the start of the study for 24 h. The third group of pigeons was treated with silymarin (35 mg/kg) plus diclofenac, beginning 12 hours after diclofenac exposure, with the silymarin treatment continuing q12h for 48 h. Blood samples were taken from the birds at times 0, 12 h, 24 h, and 48 h of the experiment for serum biochemistry analysis. The results indicated that the treatment of pigeons with silymarin reduced the serum level of AST, ALT, UA, and urea while increasing ALB and TP. Clinical observations also indicated the presence of toxication symptoms, including loss of appetite, diarrhea, and lethargy. These symptoms improved faster in the silymarin group. It can be concluded that silymarin reduces acute liver and kidney damage caused by diclofenac in pigeons.

Keywords

Diclofenac, Domestic pigeon, Serum biochemistry, Silymarin

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Abbreviations

NSAIDs: Nonsteroidal anti-inflammatory drugs
AST: Aspartate aminotransferase
ALT: Alanine transaminase
TP: Total protein
ALB: Albumin
UA: Uric acid

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Introduction

In animals, the liver is an organ in which poisons are widely accumulated.

Liver tissue is responsible for providing essential structures, such as protein, carbohydrate, fat, bile secretion, glycogen storage, and detoxification of various medications [1, 2].

NSAIDs are a class of medicines that reduce inflammation. play an important role in controlling and reducing fever and pain, and preventing blood clotting [3]. These agents are the most commonly used analgesics worldwide [4]. Adverse drug effects depend on the characteristics of the medication. Digestive system, cardiovascular, and kidney complications are among the common side effects of this pharmaceutical class [5]. Due to the arbitrary use of NSAIDs in human societies, we see drug side effects every year, which are 25% in the United Kingdom and 21% in the United States [4]. COX is an enzyme that converts arachidonic acid to prostaglandin. The main mechanism of action of NSAIDs is inhibiting this enzyme. As a result, a reduction in pain, inflammation, and fever occurs [6].

Diclofenac, sold under different names in the market, is an NSAID that is applied to treat pain and inflammatory diseases, such as gout. Oral, parenteral, or topical routes of application diminish pain within 30 minutes [3]. The most important side effects of this agent are heart disorders, kidney failure, and stomach ulcers. Moreover, its common adverse effects include abdominal pain, vomiting, gastrointestinal bleeding, headache, and dizziness. It should be noted that it is contraindicated in humans during the last three months of pregnancy [7]. As mentioned earlier, prostaglandin production from arachidonic acid through the COX pathway is inhibited by diclofenac [4]. The maximum plasma peak of diclofenac is observed 2 h after oral administration. Diclofenac is excreted through the liver and kidneys. It is metabolized in the liver to hydroxy diclofenac, which turns into sulfate and glucuronic acid, facilitating excretion through the renal system [4]. It is known so far that diclofenac causes toxicity due to the damage to the mitochondrial function and the creation of pro-oxidant radicals, which are metabolized by peroxidases [4].

In the past, several studies have been conducted on animals exposed to poisoning and their treatment with different herbal medicines [8, 9]. Silymarin is one of the plant-derived agents that has been studied many times due to its antioxidant, anti-inflammatory, immune system modulator, anti-fibrotic, antiviral, and liver-protective properties [10, 11]. Silymarin is a polyphenolic flavonoid extracted from the seeds of milk thistle (Silybum marianum), which belongs to the Asteraceae family [12]. Silymarin, as a liver-protective medication with detoxification properties, has shown good performance in studies on exposure to acetaminophen, carbon tetrachloride, arsenic, butyrophenones, and phenothiazines [7, 13]. This plant has been observed to repair the liver damage caused by sodium nitrate in rats [12].

Considering the lack of data in the literature, the present study aimed to investigate the therapeutic effects of silymarin on diclofenac-induced poisoning in pigeons and its protective role on the liver and kidney.

Result

As can be observed in Table 1 and figure 2, there was no significant difference in tested parameters between different treatment groups at the start of the experiment (hour 0). An increase in AST, ALT, and UA was found 12 h after exposure to diclofenac. The increase in AST, ALT, and UA was significantly higher in the positive control group ($p < 0.05$). In terms of these three parameters, no significant difference was observed between the treatment group and the negative control 24 and 48 h after treatment, but both groups had a significant difference with the positive control group ($p < 0.05$). Regarding urea, no significant difference was observed between the treatment group and the positive control in hours 12 and 24, but both groups showed a significant difference with the negative control group ($p < 0.05$), and 48 h after exposure to diclofenac, a significant difference was observed between silymarin and the positive control groups ($p < 0.05$).

The decrease in ALB and TP was found 12 h after exposure to diclofenac. The mean ALB level in hours 24 and 48 was not significantly different between the negative control and treatment groups, but both groups had a higher level of ALB than the positive control group and showed a significant difference ($p < 0.05$). In terms of TP in hours 12 and 48, no significant difference was observed between the treatment group and the negative control, but both groups had a significant difference with the positive control group ($p < 0.05$).

The trend of alteration in the measured parameters over time in each group showed no significant difference in the negative control group in terms of all the investigated parameters between the studied time points. On time 12, there was a rise in UA and urea, which was significant in the treatment and positive control groups between times 0 and 12. Then, the amount of UA and urea in the blood declined after 24 and 48 h in the Silymarin group, but in the positive control group, the concentration of UA was still high.

AST and ALT enzymes significantly increased in

Table 1.
Mean ± SD for kidney indices and liver enzyme concentration at different time points, post-exposure with Silymarin compared to control groups.of S. aureus in Iran.

	Treatment	0	12	24	48
Uric acid (mg/dL)	Negative control	3.93 ± 0.70 a,A	3.94 ± 0.60 a,B	3.86 ± 0.80 a,B	3.90 ± 0.78 a,B
	Positive control	4.63 ± 0.66 b, A	*17.30 ± 4.19 a, A*	*15.13 ± 3.80 a, A*	*12.20 ± 2.00 a, A*
	Silymarin	3.90 ± 0.81 b, A	12.03 ± 1.95 a, A	8.16 ± 1.95 b, B	5.70 ± 0.98 b, B
Urea (mg/dL)	Negative control	2.03 ± 0.73 a,A	2.26 ± 0.80 a,B	2.00 ± 0.81 a,B	2.50 ± 0.70 a,B
	Positive control	2.63 ± 0.66 b, A	9.73 ± 0.76 a, A	12.93 ± 3.10 a, A	10.76 ± 1.88 a, A
	Silymarin	2.83 ± 0.66 b, A	11.28 ± 1.03 a, A	8.16 ± 0.85 b, A	5.62 ± 0.61 b, B
AST (U/L)	Negative control	51.33 ± 3.21 a, A	52.33 ± 1.52 a, B	53.33 ± 5.03 a, B	53.35 ± 3.51 a, B
	Positive control	51.35 ± 1.52 b, A	149.66 ± 10.01 a, A	157.00 ± 17.08 a, A	147.66 ± 10.01 a, A
	Silymarin	51.66 ± 2.51 b, A	116.33 ± 35.92 a, A	75.33 ± 12.85 b, B	67.66 ± 2.51 b, B
ALT (U/L)	Negative control	9.33 ± 1.52 a,A	9.66 ± 2.52 a,B	10.00 ± 2.64 a,B	8.66 ± 1.52 a,B
	Positive control	10.00 ± 0.59 b, A	25.66 ± 9.29 a, A	28.33 ± 14.43 a, A	27.00 ± 14.73 a, A
	Silymarin	15.66 ± 4.04 a, B			11.33 ± 3.21 b, B
ALB (g/dL)	Negative control	1.71 ± 0.10 a, A	1.69 ± 0.10 a, A	1.71 ± 0.09 a, A	1.71 ± 0.09 a, A
	Positive control	1.21 ± 0.21 a, A	0.54 ± 0.23 b, B	0.66 ± 0.12 b, B	0.66 ± 0.12 b, B
	Silymarin	1.31 ± 0.41 a, A	0.69 ± 0.24 b, B	1.04 ± 0.05 a, A	1.22 ± 0.31 a, A
TP (g/dL)	Negative control	4.03 ± 0.45 a, A	3.93 ± 0.45 a, A	4.16 ± 0.50 a, A	3.93 ± 0.47 a, A
	Positive control	3.90 ± 0.45 a, A	2.50 ± 0.60 b, B	2.43 ± 0.49 b, B	2.46 ± 0.56 b, B
	Silymarin	3.93 ± 0.58 a,A	3.93 ± 0.58 a,A	3.00 ± 0.30 b,AB	3.60 ± 0.43 a,A

*Values are mean ± SD of three replicates.
a bThe different superscript letters in the same row indicate significant differences ($p < 0.05$).
A-BThe different superscript letters in the same column in each parameter indicate significant differences ($p < 0.05$).

the treatment group 12 h after exposure to diclofenac. AST enzyme decreased 24 h after treatment with silymarin compared to time 12 ($p < 0.05$), but ALT enzyme declined 48 h post-treatment. ALB showed a reduction after 12 h in the positive control and treatment groups. However, after 24 h, the ALB level rose in the silymarin group. Although TP decreased after 12 and 24 h in the positive control and treatment groups, it increased after 48 h in the silymarin group.

Clinical observations indicated toxication symptoms in the positive control and silymarin groups after 12 h from the onset of the experiment, which included the loss of appetite, diarrhea, and lethargy. These symptoms improved faster in the silymarin group as we did not find these symptoms 48 h after the onset of the experiment. On the other hand, the clinical signs of poisoning were still observed in the positive control group. There was no death in these groups.

Discussion

NSAIDs are utilized to treat different clinical conditions in animals. Although NSAIDs are essential to managing pain and inflammatory conditions in birds, their prescription is limited. One of the main

reasons for this issue is the shortage of research on NSAID usage in birds. NSAIDs, such as diclofenac, are applied as an antipyretic agent and a painkiller. If diclofenac is used once or several times but in a short period and a high dose, it will lead to acute poisoning [4]. In accidents and emergencies where the patient is injured and in pain, the possibility of acute diclofenac poisoning is high, especially if we do not have access to opioids and have to use a high dose of diclofenac to relieve pain [4]. Symptoms of acute poisoning include various side effects and even death within a few days [14].

The clinical signs of diclofenac poisoning include neurological symptoms, such as drowsiness, dizziness, vision problems, hearing problems, gastrointestinal problems (e.g., gastric ulcers and nausea), and renal issues (e.g., impaired urination) [15]. The results of this study showed that administering diclofenac caused some symptoms, including loss of appetite, diarrhea, and lethargy in groups II and III. However, it was observed that the symptoms in the birds in group III improved after the administration of silymarin, while the symptoms were more stable and resolved in a shorter period in comparison to the pigeons in group II. Similar results were reported in a previous study that investigated the protective effects of silymarin on hepatotoxicity and renal toxicity caused by acetaminophen in pigeons [1].

According to previous studies, it can be stated that diclofenac causes serious damage to the liver and kidneys by increasing liver enzymes and decreasing protein production, as well as increasing UA and urea [16, 17]. Diclofenac poisoning in vultures and chickens is characterized by significant increases in plasma UA and subsequent gout [17]. As mentioned in Table 1, hepatocyte injury markers (AST and ALT), were increased in diclofenac-administrated groups (II, III), while we observed a decrease in AST and ALT activities 24 h post-administration in the silymarin group (III). In line with the present study, researchers demonstrated that the administration of silymarin significantly improved the altered serum biochemical parameters [1].

Hepatic degenerative changes by diclofenac cause cellular damage [18]. Diclofenac poisoning results in varying degrees of liver damage from mild to moderate and severe. In cases of mild damage, we observe elevated liver enzymes, while in moderate damage, in addition to the increase of liver enzymes and bilirubin, decreased ALB is found. In cases of severe damage, in addition to the mentioned changes, severe jaundice is also observed [19]. AST is a non-specific liver enzyme that is also produced in other tissues, but its increase can indicate hepatotoxicity. Both AST and ALT are present in the cytosol of hepatocytes. Liver cell damage leads to a rise in the cell membrane permeability, and cytoplasmic enzymes move out of the hepatocytes, causing their increased activity in the serum [20].

Oxidative stress can be mentioned among the tissue-damaging mechanisms that are the basis of diclofenac poisoning [21]. As a result, the active form of diclofenac, which is acyl glucuronide, mediates a large part of the poisoning events caused by this medicine, reminding the role and importance of antioxidants in preventing tissue changes [22]. The current remedy for diclofenac poisoning includes preventing further exposure and administering antioxidants. It was mentioned earlier that silymarin can be considered rich in antioxidative, anti-inflammatory, anti-fibrotic, anti-viral, and protective properties against the liver [10].

The greater the severity of the damage to the liver cells, the more ALB and TP will be inhibited [16]. A decline in the ALB level was found 12 h post-administration in both groups of birds administered diclofenac. ALB is produced mainly in the parenchymal cells of the liver [23]. Therefore, liver damage can change the level of ALB in the blood. Silymarin treatment significantly increased ALB levels, and similar findings were reported by Ihedioha et al. [1]. The reduction in TP is attributed to the initial damage to the endoplasmic reticulum, leading to the loss of cytochrome P-450 enzymes and its functional failure with reduced protein synthesis and accumulation of triglycerides, resulting in fatty liver disease [24]. Treatment of group II pigeons with silymarin could normalize diclofenac-induced reductions in serum TP, indicating hepatoprotective activity.

Several studies have shown that chronic use of diclofenac in high doses can cause pathological changes in the liver and kidney tissue [4]. Diclofenac interferes with renal arterial blood flow with a resultant diminished glomerular blood supply [17]. Birds are uricotelic and 60%–80% of the total nitrogen excreted by birds is in the form of UA. Therefore, it has been proposed to measure plasma UA concentration to assess renal function in birds [25]. An increase in UA and urea was found in the diclofenac-induced groups 12 h post-administration. Loss of appetite and vomiting in birds of these groups led to dehydration and increased levels of UA and urea. As seen in Table 1, UA and urea decreased in the silymarin-treated group 24 h post-administration. Our findings were in line with earlier research, where silymarin was utilized as a nephroprotective agent, and acetaminophen was used to cause kidney injury in pigeons [1].

Based on the results of the present study, silymarin can be useful in correcting abnormal biochemical changes in serum caused by diclofenac poisoning

Figure 1.
Blood sampling from the wing vein

in pigeons and preventing its side effects. Therefore, it can be said that silymarin is a suitable treatment choice for pigeons and perhaps other birds affected by liver and kidney diseases.

Materials and Methods

Ethical approval

The present research was approved by the Ethics and Animal Rights Committee, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies (Ir.ausmt.rec.1402.15).

Animals

The pigeons in the present study were obtained from domestic bird breeding centers in the city of Amol, north of Iran. Fifteen adult pigeons of obscure sex, with an average weight of 310 g, were used for this experiment. Next, all selected pigeons were physically examined, and the birds were approved to be healthy. The birds were housed in steel birdhouses (100 cm × 60 cm × 60 cm dimensions) and were provided a standard pelletized bird diet and water. The birds were allowed to get accustomed to this condition for 14 days.

Medicine

Diclofenac sodium tablets were supplied from Tehran-darou Pharmaceutical Company, Tehran, Iran (DICOTARD®, 100 mg/kg). Silymarin tablets were supplied from Gol-darou, Esfahan, Iran, (LIVERGOL®, 70 mg/kg).

Study setting and design

Fifteen pigeons were accidentally divided into three groups (1, 2, and 3). Group 1 served as the negative control group and was given just tap water. Pigeons in groups 2 and 3 were administered diclofenac 15 mg/kg PO q12h at the onset of the experiment (hour 0) for 24 h. The third group of pigeons was further treated with silymarin 35 mg/kg, beginning 12 h after diclofenac consumption, with the silymarin treatment continuing every 12 h for 48 h. Blood samples were taken from birds at 0, 12, 24, and 48 h for serum biochemical analysis.

Sample collection

At first, 2 ml of blood from each bird in all groups was collected from the right-wing vein with a 2.5-ml syringe using a 23-gauge needle (Figure 1). Next, the collected blood was poured into test tubes to clot. To separate the clot from the serum, the samples were centrifuged for 10 min at 3000 rpm. After centrifugation, the serum supernatant was carefully separated from the clot and placed in clear and clean tubes until the analysis of biochemical factors. This operation was repeated 12, 24, and 48 h after diclofenac or water administration.

Biochemical analysis

The factors evaluated in blood serum for this study were AST, ALT, TP, ALB, UA, and urea. The preserved sera were utilized for spectrophotometric estimation (Cobas Mira Plus automatic analyzer, Roche, Switzerland) of the mentioned factors using commercial assay kits (AriaAzma, Babol, Iran).

Clinical assessment

Birds were closely monitored every 6 h for the clinical signs of acute drug poisoning (e.g., anorexia, vomiting, diarrhea, ruffled feathers, lethargy, sleeping, and death). If the mentioned clinical signs were observed in a pigeon, the cases were accurately recorded in its file.

Statistical analysis

Analysis of variance (ANOVA) with the Tukey-HSD test was used to assess significant differences in UA, urea, AST, ALT, ALB, and TP between different groups at each time point. Moreover, differences between the means of these parameters in distinct groups along exposure times were analyzed by repeated measures ANOVA and Tukey-HSD test. All results were expressed as mean ± SD. Statistical analyses were performed using SPSS Version 26 software (SPSS Inc., Chicago, IL, USA). For all analyses, $p < 0.05$ was considered statistically significant.

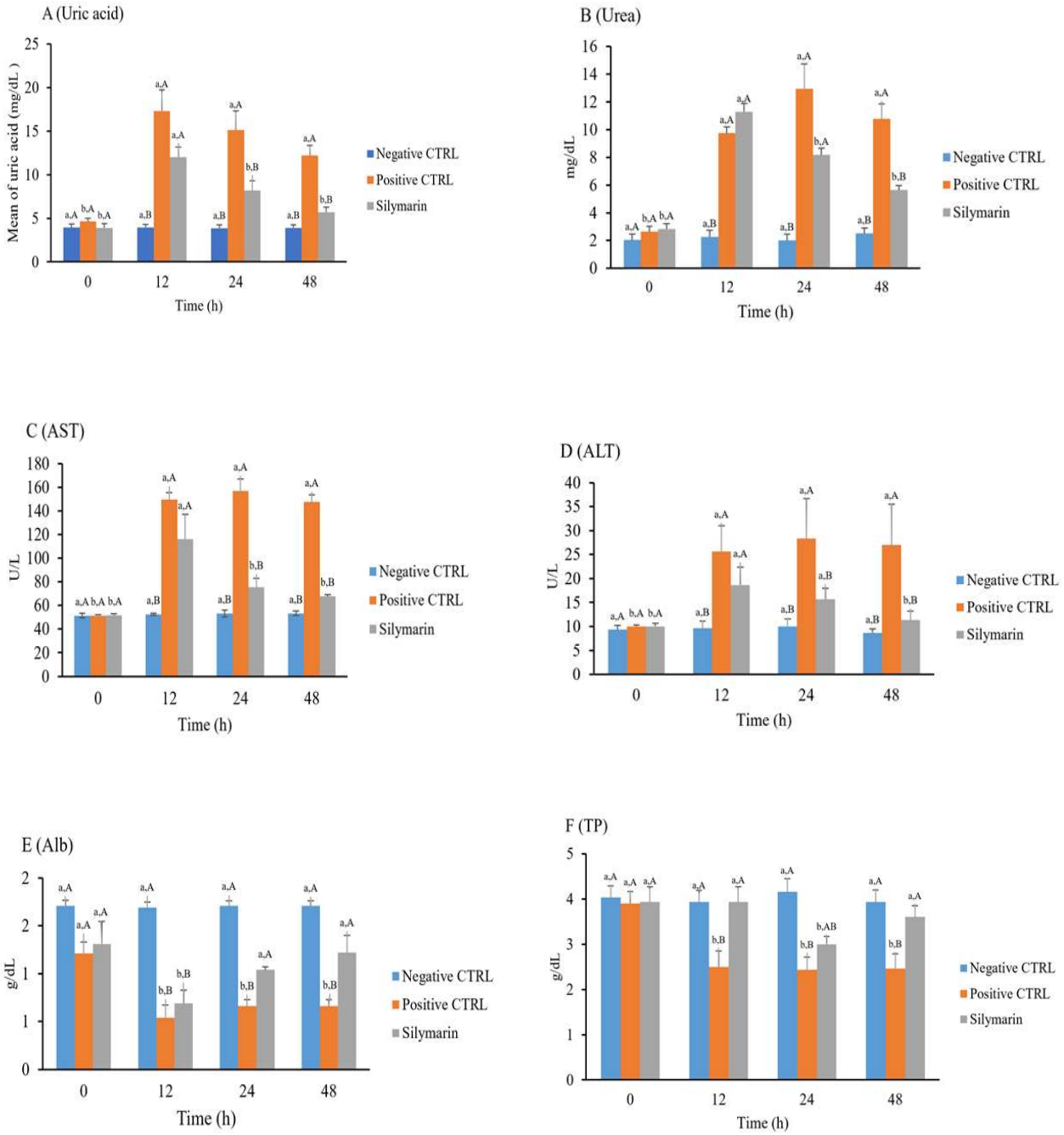

Figure 2.
Diagrams A to F indicate kidney indices and liver enzyme concentration at different time points, pre-and post-treatment with Silymarin in pigeons poisoned with diclofenac drug compared to a control group.
*Values are mean ± SE of three replicates.
a bThe different lowercase letters indicate significant differences in each group between tested times ($p < 0.05$).
A-BThe different capital letters indicate significant differences at each time point between the tested groups ($p < 0.05$).

Authors' Contributions

Nasser Vajdi, Saeed Seifi, and Shohreh Alian Samakkhah conceived and planned the experiments. Nasser Vajdi and Saeed Seifi carried out the experiments. Nasser Vajdi and Saeed Seifi contributed to sample preparation. Shohreh Alian Samakkhah contributed to the interpretation of the results. Nasser Vajdi took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Competing Interests

The authors declare that they have no conflict of interest.

Reference

1. Ihedioha JI, Anyogu DC, Ogbonna ME. The effects of silymarin on acetaminophen-induced acute hepatic and renal toxicities in domestic pigeons (Columba livia). Journal of Avian Medicine and Surgery. 2020 Dec;34(4):348-57. Doi:10.1647/1082-6742-34.4.348

2. Ahsan R, Islam KM, Mussadik A, Haque E. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in albino rats. Glob J Pharm. 2009;3(3):116–22.

3. Mallinson TE. A review of ketorolac as a prehospital analgesic. Journal of Paramedic Practice. 2017 Dec 2;9(12):522-6. Doi:10.12968/jpar.2017.9.12.522

4. Aljuhani N, Elkablawy MA, Elbadawy HM, Alahmadi AM, Aloufi AM, Farsi SH, Alhubayshi BS, Alhejaili SS, Alhejaili JM, Abdel-Halim OB. Protective effects of Ajwa date extract against tissue damage induced by acute diclofenac toxicity. Journal of Taibah University Medical Sciences. 2019 Dec 1;14(6):553-9. Doi:10.1016/j.jtumed.2019.10.002

5. Lanas A, Chan FK. Peptic ulcer disease. The Lancet. 2017 Aug 5;390(10094):613-24. Doi:10.1016/S0140-6736(16)32404-7.

6. Moore N, Pollack C, Butkerait P. Adverse drug reactions and drug–drug interactions with over-the-counter NSAIDs. Therapeutics and clinical risk management. 2015 Jul 15:1061-75. Doi:10.2147/TCRM.S79135

7. Jain A, Yadav A, Bozhkov AI, et al. Therapeutic efficacy of silymarin and naringenin in reducing arsenic-induced hepatic damage in young rats. Ecotoxicol Environ Saf. 2011;74(4):607–14. Doi:10.1016/j.ecoenv.2010.08.002

8. Razavi BM, Hosseinzadeh H. Saffron as an antidote or a protective agent against natural or chemical toxicities. Daru. 2015;23(1):31. Doi:10.1186/s40199-015-0112-y.

9. Dorri M, Hashemitabar S, Hosseinzadeh H. Cinnamon (Cinnamomum zeylanicum) as an antidote or a protective agent against natural or chemical toxicities: a review. Drug Chem Toxicol. 2018; 41(3):338–51. Doi:10.1080/01480545.2017.1417995

10. Federico A, Dallio M, Loguercio C. Silymarin/silybin and chronic liver disease: a marriage of many years. Molecules. 2017;24(2):191. Doi:10.3390/molecules22020191

11. Surai PF. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives.Antioxidants (Basel). 2015;20(4):204–47. Doi:10.3390/antiox4010204

12. Sherif IO, Al-Gayyar MM. Antioxidant, anti-inflammatory, and hepatoprotective effects of silymarin on hepatic dysfunction induced by sodium nitrite. Eur Cytokine Netw. 2013;24(3):114–21.

13. Tyagi A, Agarwal C, Harrison G, et al. Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI-CDK-cyclin cascade, and caspase 3 and PARP cleavages. Carcinogenesis. 2004;25(9):1711– 20. Doi:10.1093/carcin/bgh180.

14. Chung EY, Tat ST. Nonsteroidal anti-inflammatory drug toxicity in children: a clinical review. Pediatric emergency care. 2016 Apr 1;32(4):250-53. Doi: 10.1097/PEC.0000000000000768.

15. Aronson JK. Non-steroidal anti-inflammatory drugs (NSAIDs). Meyler's Side Effects of Drugs. 16th ed. Waltham, MA: Elsevier. 2016:236-72.

16. Rana Abdulla salih. Clinical and Histopathological Study of Diclofenac Sodium Acetylsalicylic Acid Toxic Effect on Liver of Mice. Indian Journal of Forensic Medicine & Toxicology. 2020 Dec. 31 2023 Dec. 23;15(1):2314-21.

17. Nethathe B, Chipangura J, Hassan IZ, Duncan N, Adawaren EO, Havenga L, Naidoo V. Diclofenac toxicity in susceptible bird species results from a combination of reduced glomerular filtration and plasma flow with subsequent renal tubular necrosis. PeerJ. 2021 Aug 23;9: e12002. Doi:10.7717/peerj.12002

18. Ong CK, Lirk P, Tan CH, Seymour RA. An evidence-based update on nonsteroidal anti-inflammatory drugs. Clinical medicine & research. 2007 Mar 1;5(1):19-34. Doi: 10.3121/cmr.2007.698.

19. Nouri A, Heidarian E, Nikoukar M. Effects of N-acetyl cysteine on oxidative stress and TNF-α gene expression in diclofenac-induced hepatotoxicity in rats. Toxicology mechanisms and methods. 2017 Oct 13;27(8):561-67. Doi:10.1080/15376516.2017.1334732

20. Eassawy MM, Salem AA, Ismail AF. Biochemical study on the protective effect of curcumin on acetaminophen and gamma-irradiation-induced hepatic toxicity in rats. Environmental Toxicology. 2021 May;36(5):748-63. Doi.org/10.1002/tox.23077

21. Salimi A, Neshat MR, Naserzadeh P, Pourahmad J. Mitochondrial permeability transition pore sealing agents and antioxidants protect oxidative stress and mitochondrial dysfunction induced by naproxen, diclofenac and celecoxib. Drug research. 2019 Oct;69(11):598-605. Doi: 10.1055/a-0866-9356.

22. Giridharan R, Lavinya U, Sabina EP. Suppressive effect of Spirulina fusiformis on diclofenac-induced hepato-renal injury and gastrointestinal ulcer in Wistar albino rats: a biochemical and histological approach. Biomedicine & Pharmacotherapy. 2017 Apr 1; 88:8-11. Doi:10.1016/j.biopha.2017.01.032

23. Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th ed. New York, USA: WH Freeman 2002; 231-273.

24. Topal I, Özdamar MY, Catakli T, et al. Renoprotective Effect of Taxifolin in Paracetamol-Induced Nephrotoxicity: Emerging Evidence from an Animal Model. J Clin Med 2023; 12(3): 876. Doi:10.3390/jcm12030876

25. Ritchie BW, Harrison GJ, Harrison LR. Avian medicine: principles and application. 1998 May 29.

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The Critical Control Point of *Aspergillus spp*. Aflatoxin Contamination in Smallholder Dairy Farms

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ABSTRACT

Feed and food contamination by toxigenic fungi and their aflatoxins is one of the main threats to animal and human health worldwide and in the agricultural and industrial sectors. This study evaluated the contamination magnitude by *Aspergillus* species in dairy farms, aflatoxin AFB1 in cow feeds, and aflatoxin AFM1 in milk and local cheese (Dangke). One hundred twenty-two swabs from farms, 12 roughage feeds, 16 concentrated feeds, 39 fresh cow milk, and six cheese samples were analyzed for *Aspergillus* spp., AFB1, and AFM1 contamination. *Aspergillus flavus* and *Aspergillus niger* were detected in 13.93% and 7.38% of the swab samples, respectively. The roughage feeds showed low levels of AFB1, detected in 8.33% with contamination of 7.32 µ/kg, while concentrated feed was detected in 37.5% of specimens, with contamination levels of 27.8 µg/kg. Aflatoxin AFM1 was detected in raw milk samples and represented approximately 69.2% of samples, with a mean of 7.31 µg/kg. All local cheese samples were free of AFM1. There were critical points regarding HACCP inside the farms, which play significant roles in contamination by fungi and aflatoxins. Fungal contamination and aflatoxins pose dangerous public health problems to humans, especially infants and older people. Therefore, monitoring programs for mycotoxins are critical in reducing contamination.

Keywords

AFB1, AFM1, Aspergillus spp, Food contamination, HACCP

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Abbreviations

AFB1: Aflatoxin B1
AFM1: Aflatoxin M1
HACCP: Hazard Analysis Critical Control Points
AFG: Aflatoxin Green
PDA: Potato Dextrose Agar
ELISA: Enzyme-Linked Immunosorbent Assay

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Introduction

Mycotoxins are poisonous materials formed by toxigenic fungi that attack agricultural products in the field or a storehouse in natural conditions, including bad storehouses, high moisture, high temperature, and insect infestation [1]. When set up in animal ration and feed ingredients, these contaminants might pose big trouble and risk to lactating cows when they exceed normal levels. Initially, they had a mischievous effect on animal health, such as decreased feeding efficiency, milk productivity, immunodeficiency, emaciation, laminitis, infertility, and abortion [2, 3]. Furthermore, they may affect the food supply chain when they transfer from animal feed to milk and milk products [4, 5, 6]. Fungal fossils are very resistant to high temperatures and humidity, which can disrupt milk production during processing[7]. Subsequently, people are exposed to these poisons using contaminated animal products, such as meat, milk, and dairy products. People's ex-posure to mycotoxins can have several adverse health effects, including chronic and acute diseases, as well as teratogenic, carcinogenic, and immunosuppressive effects. It might bring about death in critical cases, such as delayed chronic toxicity or high acute intoxication [8].

Considering adverse consequences on all creatures and humans, mycotoxins cause huge monetary misfortunes for some countries, especially non-industrial nations, due to the expenses posed on food safety [9]. The most common toxigenic fungi in agricultural products are species belonging to the *Aspergillus*, *Alternaria, Fusarium*, and *Penicillium genera*. *Fusarium, Aspergillus,* and *Penicillium* are considered considerable mycotoxin-producers in animal feed worldwide [10].

In addition, fungal poisons, namely aflatoxins (AFB1, AFB2, AFG1, and AFG2), shaped by fungal species in the genera Aspergillus are critical mycotoxins found routinely in all dairy rations worldwide [11]. In tropical and subtropical areas worldwide, the issue is more articulated as it relates to humidity and high climate temperatures that lean toward the growth and multiplication of fungi. Grains and plant protein sources utilized in animal diets are the principal sources of fungal contamination and aflatoxins. Aspergillus multiplicates, preferably, on commodities at 15% or higher moisture levels at 25°C-35°C. Moisture levels of more than 17.5% and temperature of 27°C-30°C are required for the highest aflatoxins production. Aflatoxins decrease the quality of ingredients by using the nutrients in the ingredients for digestion and spread [12].

There is developing proof to propose that seasonal and geological contrasts impact mycotoxins and aflatoxins formation in both food and feed [13]. Tragically, there is a need for more research in Indonesia in this regard, especially in dairy farms and animal feed parts, considering the hot and wet conditions that characterize this country as tropical. Previous studies in this region elucidated the occurrence of aflatoxins in dairy feeds and milk.

The HACCP system has long been presented worldwide to identify, assess, and control hazardous food safety factors. It is a coherent, fundamental, efficient food safety control system with a complicated structure intended to identify and control risks and critical circumstances. From one perspective, this system ensures the safety of products on the way of the pecking order from maker to the shopper, empowers recognizing all the critical points that can influence the security and safety of the final product, takes out unsafe factors, and controls the total production process [14]. The current research aims to isolate and identify the extent of *Aspergillus spp*. contamination and its toxin production in smallholder dairy farms and to determine critical control points of contamination in the environment, raw milk, and cheese processing units in Enrekang province, South Sulawesi, Indonesia.

Result

Socio-demographic of smallholder dairy farms in Enrekang Regency, Indonesia

The socio-demographic and household characteristics showed that the milking cows ranged from 1 to 14 heads, with a mean (SD) of 5 (0.78) per farm. Approximately 69% of the owners used elephant grass as roughage feed, 30.8% used rice bran as concentrated feed, and most had feed storage facilities. The owners had a chance to be educated, and most of them attended secondary school (about 84.61 %), of which a high percentage was male as described below (Table 1). Our results were similar to those in the Ethiopia [16].

Among all collected samples, about 39 (31.97%) of fungi were isolated and identified macroscopically as *Aspergillus* and segregated by colony color into subgenera *Flavi* (green colonies), *Nigri* (black colonies), *Fumigate* (blue colonies), and *Terrei* (brown colonies). Therefore, macroscopic characters alone are insufficient and inaccurate for identification. Colony color has been examined microscopically to identify some micro features of isolated samples, such as conidiophore, vesicle, and conidia. Molecular analysis is sometimes conducted to confirm the isolates and resolve the cultural limitations, but it was not carried out in the current study (Figure 2). Mycological

Table 1.
Socio-demographic features of smallholder dairy farms in Enrekang regency, Indonesia (N = 13)

Variables	Characteristics	Value
Gender	Men	11
	Women	*2*
Education	University	1
	Secondary	11
	Primary	1
Age	Mean (SD)	36 (0.64)
	30-40	3
	50-60	8
	60-70	2
Number of milking cows	Mean (SD)	5 (0.78)
Milk production/ litter/day	Mean (SD)	5 (0.86)
Type of roughage feeds	Elephant grass	9
	Elephant grass plus green corn	4
	Rice bran	4
	Soya by-products	3
Type of concentrated feed	Rice bran plus commercial concentrate	2
	Rice bran plus soya by-product	3
	Soya plus palm oil cake	1
Feed storage facilities	Yes	10
	No	3

SD: standard deviation

analysis in this study revealed that most of the samples were contaminated by diverse fungi, all of which were identified as mycotoxigenic fungi. Yeasts were also present in the samples.

A. flavus was the most frequently isolated among samples (N = 17, 13.93%), followed by A. *niger* (N = 9, 7.38%), A. *terreus* (N = 6, 4.91%), and A. *fumigatus* (N = 7, 5.74%) (Table 2). These results are similar to the report on the domination of *A. flavus* and *A. niger* in dairy animals and poultry feeds. The current study showed the contamination of 31.97% of samples by *Aspergillus* (Table 2) A high percentage was found in cows' udders (N = 12, 31.58%), followed by cage floor (N = 8, 66.67%), and milker hands (N = 5, 41.67). A low percentage was recorded in coconut mold (N=1, 8.33) and banana leaf (N = 1, 8.33%) as natural packaging.

Discussion

Socio-demographic of smallholder dairy farms in Enrekang Regency, Indonesia

The percentage of women in agricultural activity was less than men, sometimes working beside their husbands or just working was widowed or divorced. The farmers needed more information about fungi and aflatoxins, and their knowledge about contamination was fragile. All of them agreed on the storage process and stated that they stored their animal feeds in a specific place to avoid humidity and rain for less than one month. Therefore, the growth of *Aspergillus* species will be less than expected when we compare their knowledge and feed practices inside the farms.

Isolation of Aspergillus spp.

The main reason for the prevalence of these fungi is their ability to tolerate and live in a wide zone of temperatures. Much research has investigated contamination by *Aspergillus* in animal feed. Most findings concluded that *A. flavus* was the most frequent fungi, followed by other species of *Aspergillus*. No research has been published about the prevalence of *Aspergillus spp.* in cage floors, cow udder, milker hands, milk cans, hands of cheese workers, and cheese mold.

Among all isolated fungi, A. *flavus* was the most dominant, followed by A. *niger,* A. *terreus,* and A. *fumigatus*, all of which are known as mycotoxigenic fungi. Udder of cows and cage floors were highly contaminated, explaining that the floor is not properly cleaned, and when the cows slumber on the floor, the fungi transmit from one cow to another. On the other hand, the owners neglected the importance of hygiene and cleaning practices. Therefore, it is essential to practice safety systems to minimize contamination. We took samples from the milkers' hands immediately after milking, which could suggest that the fungi spread from the cow's udder to the hands or vice versa. The problem is

Isolation of Aspergillus spp.

Table 2.
Isolation and distribution of *Aspergillus* species in different swab *samplesregency*, Indonesia (N = 13)

Source of samples	No. of samples	*Aspergillus spp.*				Total
		A. flavus	*A. fumigatus*	*A. niger*	*A. terreus*	
Water	12	3	0	1	1	5
Cage floor	12	*4*	*0*	*2*	*2*	*8*
Cow udder	38	5	3	3	1	12
Milker hand	12	2	1	1	1	5
Milk cans	12	2	1	0	1	4
Cheese worker hand	12	1	1	1	0	3
Coconut mold	12	0	0	0	1	1
Banana leaf	12	0	0	1	0	1
Total	**122**	**17**	**6 (4.91%)**	**9**	**7 (5.74%)**	**39 (31.97 %)**

Figure 1.
Collection of samples in farm sites

that these fungi transmit contamination to milk and milk products directly or indirectly, and cause adverse health impacts on workers' and animals' health regularly inside farms [17]. In a study, these findings disagreed with the present results. The results of another study on the prevalence of *Aspergillus* in well water in dairy farms revealed that water was contaminated by A. *flavus*, A. *niger*, and A. *terreus*. These results were similar to our findings, and this similarity may be due to environmental conditions in the two countries.

Aspergillus was isolated from animal feeds in Indonesia in another place in Bogor, West Java. The results demonstrated the presence of A. *flavus* in feed samples [18]. Another study was conducted on *Aspergillus* in agricultural commodities in Indonesia, and their results revealed toxigenic *Aspergillus* [19]. Natural weather conditions, including high humidity, rainfall, and moderate temperature in Indonesia, support the growth of fungi, especially *Aspergillus* spp. In South Sulawesi, there was no previous study on the isolation of fungi from the feed of dairy farms or farm environment, making this study the first research in this area, which has considerable dairy farms. Investigations showed no contamination in the majority of roughage feed samples, with only one sample (8.33%) being positive for AFB1 and the mean of contamination being 7.32 µg/kg (Table 3), which is above legal limits set by the European Union (5 µg/kg). However, it is lower than the National Indonesian Standard (50 µg/kg). The present results supported those obtained in

Table 3.
Levels of AFB1 contamination in different animal feed types

	Animal feed type				
	Elephant grass	Rice bran	Soya by-products	Commercial concentrate	Palm oil cake
AFs (µg/kg)	7.32	32.81	-	44.08	6.3
EU limits 20 (µg/kg)	LOD	EOD	LOD	EOD	*LOD*
SNI 200 (µg/kg)	LOD	LOD	LOD	LOD	LOD

LOD: Low Limits of Detection, LOD is low in terms of the detection limits. EOD exceeds the limits of detection

Ethiopia, which reported that about 52% of feed samples were above EU limits. On the other hand, the findings here partially agreed with those obtained in Italy, showing a lower degree of AFB1 contamination [20].

However, the owners did not store roughage feed. They introduced it directly from the field to the animals, which explains that the fungi grow on feed in the field, not during storage. Mold growth needs suitable humidity and temperature. To prevent mold growth and contamination, we should focus on the natural and chemical methods that reduce the multiplication of fungi in the field during harvest, transportation, and storage.

Concentrated feeds in this study revealed low and high AFB1 contamination levels of 6.3 and 44.08 µg/kg, respectively. These incidents are different based on concentrated feed type. Palm oil cake showed low incidents, while rice bran and commercial concentrated feed showed high levels. High AFB1 in concentrate can be attributed to the proven and scientific facts that concentrated feed has high levels of fat, carbohydrate, and protein favorable for Aspergillus species multiplication and aflatoxin production [12]. These findings align with another study (Omeiza et al. 2018) in which the authors detected AFB1 in animal feeds with a concertation range of 10-20 µg/kg. Many factors can play a role in the contamination of dairy cattle feed, such as the type of feed, feed processing, storage and handling, geographic conditions, and owners' awareness of the risk of aflatoxin. The owners in the present study area had no idea about aflatoxins. Therefore, the lack of sufficient knowledge of aflatoxins might lead to their high occurrence in the feed of animals besides milk and milk products [16].

Regarding the analysis of the factors associated with aflatoxins, many factors showed a

Table 4.
Logistic regression analysis of factors associated with aflatoxins

Factors	AFs			
	≤ 5 µg/kg	≥ 5 µg/kg	OR	P value
Level of education				
Primary	1	0		
Secondary	0	5	0.0	0.99
University	0	1		
Gender				
Female	0	2		
Male	0	0	0.0	0.99
Type of roughage feed				
Elephant grass	0	0	0.0	0.99
Type of concentrated feed				
Rice bran	7	3		
Soya by-products	1	0		
Commercial concentrate	0	2	69.95	0.99
Palm oil cake	0	1		
Feed Storage				
On floor	1	2	0.0	1.00
On Special place	3	7		

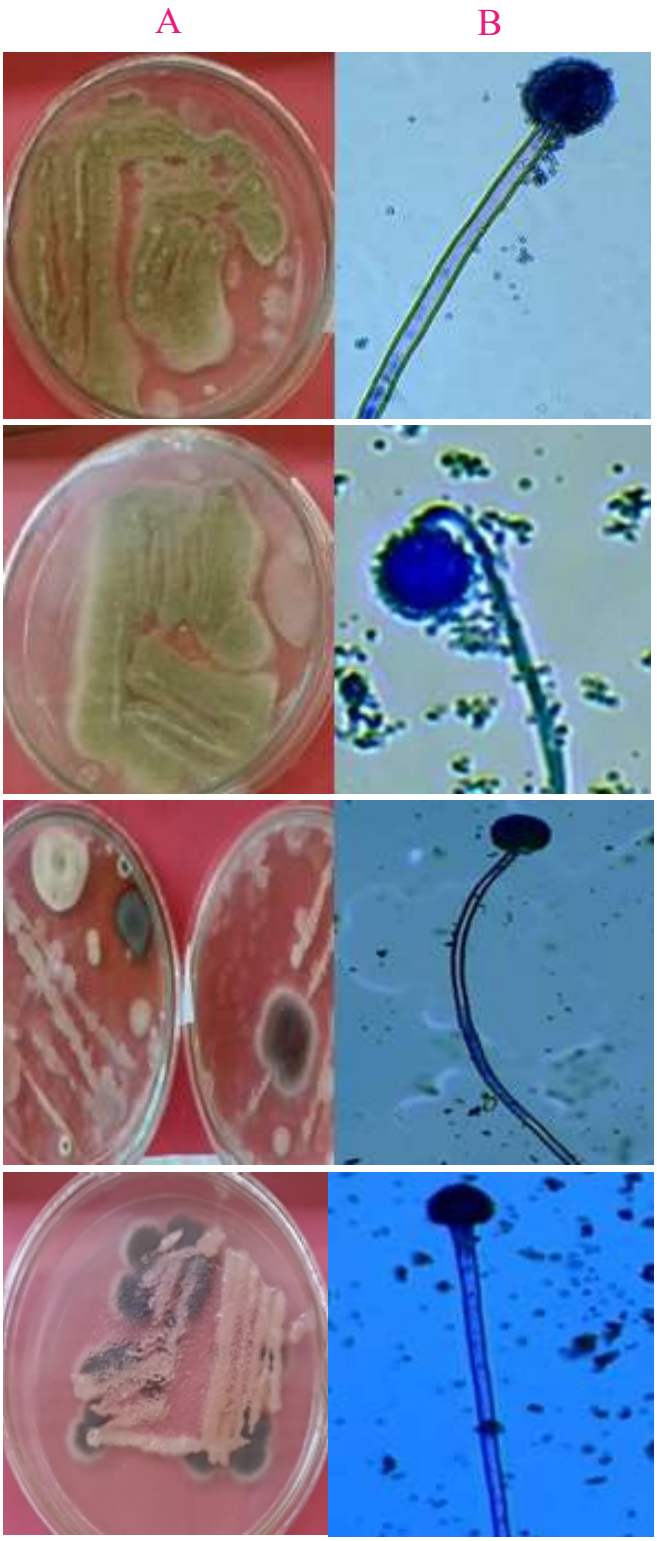

Figure 2.
(A) macroscopic characters and (B) microscopic characters of *Aspergillus spp.*

strong relationship with aflatoxin. This study showed that only concentrated feed positively impacts aflatoxin, and its aflatoxin content is about six times more than roughage (Table 4). These results partially support those who reported that concentrated feed has increased aflatoxin seven times more and disagree with those regarding education level, gender, feed storage, and type of roughages. None of these factors had a positive effect on aflatoxin content.

Aflatoxin AFM1 was found in 69.2% of raw milk samples with contamination levels from 6.14 to 10.02 µg/kg with a mean of 7.31 µg/kg. These results disagree with those recorded in Albania, which showed about 0.022 to 1 µg/kg of AFM1 in milk [21]. A study in the Amazon region found AFM1 of about 0.06 µg/kg, which is less than our results [22]. These differences are related to feed, weather, and animal physiological status as the concentration of AFM1 in milk was higher during the early lactation period and decreased during the late lactation stage in dairy cows. All of the tested local cheese Dangke samples were free from aflatoxin AFM1, which might be due to the method of cheese processing that uses the natural plant enzyme papain extracted from the papaya tree as a coagulant. This finding disagrees with many researchers reporting the presence of AFM1 in different kinds of cheese fields [23, 24].

Regarding HACCP, these results identified many critical points and risks inside the farm that affect the quality of milk and, subsequently, the health of both animals and humans. Cows' udder contained high *Aspergillus spp*. followed by cage floor, milker hands, milk cans, and cheese worker hands. All of these are critical control points, and it is possible to control them to minimize the magnitude of biological and chemical hazard risks in the food supply chain and ensure food safety by implementing good management practices in dairy farms. The point is that milk quality starts from milking until the final product. Many studies have been conducted on implementing the HACCP program in dairy companies and dairy products [25].

In conclusion, the results of the present study indicated the high contamination of the farm environment by mycotoxigenic fungi, especially in cow's udder, cage floor, water, milker hands, milk cans, and cheese worker hands, especially *Aspergillus spp.* Considerable levels of contamination by aflatoxins AFB1 and AFM1 in animal feeds and milk were recorded. We found that concentrated feed type was significantly associated with high aflatoxin contamination levels. All positive feed and milk samples are subject to Indonesian legislation. It is essential to focus on implementing good practices for feed production from the field during cultivation, harvest, transport, processing, storage, and feeding procedures.

The farmers should be trained and educated about the health risks of aflatoxin for their animals and humans, and how to control and manage by implanting the HACCP program, in addition

to adopting hygiene practices and cleaning milker hands, farm floor, and cow udder and sanitation before and after milking to reduce contamination levels and produce clean milk. Therefore, further research and investigations are needed on aflatoxigenic fungi in dairy farms and their feed and produced milk to provide a more comprehensive approach to one health program strategy.

Materials and Methods

Study area

The study areas were intentionally chosen to serve the research aims. The site was located about 1300 m above sea level, with a day-to-day typical temperature of approximately 27°C-34°C. The climate of this area is a tropical rainstorm described by the rainy season from November to June and the dry season from July to November. Dairy cows are mostly kept in a zero-grazing system called 'stall feeding,' and are supplemented with concentrated feeds. The expected milk production in this area was estimated to be around 10-15 liter/head/day.

Sampling

Thirty-nine raw milk samples and six local cheese samples were collected from smallholder farms from August to September 2023. Simultaneously, different types of concentrated and roughage feeds were collected, from which the roughage (N = 12), commercial concentrate (N = 2), soya bean (N = 4), palm oil meal (N = 1), and rice bran (N = 9).

At the same time, a total of 122 swabs from water, cage floor, milker hands, cow udders, milk cans, cheese maker hands, coconut mold, and banana leaf samples were collected (Figure 1). The samples were kept in a cool ice box at 4°C, transported to the laboratory, and stored until analysis. A structured questionnaire was used to assess farmers' knowledge, their practices of animal feeds, and farmers' experience with aflatoxin and fungi in feed, in addition to animal feed handling and storage. The samples were taken from all farms around the research area, and the results were represented and generalized to all communities there.

Fungal analysis

Equipment and selected media were correctly autoclaved before use. Culturing and isolation of the swabs were completed in sanitized conditions, and laboratory windows and doors were kept shut. Two plates of PDA were utilized for each swab sample; subsequently, the media was placed in 9 cm plates and left to solidify at room temperature. Each swab sample was spread on the surface of the plates in duplicate, and each plate was continuously labeled with the code name of the farm from which the swab was taken and the swab name. After the culture process, the dishes were incubated at 30^{0}C for 1-4 days, and until the third day, changes were noted and recorded each day.

Controls were prepared using two sterile PDA dishes, which were used to test the general conditions and environment of the laboratory. *Aspergillus* species were identified based on their colonial morphology and colony color after incubation. As described, the microscopic characteristics of isolates were examined using the lactophenol cotton blue staining [25]. One drop of the dye was placed on a prepared slide, and a small piece of the culture was taken and set in the decline of the dye using a mounting needle. The same needle was used to spread the culture. A cover slip was then delicately and gently put on the spread culture with delicate pressure to remove air bubbles. Afterward, the slide was mounted and observed under the ×40 objective lens. Identifying *Aspergillus spp.* depends on septate hyphae and rough and colorless conidiophores that end in vesicles with the whole surface covered with either uni- or biseriate sterigmata.

Isolation of aflatoxins B1 and M1 from animal feed, milk, and cheese

The samples were analyzed for AFB1 and AFM1 in animal feeds, milk, and cheese using a specific ELISA kit (Romer Labs, AgraQuant total Aflatoxin, Austria). Five mL from each raw milk sample was incubated for 30 min at 4°C and centrifuged at 3000 g for 10 min. Next, the serum of milk under the fat layer was taken and then immediately assayed for AFM1 using a specific ELISA kit. A volume of 5 g of ground samples of cheese and feeds (roughage and concentrated) was taken separately in a clean pitcher, and 25 ml of 70% methanol extraction solution (extraction ratio of 1:5 of sample to extraction) was added. Raw milk samples were prepared as described above.

Analysis of aflatoxins B1 and M1 in samples by competitive ELISA

All samples were analyzed for AFB1 and AFM1 in animal feeds, milk, and cheese using a specific ELISA kit (Romer Labs, AgraQuant total Aflatoxin, Austria). The maximum and minimum amounts were 4 and 40 ppb, respectively, for AFM1 and AFB1 with high specificity and sensitivity. The kit materials were stored at 2°C-8°C. Before starting the test, the materials were incubated for 1 h at room temperature. The kit test materials were used according to the manufacturer's instructions: About 200 µL of the conjugate solution was pipetted and moved into the dilution wells (supplied with the kit). Next, the samples (100 µL) were pipetted into all dilution wells (100 µL/well/sample). Standard samples were pipetted in duplicate (100 µL/well/standard). The solution was mixed well, 100 µL was moved from the dilution wells into antibody-coated wells, and the plate containing the samples was incubated at room temperature for 15 min. The unbound conjugate was washed using a washing solution five times (supplied with the kit). After the washing step, the washed wells were gently dried.

The aflatoxin substrate solution was added to the antibody-coated wells, and the plate was incubated again at room temperature for 5 min. The reaction was allowed to proceed in the dark, at the end of which a blue color developed. The reaction was stopped by adding 100 µL stop solution to the antibody-coated wells, and the color changed from blue to yellow. The absorbance was measured at 450 nm with a differential filter at 630 nm using an ELISA plate reader, and the absorption intensity was found to be inversely proportional to the aflatoxin concentration in samples. The aflatoxin soft worksheet program supplied with the kit was used to calculate the AFB1 and M1 concentrations in the samples.

Statistical analysis

Data were expressed as mean ± standard deviation by descriptive statistics, and the feed samples were calculated as a percentage using the SPSS software (version 26). In addition, logistic regression analysis was conducted, and an odd ratio with 95% confidence intervals was used to test the relationship between predictors and expected or outcome variables. Differences were considered statistically significant at $p < 0.05$.

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Authors' Contributions

S.Y.M.S., and R.M. conceptualize the manuscript. S.Y.M.S., and R.M. wrote the original draft. S.Y.M.S., R.M., S.B., J.M., and R.W.K. revised the original draft. S.Y.M.S., R.M., S.B., J.M., and R.W.K. wrote, reviewed, and edited the manuscript. R.M. per-formed supervision. All authors read and approved the final manuscript for publication.

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Competing Interests

The authors declare that they have no conflict of interest.

Reference

1. Aasa AO, Fru FF, Adelusi OA, Oyeyinka SA, Njobeh PB. A review of toxigenic fungi and mycotoxins in feeds and food commodities in West Africa. World Mycotoxin J. 2023;16(1):33–47. Doi: 10.3920/WMJ2021.2766.

2. Janik E, Niemcewicz M, Ceremuga M, Stela M, Saluk-bijak J, Siadkowski A, Bijak M. Int J Mol Sci. 2020;8187:1–21. Doi: 10.3390/ijms21218187.

3. Yang C, Song G, Lim W. Effects of mycotoxin-contaminated feed on farm animals. J Hazard Mater. 2020;389(October 2019):122087. Doi: 10.1016/j.jhazmat.2020.122087.

4. Claudious G, Sheperd M, Charles MT, Prosper J, Jerikias M. Isolation of *AspergillusFlavus* from Dairy Cattle Feed and Assessment of Aflatoxin M1 in Milk from Small Dairy Farms around Harare, Zimbabwe. HSOA Advances in Microbiology Research. 2019;3(009):2–7. Doi: 10.24966/AMR-694X/100009.

5. Izzo L, Mikušová P, Lombardi S, Sulyok M, Ritieni A. Analysis of Mycotoxin and Secondary Metabolites in Commercial and Traditional Slovak Cheese Samples. Toxins. 2022;14(2):1–12. DOI: 10.3390/toxins14020134.

6. Pereira CS, Cunha SC, Fernandes JO. Prevalent mycotoxins in animal feed: Occurrence and analytical methods. Toxins. 2019;11(5). Doi: 10.3390/toxins11050290.

7. Djekic I, Petrovic J, Jovetic M, Redzepovic-Djordjevic A, Stulic M, Lorenzo JM, Iammarino M, Tomasevic I. Aflatoxins in milk and dairy products: Occurrence and exposure assessment for the serbian population. Applied Sciences. 2020;10(21):1–17. Doi: 10.3390/app10217420.

8. Omotayo OP, Omotayo AO, Babalola OO, Mwanza M. Comparative study of aflatoxin contamination of winter and summer ginger from the North West Province of South Africa. Toxicol Rep. 2019;6(December 2018):489–95. Doi: 10.1016/j.toxrep.2019.05.011.

9. Ochieng PE, Scippo ML, Kemboi DC, Croubels S, Okoth S, Kang'ethe EK, et al. Mycotoxins in poultry feed and feed ingredients from sub-saharan africa and their impact on the production of broiler and layer chickens: A review. Toxins. 2021;13(9). Doi: 10.3390/toxins13090633.

10. Javadikasgari H, Soltesz EG, Gillinov AM. Surgery for Atrial Fibrillation: Atlas of Cardiac Surgical Techniques. 2nd ed. Philadelphia, USA: Elsevier; 2018. Doi: 10.1016/B978-0-323-46294-5.00028-5.

11. Rodríguez-Blanco M, Ramos AJ, Sanchis V, Marín S. Mycotoxins occurrence and fungal populations in different types of silages for dairy cows in Spain. Fungal Biol. 2021;125(2):103–14. Doi: 10.1016/j.funbio.2019.08.006.

12. Motbaynor A, Kassaye D, Keffale M, Wasihun P. Magnitude of Aflatoxigenic Aspergillus Species, Level of *Aflatoxin* B1, and Associated Factors in Stored Feed at Poultry Farms in Dire Dawa, Ethiopia. Vet Med Int. 2021;2021:1–11. Doi: 10.1155/2021/6638083.

13. Adekoya I, Njobeh P, Obadina A, Landschoot S, Audenaert K, Okoth S, Boevre MD, Saeger SD. Investigation of the metabolic profile and toxigenic variability of fungal species occurring in fermented foods and beverage from nigeria and South Africa using UPLC-MS/MS. Toxins. 2019;11(2). Doi: 10.3390/toxins11020085.

14. Parkinson G. Modern Science. Surrealism. Cambridge, UK: Cambridge University Press 2021. DOI: 10.1017/9781108862639.005.

15. Tadele F, Demissie B, Amsalu A, Demelash H, Mengist Z, Ambelu A, Yenew C. Aflatoxin contamination of animal feeds and its predictors among dairy farms in Northwest Ethiopia: One Health approach implications. Frontiers in veterinary sciene. 2023;1–8. Doi: 10.3389/fvets.2023.1123573.

16. Sadam SMY, Malaka R, Baco S, Mustabi J. Aflatoxin M1 in Milk: Occurrence and Its Risk Association: A Review. Hasanuddin Journal of Animal Science (HAJAS). 2022;4(2):68–81. Doi: 10.20956/hajas.v4i2.22097.

17. Sukmawati D, Setyaningsih A, Handayanik T, Rahayu S, Rustam Y, Moersilah M, et al. Isolation and characterization of aflatoxigenic *Aspergillus spp.* from maize of livestock feed from Bogor. IOP Conference Series: Materials Science and Engineering. Institute of Physics Publishing. 2018;434:1–8. Doi: 10.1088/1757-899X/434/1/012105.

18. Anidah, Rahayu WP, Nurjanah S, Retnowati I. Molecular characterization of *aspergillus* flavus toxigenicity in agricultural commodities in Indonesia. Biotropia. 2023;30(2):195–205. Doi: 10.11598/btb.2023.30.2.1842.

19. Ceniti C, Costanzo N, Spina AA, Rodolfi M, Tilocca B, Piras C, Britti D, Morittu VM. Fungal Contamination and Aflatoxin B1 Detected in Hay for Dairy Cows in South Italy. Front Nutr. 2021;8:704976. Doi: 10.3389/fnut.2021.704976.

20. Topi D, Spahiu J, Rexhepi A, Marku N. Two-year survey of aflatoxin M1 in milk marketed in Albania, and human exposure assessment. Food Control. 2022 Jun 1;136.

21. Diogenes LCG, Kluczkovski AM, Goes AFF, Pinto S de CB, Araujo CF, Kluczkovski-Junior A. Occurrence of Aflatoxin M1 and Risk Assessment in Bovine Milk from the Amazon Region. J Food Res. 2023;12(2):23. Doi: 10.1016/j.foodcont.2015.01.025.

22. Sarmast E, Fallah AA, Jafari T, Mousavi Khaneghah A. Impacts of unit operation of cheese manufacturing on the aflatoxin M1 level: A global systematic review and meta-analysis. Lwt. 2021;148:111772. Doi: 10.1016/j.lwt.2021.111772.

23. Mousavi Khaneghah A, Moosavi M, Omar SS, Oliveira CAF, Karimi-Dehkordi M, Fakhri Y, Huseyn E, Nematollahi A, Faharani M, Sant'Ana AS. The prevalence and concentration of aflatoxin M1 among different types of cheeses: A global systematic review, meta-analysis, and meta-regression. Food Control. 2021;125:107960. Doi: 10.1016/j.foodcont.2021.107960.

24. Oktavianty O, Hamdala I, Putro WW. Risk Analysis of Environmentally Friendly Milk Production Process with GMP and HACCP. International Conference on Innovation and Technology. 2021:93–101. Doi: 10.2991/aer.k.211221.012.

25. Omeiza GK, Kabir J, Kwaga JKP, Kwanashie CN, Mwanza M, Ngoma L. A risk assessment study of the occurrence and distribution of aflatoxigenic *Aspergillus flavus* and aflatoxin B1 in dairy cattle feeds in a central northern state, Nigeria. Toxicol Rep. 2018;5:846–856. Doi: 10.1016/j.toxrep.2018.08.011.

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RESEARCH ARTICLE

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Effect of apple cider vinegar and vitamin A on hematological parameters and total immunoglobulin G in sheep: a pilot study

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ABSTRACT

Apple cider vinegar is a fermented compound that contains acetic acid, flavonoids, phenolic compounds, organic acids, minerals, and vitamins. Vitamin A is involved in the development of the immune system and plays regulatory roles in cellular immune responses and humoral immune processes. The aim of this study was to investigate the effect of apple cider vinegar and vitamin A on hematological parameters and immunoglobulin G levels in Gezel lambs. 10 healthy Gazel lambs were used for the study. The lambs were randomly grouped into three groups (control (n = 3), Apple cider vinegar (n = 4), and Vitamin A (n = 3) group). Vitamin A was administrated at a dose of 44,000 IU/kg every ten days for four treatments. ACV was administered orally by drenching (0.5 ml/kg of 6% ACV solution (600 mg ACV), every day, for 40 days). Hematological parameters were determined using standard methods. Total immunoglobulin G concentration was assayed using the turbidimetric immunoassay method. The data obtained before and after drug administration were analyzed by paired T-test and the data of different groups were analyzed using Independent-sample T-test.White blood cells, lymphocytes, and IgG in lambs were significantly increased after administration of Apple cider vinegar ($p < 0.05$). IgG and lymphocytes were significantly higher in lambs under oral administration of Apple cider vinegar compared to the lambs in the control group ($p < 0.05$). White blood cells, neutrophils, and IgG were significantly increased in lambs after vitamin A administration ($p < 0.05$). Neutrophils and IgG were significantly higher in lambs under vitamin A injection compared to the lambs in the control group ($p < 0.05$). Administration of vitamin A and apple cider vinegar in sheep is safe. They also improve the immune system.

Keywords

apple cider vinegar, hematological parameter, immunoglobulin G, lamb, vitamin A

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Abbreviations

APC: apple cider vinegar
RBC: red blood cell
WBC: white blood cell
TP: total protein
PCV: packed cell volume
Hb: Hemoglobin
IgG: immunoglobulin G

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Introduction

Apple cider vinegar (ACV) is a fermented compound that contains acetic acid, flavonoids, phenolic compounds, organic acids, minerals, and vitamins [1, 2]. ACV is produced through a two-stage fermentation process. First, the sugar is converted into alcohol by yeasts, then the alcohol is converted into acetic acid by acetic acid bacteria[3].

Animal experiments have reported that ACV has a variety of pharmacological activities, including antioxidant, anti-inflammatory, anti-diabetic, antifungal, antimicrobial, anti-hypertensive, and anti-hyperlipidemic actions [4-8]. A study showed that using ACV in a fish diet improves immunological parameters and gene expression related to immunity, antioxidant system, and growth performance[9]. Also, a study showed that using ACV in a fish diet increases total immunoglobulin and lysozyme activity[10]. In addition, a study has reported that dietary supplementation with ACV has beneficial effects on performance and immune response in broiler chickens[11]. It has been shown that ACV has an anthelmintic impact against gastrointestinal parasites in sheep[12], and increases the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase[13].

Vitamin A is known as an anti-inflammation vitamin because of its critical role in enhancing immune function[14]. Vitamin A is involved in the development of the immune system and plays regulatory roles in cellular immune responses and humoral immune processes[14].

Thus, the aim of this study was to assess the effect of ACV and vitamin A on hematological parameters and immunoglobulin G in Gezel lambs.

Result

By administering ACV and Vitamin A at the mentioned doses, no disorders and lesions have occurred in the lambs.

ACV

RBC, PCV, Hb, fibrinogen, TP, neutrophils, band neutrophils, eosinophils, and monocytes in lambs before and after administration of ACV were not statistically different.

WBC, lymphocytes, and IgG in lambs were significantly higher after administration of ACV compared to the control group ($p < 0.05$).

RBC, PCV, Hb, fibrinogen, TP, WBC, neutrophils, band neutrophils, eosinophils, and monocytes were not statistically different between lambs under oral administration of ACV and lambs in the control group.

IgG and lymphocytes were significantly higher in lambs under oral administration of ACV than in lambs in the control group ($P<0.05$).

The results of the study are summarized in Tables 1 and 2.

Vitamin A

RBC, PCV, Hb, fibrinogen, TP, band neutrophils, lymphocytes, eosinophils, and monocytes in lambs before and after administration of vitamin A were not statistically different.

WBC, neutrophils, and IgG were significantly higher in lambs after vitamin A administration compared to the control group ($p < 0.05$).

RBC, PCV, Hb, fibrinogen, TP, WBC, band neutrophils, lymphocytes, eosinophils, and monocytes were not statistically different between lambs under vitamin A injection and lambs in the control group.

Neutrophils and IgG levels were significantly higher in lambs under vitamin A injection than in the control group ($p < 0.05$).

The results of the study are summarized in Tables 1 and 2.

Discussion

A study on the effects of ACV on haemato-biochemical parameters in rate showed that there are no significant variations between the groups in post-treatment values of PCV, Hb, RBC, and absolute eosinophil counts [15]. In our study, these mentioned parameters were not different before and after administration of ACV and also they were not different between the test article groups and the control group. Also, another study found no difference in RBC between control and case groups after adding a combination of onion, garlic, and ACV to broiler chicken diets[16]. A study showed that there is no significant impact of ACV supplementation on broiler serum total protein levels[17]. This finding is similar to our finding. Also, a study showed that ACV has no effect on RBC, Hb, and PCV in humans [18]. The same results are presented in our study.

A study on the effects of an herbal mixture (onion, garlic, and ACV) on hematological parameters of broiler chickens showed that the herbal mixture resulted in significant increases in PCV and Hb values[16]. This finding is contrary to our results, and probably the results obtained in the mentioned study were not caused by the effect of ACV, or maybe the difference between the results of our study and that study is due to differences between species.

Table 1.
Hematological parameters and IgG in lambs before and after administration of vitamin A and ACV

Parameter	A day before study			A day after study			P value
	Min	Max	Mean ±SE	Min	Max	Mean ±SE	
Case group under vitamin A injection							
RBC (104/µl)	1025	1190	1105 ± 47.69	1125	1155	1142 ± 9.06	0.464
PCV (%)	30	35	32.67 ± 1.45	33	34	33.34 ± 0.33	0.635
Hb (g/dl)	10	11.66	10.88 ± 0.48	11	12	11.44 ± 0.29	0.336
TP (g/dl)	6.2	6.66	6.42 ± 0.13	6.4	7.1	6.67 ± 0.21	0.134
Fibrinogen (mg/dl)	100	200	166.7 ± 33.34	100	400	266.7 ± 88.2	0.225
WBC/µl	4446	10349	7248.4 ± 1710	8511	14150	10917.7 ± 1679.3	0.006*
Neutrophil /µl	1269	3895	2421.4 ± 774.9	4514	5382	4898.7 ± 255.3	0.041*
Band neutrophil/µl	30	100	66.7 ± 20.2	112	207	143.7 ± 31.7	0.055
Lymphocyte/µl	2976	5842	4439.4 ± 827.89	3519	7320	5113 ± 1139.2	0.259
Eosinophil /µl	124	307	200.4 ± 54.96	244	724	449.4 ± 142.8	0.105
Monocyte/µl	47	205	120.7 ±45.9	122	517	313 ±114.2	0.107
IgG (g/l)	1.19	1.30	1.24 ± 0.031	1.34	1.40	1.36 ± 0.018	0.020*
Case group under oral administration of ACV							
RBC (104/µl)	952	1260	1128 ± 76.03	982	1342	1143 ± 74.95	0.804
PCV (%)	28	37	32.7 ± 1.89	29	40	35.5 ± 2.39	0.102
Hb (g/dl)	9.33	12.33	10.9 ± 0.61	9.6	13.33	8.5 ± 2.4	0.470
TP (g/dl)	5.7	6.5	6.25 ± 0.18	6.1	7.4	6.52 ± 0.29	0.340
Fibrinogen (mg/dl)	100	300	187.5 ± 42.6	100	100	100 ± 0	0.133
WBC/µl	3330	8702	5242 ± 1187.5	5458	12470	8811.5 ± 1459.2	0.01*
Neutrophil /µl	1239	2790	1732.2 ± 356.6	910	4810	3005 ± 813.1	0.105
Band neutrophil/µl	62	80	67.7 ± 4.2	60	100	80 ± 8.16	0.091
Lymphocyte/µl	1909	5460	3217.2 ± 780.1	4250	6900	5337.5 ± 571.3	0.007*
Eosinophil/µl	80	186	121.5 ± 22.9	130	400	232.5 ± 58.36	0.053
Monocyte/µl	40	186	104 ± 30.8	108	260	156.5 ± 35.8	0.063
IgG (g/l)	1	1.25	1.08 ± 0.059	1.55	1.90	1.73 ± 0.074	0.001*
Control group							
RBC (104/µl)	1021	1184	1118.4 ± 49.7	1187	1274	1220 ± 27.09	0.096
PCV (%)	30	35	33.4± 1.7	35	38	36 ± 1	0.208

Table 1 cont.

Hb (g/dl)	10	11.66	11 ± 0.54	11.66	12.66	12.1 ± 0.293	0.107
TP (g/dl)	5.8	6.6	6.3 ± 0.25	6.8	7.3	6.97 ± 0.16	0.081
Fibrinogen (mg/dl)	150	300	200 ± 50	200	300	266.7 ± 66.7	0.181
WBC /µl	3851	9960	6722.7 ± 1772.96	5484	7400	6561.4 ± 565.8	0.909
Neutrophil /µl	1692	3840	2677.4 ± 626.3	2557	3040	2765.7 ± 143.2	0.872
Band neutrophil /µl	47	60	51.4 ± 4.3	40	70	53.4 ± 8.8	0.724
Lymphocyte/µl	1909	5460	3623 ± 1026.9	2500	3750	3283.4 ± 394.05	0.675
Eosinophil/µl	141	360	217 ± 71.5	232	340	274 ± 33.4	.278
Monocyte /µl	62	240	154 ± 51.4	155	200	185 ± 15	0.507
IgG (g/l)	1.18	1.25	1.2 ± 0.023	1.20	1.29	1.24 ± 0.026	0.225

*$p < 0.05$, RBC: red blood cell, PCV: packed cell volume, Hb: hemoglobin, TP: total protein, WBC: white blood cell, IgG: immunoglobulin G, min: minimum, max: maximum, SE: standard error.

In our study, the significant increase in WBC count after the administration of ACV was mainly due to the substantial increase in lymphocyte count. However, the significant increase in the lymphocyte count in the ACV-administered group compared to the control group did not lead to an increase in WBC count in the ACV-administered group compared to the control group. A study showed that the addition of an herbal mixture (onion, garlic, and ACV) to the diet of broiler chickens increased lymphocyte counts [16]. The improvement of the immune system due to the administration of ACV has been mentioned in several studies.[9, 10, 19, 20]. Since lymphocytes play a role in humoral immunity, perhaps the improvement of the immune system caused by ACV is responsible for the increase in lymphocytes. However, this requires further study.

In our study, it was shown that immunoglobulin G increased after the administration of vitamin A and ACV. It also increased in the ACV and vitamin A-administered group compared to the control group. These findings show that vitamin A and ACV (as mentioned above) are useful in improving the immune system in lambs. An increase in total immunoglobulin has been reported in fish after administration of ACV [10, 20, 21]. In a study, it was shown that administration of vitamin A leads to an

Materials and Methods

Selection of lambs, grouping and sampling

To perform this study, ten healthy male Gazel lambs were selected. Their health was confirmed by veterinary examinations. The age (6 months) and weight (20 kg) of the selected lambs were similar. Before starting the study, the selected lambs underwent a week of adaptation to the new environment. During the study, lambs were fed alfalfa and hay ad libidum and were observed for any clinical signs of disease throughout the study.

The lambs were randomly grouped into three groups:

1- Control group: 3 lambs were assigned to this group, and no medication was administered to them.

2- Case group under vitamin A injection: 3 lambs were assigned to this group and vitamin A was administered to them (44,000 IU/kg Retinavit®, intramuscularly (IM), every ten days, 4 treatments).

3- Case group under oral administration of ACV: 4 lambs were assigned to this group, and ACV was administered orally by drenching (0.5 ml/kg of 6% ACV solution (600 mg ACV), every day, for 40 days).

Blood samples were taken from the jugular vein of all lambs before and after the study.

Laboratory measurement

Red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), fibrinogen, total protein (TP), white blood cell (WBC) count, and absolute count of neutrophils, band neutrophils, lymphocytes, eosinophils, and monocytes were determined by routine hematology tests.

Total immunoglobulin G (IgG) concentration was assayed by turbidimetric immunoassay method as previously described [25, 26].

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Table 2.

Hematological parameters and IgG in lambs after administration of Vitamin A and ACV compared to control group

Parameter	Case group under vitamin A injection			P value	Control group			P value	Case group under oral administration of ACV		
	Min	Max	Mean ±SE		Min	Max	Mean ±SE		Min	Max	Mean ±SE
RBC(104/µl)	1125	1155	1142.7 ± 9.06	0.053	1187	1274	1220.4 ± 27.09	0.440	982	1342	1143± 74.9
PCV (%)	33	34	33.4 ± 0.33	0.065	35	38	36 ± 1	0.872	29	40	35.5 ±2.39
Hb (g/dl)	11	12	11.44 ± 0.29	0.186	11.66	12.66	12.1 ± 0.29	0.560	9.6	13.33	11.51± 0.77
TP (g/dl)	6.4	7.1	6.67 ± 0.21	0.413	6.8	7.3	6.8 ± 0.06	0.383	6.8	7.4	6.52 ±0.29
Fibrinogen (mg/dl)	100	400	266.7 ± 88.19	0.742	200	400	233.4 ± 33.4	0.057	100	100	100 ± 0
WBC/µl	8511	14151	10917.7 ± 1679.3	0.070	5484	7400	6561 ± 565.8	0.808	1270	9320	6011.5 ± 1772
Neutrophil/µl	4514	5382	4898.7 ± 255.3	0.002*	2557	3040	2765.7 ± 143.2	0.815	910	4810	3005 ± 813.1
Band neutrophil/µl	112	207	143.7 ± 31.7	0.051	40	70	53.4 ± 8.8	0.080	60	100	80 ±8.1
Lymphocyte/µl	3519	7320	5113 ± 1139.2	0.204	2500	3750	3283 ± 394.05	0.041*	4250	6900	5337.5 ± 571.3
Eosinophil/µl	244	724	449.4 ± 142.8	0.298	232	340	274 ± 33.4	0.601	130	400	232.5 ± 58.3
Monocyte /µl	122	517	313 ± 114.2	0.329	155	2	185 ± 15	0.548	108	260	156.5 ± 35.8
IgG (g/l)	1.34	1.40	1.36 ± 0.018	0.022*	1.25	1.29	1.24 ± 0.026	0.004*	1.55	1.90	1.73± 0.07

*$p <$ 0.05, RBC: red blood cell, PCV: packed cell volume, Hb: hemoglobin, TP: total protein, WBC: white blood cell, IgG: immunoglobulin G, min: minimum, max: maximum, SE: standard error.

Statistical analysis

The data obtained before and after drug administration were analyzed by paired T-Test in SPSS software. Also, the data of different groups were analyzed using the independent-sample t-test. $p < 0.05$ was considered significant.

Conclusion

Administering vitamin A and ACV in sheep is safe. They also improve the immune system. Vitamin A and ACV increase total IgG, and also vitamin A increases neutrophils and ACV increases lymphocytes. In general, they can be used to improve the immune system in sheep.

Authors' Contributions

Conceived and designed the experiments: M.T., and E.F. Performed the experiments: M.T., and M.A.. Wrote the paper: A .Sh.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. Morgan J, Mosawy S. The potential of apple cider vinegar in the management of type 2 diabetes. International Journal of Diabetes Research. 2016;5(6): 129-34. Doi: 10.5923/j.diabetes.20160506.02.

2. Tripathi S, Mazumder PM. Apple cider vinegar (ACV) and their pharmacological approach towards Alzheimer's disease (AD): A review. Indian J. Pharm. Educ. Res. 2020;54: s67-s74. Doi:10.5530/ijper.54.2s.62.

3. Samad A, Azlan A, Ismail A. Therapeutic effects of vinegar: a review. Current Opinion in Food Science. 2016;8: 56-61. Doi:10.1016/j.cofs.2016.03.001.

4. Hadi A, Pourmasoumi M, Najafgholizadeh A, Clark CC, Esmaillzadeh A. The effect of apple cider vinegar on lipid profiles and glycemic parameters: a systematic review and meta-analysis of randomized clinical trials. BMC Complementary Medicine and Therapies. 2021;21(1): 179. Doi:10.1186/s12906-021-03351-w.

5. Iman M, Moallem SA, Barahoyee A. Effect of apple cider vinegar on blood glucose level in diabetic mice. Pharmaceutical Sciences. 2014;20(4): 163-168.

6. Nazıroğlu M, Güler M, Özgül C, Saydam G, Küçükayaz M, Sözbir E. Apple cider vinegar modulates serum lipid profile, erythrocyte, kidney, and liver membrane oxidative stress in ovariectomized mice fed high cholesterol. The Journal of Membrane Biology. 2014;247: 667-673. Doi:10.1007/s00232-014-9685-5.

7. Shishehbor F, Mansoori A, Sarkaki A, Jalali M, Latifi S. Apple cider vinegar attenuates lipid profile in normal and diabetic rats. Pakistan journal of biological sciences: PJBS. 2008;11(23):2634-2638. Doi:10.3923/pjbs.2008.2634.2638.

8. Mota ACLG, de Castro RD, de Araújo Oliveira J, de Oliveira Lima E. Antifungal activity of apple cider vinegar on Candida species involved in denture stomatitis. Journal of Prosthodontics. 2015;24(4): 296-302. Doi:10.1111/jopr.12207.

9. Ahmadifar E, Dawood MA, Moghadam MS, Sheikhzadeh N, Hoseinifar SH, Musthafa MS. Modulation of immune parameters and antioxidant defense in zebrafish (Danio rerio) using dietary apple cider vinegar. Aquaculture. 2019;513: 734412. Doi:10.1016/j.aquaculture.2019.734412.

10. Ahmadniaye Motlagh H, Javadmanesh A, Safari O. Improvement of non-specific immunity, growth, and activity of digestive enzymes in Carassius auratus as a result of apple cider vinegar administration to diet. Fish physiology and biochemistry. 2020;46(4): 1387-1395. Doi:10.1007/s10695-020-00797-6.

11. Jahantigh M, Kalantari H, Ayda Davari S, Saadati D. Effects of dietary vinegar on performance, immune response and small intestine histomorphology in 1-to 28-day broiler chickens. Veterinary Medicine and Science. 2021;7(3): 766-772. Doi:10.1002/vms3.408.

12. Hayajneh FM, Titi HH, Alnimer MA, Irshaid R. Evaluation of anthelmintics resistance against gastrointestinal parasites infection in awassi sheep in Jordan and the use of alternative herbal anthelmentics. Am. J. Anim. Vet. Sci. 2019;14(2); 122-126. Doi:10.3844/ajavsp.2019.122.126.

13. Halima BH, Sarra K, Houda BJ, Sonia G, Abdallah A. Antidiabetic and antioxidant effects of apple cider vinegar on normal and streptozotocin-induced diabetic rats. International Journal for Vitamin and Nutrition Research. 2019. Doi:10.1024/0300-9831/a000246.

14. Huang Z, Liu Y, Qi G, Brand D, Zheng SG. Role of vitamin A in the immune system. Journal of Clinical Medicine. 2018;7(9): 258. Doi:10.3390/jcm7090258.

15. Ngwu GI, Opara BA, Ngwu MI, Atuogba NC, Ugwuoke WI, Ossai NI, et al. Effects of apple cider vinegar on haemato-biochemical parameters of gestating rats and morphometric indices of their pups at delivery. Comparative Clinical Pathology. 2021;30(6): 953-960. Doi:10.1007/s00580-021-03294-1.

16. Abdulkareem ZA, Mohammed NI, Abdollahi A, Ahmed OR, Ghaffar OR, Khdir HA, et al. Effects of garlic, onion, and apple cider vinegar as a herbal mixture on performance and blood traits of broilers inoculated with chicken infectious anemia virus. Heliyon. 2023;9(7). Doi:10.1016/j.heliyon.2023.e17768.

17. Hayajneh F. Natural feed additives for broiler chickens. South African Journal of Animal Science. 2019;49(5): 869-875. Doi:10.4314/sajas.v49i5.9.

18. Hosseini ZSM, Hosseini J, Nabati S, Hasanshahi G, Mahmoodi M. Survey on the anti-diabetic effects of vinegar on some biochemical factors in type 2 diabetic patients. Clinical Biochemistry-New York. 2011;44(13): 2. Doi:10.1016/j.clinbiochem.2011.08.543.

19. Pourmozaffar S, Hajimoradloo A, Miandare HK. Dietary effect of apple cider vinegar and propionic acid on immune-related transcriptional responses and growth performance in white shrimp, Litopenaeus vannamei. Fish & shellfish immunology. 2017;60: 65-71. Doi:10.1016/j.fsi.2016.11.030.

20. Ahmadniaye Motlagh H, Sarkheil M, Safari O, Paolucci M. Supplementation of dietary apple cider vinegar as an organic acidifier on the growth performance, digestive enzymes and mucosal immunity of green terror (Andinoacara rivulatus). Aquaculture Research. 2020;51(1): 197-205. Doi:10.1111/are.14364.

21. Safari R, Hoseinifar SH, Nejadmoghadam S, Khalili M. Apple cider vinegar boosted immunomodulatory and health promoting effects of Lactobacillus casei in common carp (Cyprinus carpio). Fish & shellfish immunology. 2017;67: 441-448. Doi:10.1016/j.fsi.2017.06.017.

22. Semba R, Scott A, Natadisastra G, West Jr K, Sommer A. Effect of vitamin A supplementation on immunoglobulin G subclass responses to tetanus toxoid in children. Clinical and Diagnostic Laboratory Immunology. 1994;1(2): 172. Doi: 10.1128/cdli.1.2.172-175.1994.

23. Semba RD. The role of vitamin A and related retinoids in immune function. Nutrition reviews. 1998;56(1): S38-S48.

24. Twining SS, Schulte DP, Wilson PM, Fish BL, Moulder JE. Vitamin A deficiency alters rat neutrophil function. The Journal of Nutrition. 1997;127(4): 558-565.

25. Davis DG, Schaefer DM, Hinchcliff KW, Wellman ML, Willet VE, Fletcher JM. Measurement of serum IgG in foals by radial immunodiffusion and automated turbidimetric immunoassay. Journal of Veterinary Internal Medicine. 2005;19(1); 93-96. Doi:10.1111/j.1939-1676.2005.tb02664.x.

26. Ferris RA, McCue PM. How to use a quantitative turbidimetric immunoassay to determine immunoglobulin G concentrations in neonatal foals, Proceedings of the... annual convention, 2009.

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Cross Immunity of a Sonicated Trivalent Avian Colibacillosis Vaccine to Pathogenic Escherichia coli O26 and O78 Strains in Broiler Chickens

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ABSTRACT

Colibacillosis outbreaks are a global issue affecting the poultry industry. There is no cross-immunity among the strains of APEC. If a vaccine induces cross-immunity, it will play a key role in preventing colibacillosis. Herein, a sonicated trivalent colibacillosis vaccine containing O78:K80, O2:K1, and O1:K1 serotypes, with Alum as an adjuvant, was used to assess cross-immunity against E. coli O26. Ninety-six broiler chickens were randomly assigned to four groups: Group A was vaccinated and exposed to O78; Group B was unvaccinated but exposed to O78; Group C was vaccinated and exposed to O26; Group D was unvaccinated but exposed to O26. At 14 days old, chickens in groups A and C received a single dose of the vaccine, while groups B and D received normal saline subcutaneously. At 35 days old, all groups were challenged with O78 and O26 as described above. Clinical signs and lesions, isolation of the bacterium, weight gain, food intake, FCR, and antibody titers against the O antigens of the vaccine strains and O26 were recorded. The results indicated that 2 weeks post-vaccination, titers to the O antigens of the vaccine strains were significantly higher in the vaccinated groups than in the unvaccinated groups ($p \leq 0.05$). Following the challenge, no significant difference was observed in food intake and FCR between the groups ($p > 0.05$); however, the growth rate in group A was significantly higher than in group B ($p \leq 0.05$). At 42-49 days old, the vaccinated groups had the highest growth rate, which was statistically significant compared to the unvaccinated groups; and FCR in group A was significantly better than in group B ($p \leq 0.05$). In conclusion, it appears that in addition to homologous immunity, the vaccine also induces cross-immunity against O26.

Keywords

Colicepticemia, Heterologous, Homologous, Prevention, Poultry

Abbreviations

APEC: Avian Pathogenic Escherichia coli
FCR: Feed Conversion Ratio

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Introduction

Colibacillosis, especially colisepticemia as the acute form, is one of the most common bacterial diseases in the poultry industry with economic losses worldwide [1-3]. Colibacillosis which is caused by APEC, often occurs simultaneously with other diseases and due to predisposing factors, such as avian vaccination, particularly infectious bronchitis vaccines, and stress [2, 4]. Several methods have been applied to prevent and control colibacillosis, such as better management of the litter, improving drinking water, and better ventilation in farms, and also using antibiotics, bacteriophages, and nutrient synergy [1, 2, 5]. Despite these efforts, incidences and economic losses due to colibacillosis continue in poultry houses all over the world. Several studies indicated that colibacillosis can be prevented in poultry by vaccination and different vaccines, including inactivated, live, recombinant, mutant, and molecular vaccines, which have been prepared and tested experimentally [1, 6, 7]. The great diversity among APEC serogroups and the different mechanisms and stages of infection by the serogroups [1] are the main reasons vaccines have not been able to induce cross-immunity. Melamed et al. reported that by using the ultrasonic inactivation method for preparing an inactivated vaccine against APEC infection a certain degree of heterologous protection is possible because of the expression of some of the important internal immunogenic determinants [7].

Although APEC has diverse serogroups, the ones isolated from diseased birds in most countries are O78, O2, and O1 [1, 2, 7]. Moreover, we know that antigen particulates can increase the activation of antigen-presenting cells [8]. Alum [9], if used as an adjuvant, may enhance the efficacy of a vaccine because of its particulate form. It may also enable the use of a potential inactivated colibacillosis vaccine in broiler chickens due to its safety [8]. *E. coli* O26 strain is one of the APECs that has been isolated from chickens in Iran and other countries [10-12].

In the present study, we used a sonicated trivalent avian colibacillosis vaccine, including the O2:K1, O78:K80, and O1:K1 serotypes of APEC to evaluate the cross-immunity of this vaccine against O26 strain in broiler chickens.

Result

Clinical signs, gross lesions, mortality rate, and isolation of E. coli

Subsequent to the challenge, both the vaccinated and unvaccinated cohorts displayed a period of lethargy and listlessness, spanning approximately one day. Notably, avian subjects within the unvaccinated clusters, especially group B which was challenged with the O78 strain, exhibited more pronounced clinical signs. Dullness, lethargy, lack of movement, decreased appetite, hanging of stool on the anus, diarrheal stool, green stool in the bedding, and decreased reaction to movements were observed.

A chicken, that died in the unvaccinated group 8 days after challenge with the O78 strain and also three other euthanized chickens from different groups had gross lesions, including septicemia, pericarditis, perihepatitis, peritonitis, breast blister, emaciation, and airsacculitis (Table 1). *E. coli* bacteria were isolated from the liver and heart blood of two dead chickens that belonged to the unvaccinated group challenged with the O78 strain.

Growth rate

The analysis indicated no significant difference in weight gain between the examined groups during the periods of 14, 14-21, 21-28, and 28-35 days. However, a significant difference was observed among the groups in the age period of 35-42 days. The vaccinated group challenged with O78 exhibited the highest weight gain, while the unvaccinated group challenged with O78 demonstrated the lowest weight gain ($p \leq 0.05$). Furthermore, in the age period of 42-49 days, there was a significant difference between vaccinated and unvaccinated groups challenged with O26 and also between vaccinated and unvaccinated groups challenged with O78 ($p \leq 0.05$) (Table 2).

Feed consumption

Before the challenge, across some age periods, various groups exhibited equivalent consumption patterns, and from a statistical standpoint, no noteworthy distinctions were identified between the analyzed cohorts. After the challenge, at 35-42 days old, food consumption in group B decreased, but there was no significant difference with other groups ($p > 0.05$) (Table 3).

Feed conversion ratio

During the age intervals of 14-21, 21-28, 28-35, and 35-42 days, no statistically significant differences were observed between the examined cohorts. However, a notable disparity in FCR emerged during the 42-49-day period. The unvaccinated group challenged with O78 exhibited the highest FCR, whereas the vaccinated group exposed to O26 demonstrated the most favorable FCR outcome (Table 4).

Microagglutination test to O antigen

Before vaccination, antibodies to the O antigens

Table. 1
Post-mortem lesions and bacteria titers in the vaccinated broiler chicks challenged with O26 and O78

No	group	Autopsy symptoms	Isolation of *E.coli*
1	Carcass belonged to the vaccinated group challenged with O78	Perihepatitis	-
2	Carcass belonged to the unvaccinated group challenged with O78	Breast blister, emaciation, pericarditis, perihepatitis	+
3	Carcass belonged to the vaccinated group challenged with O26	Breast blister, pericarditis	-
4	Deceased carcass belonged to the unvaccinated group challenged with O78	Septicemia, pericarditis, airsacculitis	+

- Isolation of *E. coli* was negative
+ Isolation of *E. coli* was positive

Table. 2
Mean growth rate (g) of the chickens of different ages vaccinated and unvaccinated and challenged with O26 and O78

groups[1]	Age (day-old)					
	14	14-21	21-28	28-35	35-42	*42-49*
A	288.25 ± 4.77	328.41 ± 9.82	436.71 ± 13.30	498.16 ± 21.45	431.12 ± 41.00b	515.70 ± 41.44bc
B	307.21 ± 7.52	344.25 ± 10.62	440.00 ± 14.20	505.66 ± 24.00	284.25 ± 23.06a	*398.25 ± 46.65a*
C	286.37 ± 8.76	309.10 ± 9.48	426.16 ± 14.00	531.54 ± 13.08	389.00 ± 20.49ab	586.60 ± 33.30c
D	289.54 ± 6.12	328.54 ± 9.07	448.21 ± 11.00	507.08 ± 10.60	382.37± 15.32a	464.41 ± 34.31ab
P-value	**0.13**	**0.09**	**0.70**	**0.60**	**0.002**	**0.008**

1 The chickens of groups A and C received vaccine, while the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with different letters are significantly different.

Table 3.
Mean Feed consumption ± standard error of the chickens of different ages vaccinated and unvaccinated and challenged with O26 and O78

groups[1]	Age (day-old)				
	14-21	21-28	28-35	35-42	42-49
A	502.53 ± 2.00	758.33 ± 4.16	937.50 ± 0.00	887.50 ± 0.00	1225.00 ± 0.00
B	502.60 ± 1.80	760.43 ± 2.06	937.50 ± 0.00	800.00 ± 25.00	1314.26 ± 57.13
C	500.03 ± 9.53	762.53 ± 3.60	937.50 ± 0.00	862.50 ± 19.09	1257.13 ± 57.13
D	509.60 ± 1.80	760.43 ± 2.06	937.50 ± 0.00	865.83 ± 28.33	1257.13 ± 57.13
P-value	**0.46**	**0.82**	**1**	**0.08**	**0.65**

[1]A and C are the vaccinated groups challenged 16 and 26 days post-vaccination, respectively. B and D are the unvaccinated groups challenged 16 and 26 days post-vaccination, respectively.
*The data shown with the same letter are not significantly different.

of the vaccine strains were negative. Statistical analysis revealed significant differences between the examined groups on days 28 and 42 ($p \leq 0.05$). On 28 days, the vaccinated group challenged with O78 exhibited the highest titer, while the unvaccinated group challenged with O78 demonstrated the lowest titer. Similarly, at the age of 42 days, the vaccinated group treated with O26 displayed the highest microagglutination rate, whereas the unvaccinated group treated with O26 exhibited the lowest microagglutination rate (Table 5).

Table. 4
Mean FCR ± standard error of the broiler chickens of different ages vaccinated and unvaccinated and challenged with O78 and O26

groups[1]	Age (day-old)				
	14-21	21-28	28-35	35-42	42-49
A	1.54 ± 0.08	1.70 ± 0.02	1.86 ± 0.11	2.06 ± 0.09	2.64 ± 0.11ab
B	1.48 ± 0.02	1.73 ± 0.03	1.90 ± 0.06	2.87 ± 0.32	3.33 ± 0.31c
C	1.61 ± 0.02	1.80 ± 0.07	1.76 ± 0.03	2.25 ± 0.18	2.15 ± 0.13a
D	1.56 ± 0.08	1.70 ± 0.01	1.86 ± 0.11	2.27 ± 0.02	2.73 ± 0.20ab
P-value	**0.50**	**0.38**	**0.11**	**0.15**	**0.02**

[1] The chickens of groups A and C received vaccine, while the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with the same letter are not significantly different.

Table. 5
Geometric mean antibody titers to the O antigen of the O2, O78, O1, and O26 strains of APEC in the vaccinated broiler chickens

groups[1]	Age (day-old)		
	21	28	42
A	0.85 ± 0.21	4.25 ± 0.27b	8.2 ± 0.36b
B	0.65 ± 0.15	2.41 ± 0.25a	7.78 ± 0.82b
C	0.45 ± 0.15	3.5 ± 029b	9.59 ± 0.33c
D	0.54 ± 0.15	2.5 ± 0.25a	5.5 ± 0.89a
P-value	**0.43**	**0.000**	**0.000**

[1] The chickens of groups A and C received vaccine, and the chickens of groups B and D received sterile normal saline. Groups A and B were challenged with the O78:K80 strain, and groups C and D with the O26 strain.
*The data shown with the same letter are not significantly different.
*The data shown with the same letter are not significantly different.

Discussion

There is a need for an ideal APEC vaccine that can provide cross-protection against multiple APEC serotypes [13]. The results of the present study indicated that the alum adjuvanted-sonicated trivalent avian colibacillosis vaccine can induce homologous and heterologous immunity 21 days post-vaccination in a single dose. After the challenge, the incidence of the clinical signs and gross lesions decreased in the vaccinated challenged groups.

Common gross lesions of colibacillosis in broiler chickens are pericarditis, perihepatitis, and airsacculitis [1]. According to a study in Egypt (2021), chickens infected with *E. coli* strains O78 and O26 exhibited pronounced clinical manifestations compared to those infected with other strains. Consequently, these two strains are noteworthy for their potential to induce colibacillosis in poultry [14]. In this study, following challenges, in contrast to the challenged vaccinated group, the dead chickens of the unvaccinated group challenged with O78 typically developed pericarditis, perihepatitis, and airsacculitis, with *E. coli* bacteria isolated from the carcasses. Colibacillosis is characterized by variable polyserositis, but none of them are pathognomonic signs, and isolation of APEC is needed for diagnosis [2, 3, 15]. The challenge of chickens with O26 in both unvaccinated and vaccinated groups, despite the incidence of clinical signs, did not lead to mortality, which may be due to the low pathogenicity of the O26 strain. APEC has numerous strains with widespread pathogenic bacteria in poul-

try [16].

The weight loss of affected chickens is one of the reasons for the economic importance of colibacillosis. Colibacillosis causes the affected chickens to lose about 84 g/bird of average body weight [3]. The present study showed that the weekly mean body weight gain of challenged unvaccinated chickens in both groups which were challenged with O78 and O26 significantly decreased in comparison with the challenged vaccinated groups ($p \leq 0.05$). Colibacillosis reduces feed intake in affected chickens [17]. In this study food consumption insignificantly dropped immediately after a challenge on 35-42 days old. However, the chickens in all groups were compensated throughout the study.

Avian colibacillosis usually increases FCR [18]. The results of FCR at 42-49 days old indicated that the increase in food consumption in the vaccinated groups, especially in the vaccinated group challenged with O78, significantly raised the body weight of the chickens. The significant difference in FCR between the vaccinated group challenged with O78 and the unvaccinated group challenged with O78 means that although food intake increased one week after the challenge, it failed to improve the body weight of chickens in the unvaccinated challenged groups, especially in the unvaccinated group challenged with O78. The results related to FCR are in line with the findings of Amen et al. (2023) [19].

Regarding the two groups challenged with the O26 strain, although the FCR difference at the age of 42-49 days was not significant between the two groups, the FCR of the vaccinated group was better than the unvaccinated group and compared to all groups, this group had the highest growth rate at 42-49 days old. It seems that the pathogenicity of E. coli strains affects the FCR. As described above, APEC has numerous strains with widespread pathogenic bacteria in poultry [16].

The results of the microagglutination test showed that the O antigens of the three strains of the vaccine-induced immune responses in the vaccinated chickens 14 days post-vaccination. The O antigens in E. coli bacteria are among the highly immunogenic antigens [20]. The results also confirmed the challenge because antibody titers against O78 and O26 antigens rose in the challenged chickens.

Future vaccine development requires a multi-dimensional approach, focusing on identifying conserved antigens that confer broad protection across different APEC serotypes or incorporating such antigens. Multivalent vaccines targeting multiple serogroups or incorporating diverse antigens may offer enhanced efficacy and broader coverage [21].

Conclusion

The results demonstrated that it is possible to produce heterologous immunity with the alum-adjuvanted sonicated trivalent avian colibacillosis vaccine. These results support the data reported by Melamed et al. (1991) that demonstrated that chickens vaccinated with the sonicated O2:K1 strain were protected from challenges with the heterologous O78:K80 strain. Sonication increases the presentation of internal and common antigens to the immune system [22].

Materials and Methods

E. coli strains

Two strains of APEC, including O78:K80 and O26, were used in this study. These strains had been isolated from affected broiler chickens in Iran and their pathogenicity had been confirmed by experimental studies (Microbiology Laboratory, Faculty of Veterinary Medicine, University of Tehran).

Sonicated trivalent avian colibacillosis vaccine

The vaccine contains three inactivated serotypes of *E. coli,* including O78:K80, O2:K1, and O1:K1, and alum as an adjuvant.

Experimental design

A total of 96 broiler chickens (Ross 308®) were randomly assigned to four groups of 24 chicks each (female and male chicks were equal in each group). Each group had three subgroups of eight chicks per cage. Groups A and C were vaccinated, while groups B and D were not vaccinated (received normal saline instead of the vaccine). All the four groups were challenged 21 days post-vaccination. Groups A and B were challenged with the O78:K80 strain, while groups C and D were challenged with the O26 strain.

On day 14 of life, just before vaccination, and 7, 14, 21, 27, and 36 days after vaccination, the growth rate of chickens in the different groups was recorded. The mortality rate, feed consumption, and FCR were also assessed. After vaccination and challenge, the clinical signs and gross lesions of euthanized, moribund birds and dead chickens were recorded and also, using colony morphology and biochemical features attempts were made to isolate *E. coli* bacteria from the heart blood of fresh dead chickens [23]. Please replace the highlighted phrase with " using colony morphology and biochemical features.

Vaccination

Chickens in groups A and C received sonicated trivalent avian colibacillosis vaccine in the dorsal cervical region via the subcutaneous route on day 14 (0.5 ml/chick). Groups B and D received sterile normal saline at the same site and via the same route (0.5 ml/chick) (Table 6).

Challenge

Chickens of groups A and B were subcutaneously [10, 24] challenged 21 days post-vaccination with 0.5 ml of a suspension containing 1.5×109 CFU/ml of O78:K80, while chickens of groups C and D were challenged with 0.5 ml of a suspension containing 1.5×109 CFU/ml of O26 (Table 6).

Microagglutination test to O antigen

Blood samples were taken randomly from the chickens on days 14, 21, 28, and 42 for serology study. A volume of 50 µL of normal saline was placed in each well of a rounded-bottom microtiter plate. In the first well, 50 µL of serum sample was added and the mixture was serially diluted. Next, 50 µL of E. coli O antigens, including O2, O78, and O1 antigens or O26 antigens, was added to all nine wells. After overnight incubation at 4°C, the results were recorded.

Statistical analysis

Table. 6
Experimental design

Chikens[a]	Vaccination	Challenge
Groups	Age (day)	Age (day)
	14	35
A	Vb	+d
B	NVc	+
C	V	+
D	NV	+

[a]The chickens of groups A and C received sonicated trivalent avian colibacillosis vaccine via subcutaneous injection in the dorsal cervical region (0.5 ml/chick); the chickens of groups B and D received sterile normal saline (0.5 ml/chick).
Groups A and B were challenged with strain O78:K80 and groups C and D with strain O26.
[b]V: vaccinated, NV: unvaccinated, +: chickens were challenged.

Authors' Contributions

Sima Alempour Rajabi and Zolfaghar Rajabi conceived and planned the experiments. Sima Alempour Rajabi carried out the experiments. Sima Alempour Rajabi and Zolfaghar Rajabi planned and carried out the simulations. Sima Alempour Rajabi contributed to sample preparation. Sima Alempour Rajabi and Zolfaghar Rajabi contributed to the interpretation of the results. Sima Alempour Rajabi and Zolfaghar Rajabi took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. Dziva F, Stevens MP. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic Escherichia coli in their natural hosts. Avian Pathology. 2008; 37(4):355-66. Doi: 10.1080/03079450802216652.

2. Dhama K, Chakraborty S, Barathidasan R, Tiwari R, Rajagunalan S, Singh SD. Escherichia coli, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance: A review. Research Opinions in Animal and Veterinary Sciences. 2013; 3(6):179-94.

3. Nolan LK, Vaillancourt JP, Nicolle LB, Logue CM. Colibacillosis. In: Swayne DE, Boulianne M, Logue CM, Mcdougald LR, Nair V, Suarez DL. Diseases of poultry. 14th edition. Blackwell Publishing. 2020; 770-830.

4. Matthijs MG, Van Eck JH, Landman WJ, Stegeman JA. Ability of Massachusetts-type infectious bronchitis virus to increase colibacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent field virus. Avian Pathology. 2003; 32(5):473-81. Doi: 10.1080/0307945031000154062.

5. Barbour EK, Mastori FA, Shaib HA, Yaghi RH, Tayeb IT, Sleiman FT, Kassaify ZG, Sawaya RK, Nour AM, Sabra AH, Harakeh S. Efficacy of a Nutrient Synergy Against Colibacillosis in Poultry. International Journal of Applied Research in Veterinary Medicine. 2009; 7(1-2):43-9.

6. Kwaga JK, Allan BJ, Van der Hurk JV, Seida H, Potter AA. A carAB mutant of avian pathogenic Escherichia coli serogroup O2 is attenuated and effective as a live oral vaccine against colibacillosis in turkeys. Infection and immunity. 1994; 62(9):3766-72. Doi: 10.1128/iai.62.9.3766-3772.1994.

7. Melamed D, Leitner G, Heller ED. A vaccine against avian colibacillosis based on ultrasonic inactivation of Escherichia coli. Avian diseases. 1991; 17-22.

8. Hem SL, HogenEsch H. Aluminum-containing adjuvants: properties, formulation, and use. In: Singh M. Vaccine adjuvants and delivery systems. Hoboken, NJ: John Wiley & Sons inc. 2007; 81-114.

9. Gupa RK, RK, Rost BE. Aluminum compounds as vaccine adjuvants. In: O'Hagan DT. Vaccine adjuvants. Springer New York: HumanaPress. 2000; 65-89.

10. Kholdi S, Motamedifar M, Fani F, Mohebi S, Bazargani A. Virulence factors, serogroups, and antibiotic resistance of Shiga-toxin producing Escherichia coli from raw beef, chicken meat, and vegetables in Southwest Iran. Iranian Journal of Veterinary Research. 2021; 22(3):180-187. Doi: 10.22099/IJVR.2021.39266.5706.

11. Rahman MM, Husna A, Elshabrawy HA, Alam J, Runa NY, Badruzzaman AT, Banu NA, Al Mamun M, Paul B, Das S, Rahman MM. Isolation and molecular characterization of multidrug-resistant Escherichia coli from chicken meat. Scientific Reports. 2020;10(1):21999. Doi:10.1038/s41598-020-

78367-2.

12. Tairov J, Salimov K. Cultural, biochemical, and pathogenic properties of Escherichia coli isolated from birds. InE3S Web of Conferences 2023 (Vol. 452, p. 01023). EDP Sciences. Doi:10.1051/e3sconf/202345201023.

13. Kathayat D, Lokesh D, Ranjit S, Rajashekara G. Avian Pathogenic Escherichia coli (APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. Pathogens. 2021; 10(4):467.Doi/10.3390/pathogens10040467.

14. Elnagar R, Elkenany R, Younis G. Interleukin gene expression in broiler chickens infected by different Escherichia coli serotypes. Veterinary World. 2021; 14(10):2727-2734. Doi: 10.14202/vetworld.2021.2727-2734.

15. Lutful Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International journal of environmental research and public health. 2010; 7(1):89-114. Doi: 10.3390/ijerph7010089.

16. Hu J, Afayibo DJ, Zhang B, Zhu H, Yao L, Guo W, Wang X, Wang Z, Wang D, Peng H, Tian M. Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic Escherichia coli (APEC). Frontiers in Microbiology. 2022; 13:1049391. Doi: 10.3389/fmicb.2022.1049391.

17. Pham VH, Abbas W, Huang J, Guo F, Zhang K, Kong L, Zhen W, Guo Y, Wang Z. Dietary coated essential oil and organic acid mixture supplementation improves health of broilers infected with avian pathogenic Escherichia coli. Animal Nutrition. 2023; 12:245-62. Doi: 10.1016/j.aninu.2022.09.010.

18. El-Tahawy AO, Said AA, Shams GA, Hassan HM, Hassan AM, Amer SA, El-Nabtity SM. Evaluation of cefquinome's efficacy in controlling avian colibacillosis and detection of its residues using high performance liquid chromatography (HPLC). Saudi Journal of Biological Sciences. 2022; 29(5):3502-10. Doi:10.1016/j.sjbs.2022.02.029.

19. Amen O, El Hendy AH, Elfeil WK, Hussein AG. Evaluating the efficacy of commercial Escherichia coli killed vaccine in broiler chickens. Journal of Advanced Veterinary Research. 2023; 13 (6):1037-43.

20. Liu B, Furevi A, Perepelov AV, Guo X, Cao H, Wang Q, Reeves PR, Knirel YA, Wang L, Widmalm G. Structure and genetics of Escherichia coli O antigens. FEMS microbiology reviews. 2020; 44(6):655-83. Doi: 10.1093/femsre/fuz028.

21. Paudel S, Apostolakos I, Vougat Ngom R, Tilli G, de Carvalho Ferreira HC, Piccirillo A. A systematic review and meta-analysis on the efficacy of vaccination against colibacillosis in broiler production. Plos one. 2024; 19(3):e0301029.

22. Melamed D, Leitner G, Heller ED. A vaccine against avian colibacillosis based on ultrasonic inactivation of Escherichia coli. Avian diseases. 1991; 1:17-22. Doi: 10.2307/1591289.

23. Lee, MD and Arp, LH (1998). Colibacillosis.In: Swayne, DE; Glisson, JR; Jackwood, MW; Pearson, JE; Reed, WM; editors. Isolation and identification of avian pathogens.4th ed. American Association of Avian Pathologists. 14-16.

24. Gibbs PS, Petermann SR, Wooley RE. Comparison of several challenge models for studies in avian colibacillosis. Avian diseases. 2004; 48(4):751-8. Doi: 10.1637/7176-030404R.

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Identification of Effective Genes in Feline Infectious Peritonitis and Drug Repurposing Using Systems Biology Approach

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ABSTRACT

FIP is a systemic infectious disease of cats of coronavirus origin. The lack of clear signs of the presence of the virus before clinical form presentation, and the absence of easy and inexpensive diagnostic tests to confirm virus presence are among the problems for controlling and preventing the spread of the virus. In addition, there is not yet any approved medications or treatment protocols for this disease. In this paper, the gene co-expression network was first reconstructed and modulated using the STRING database and Cytoscape software. The GO and pathways of the modules were obtained using the DAVID and KEGG databases. The most important possible pathways are proteasome, protein processing in the endoplasmic reticulum, protein export, aminoacyl-tRNA biosynthesis, phagosome, tuberculosis, and T cell receptor signaling pathway. In the other part of the study, the gene-drug network regeneration strategy was used to identify a potential medicine reconstructed using the DGIdb database and Cytoscape software using the drug-gene network. BORTEZOMIB, CARFILZOMIB, OPROZOMIB, IXAZOMIB CITRATE, MARIZOMIB, BCG VACCINE, IC14, NELFINAVIR, and RITONAVIR are some of our recommended drugs for this disease. Although our computational strategy predicts repurposable candidate drugs against FIP, more detailed experimental trials and clinical analyses of drug performance, toxicity, and validation are necessary to achieve an accurate and improved treatment protocol.

Keywords

Feline infectious peritonitis, Systems biology, Gene co-expression, Drug repurposing, Coronaviridae

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Abbreviations

BP: Biological processes
CC: Cellular components
FCoV: Feline coronavirus
FIP: Feline infectious peritonitis
FIPV: Feline infectious peritonitis virus
GO: Gene ontology

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Introduction

FIP is a severe disease primarily affecting younger cats [1, 2]. Diagnosis is challenging due to the lack of definitive tests and specific symptoms [3]. It is caused by FCoV, an RNA virus belonging to the Alphacoronavirus genus with serotypes 1 and 2 [4]. Serotype 1 is more prevalent [2], but research focuses on serotype 2 due to easier laboratory propagation. FCoV can persist in dry environments for up to seven weeks and spreads indirectly [5]. Early detection of FIP is problematic as clinical signs and imaging lack specificity. Real-time polymerase chain reaction tests for viral RNA are inconclusive. Prevention outweighs treatment options due to rapid progression and diagnostic limitations [5–7]. However, efforts to develop a vaccine have been hindered by antibody-dependent enhancement [8]. Therefore, identifying effective treatments is critical.

This study employs computational biology to analyze gene expression data (dataset GSE152676) from healthy and FIP-infected cats. GO analysis using the DAVID database identifies relevant biological processes [9]. The present research aims to pinpoint FIP-related genes and potential drug targets efficiently. By leveraging a drug-gene interaction network, the study rapidly identifies candidate medications through bioinformatics and gene co-expression network analysis. This approach enhances our understanding of FIP mechanisms and aids in developing effective treatments [10].

In this study, gene expression data for FIP was obtained from the NCBI database using the GSE152676 dataset [11], which includes data from healthy and FIP-affected cats. The dataset comprises 19,493 transcripts obtained via Illumina sequencing. Significant gene expression differences ($p < 0.001$) between healthy and FIP groups were identified, resulting in 1,332 genes for network construction.

The gene co-expression network was constructed using the String database (version 11.5) [12], focusing on 1,332 genes from the previous step, specifying Felis catus as the species of interest. The network settings emphasized co-expression interactions with a confidence score of 0.4, excluding unconnected nodes. High-confidence data were obtained by adjusting interaction settings. The network was visualized and refined using Cytoscape software [13] and employing graph theory principles to reconstruct the main gene co-expression network (Figure 1).

Clustering

The cluster analysis of biological networks has become one of the most remarkable strategies for identifying functional modules and predicting network biomarkers and protein complexes. The visualization of the clustering outcome is highly important for demonstrating the structure of biological networks [14]. The ClusterVis [15] plugin (version 1.0.3) was used to cluster the main network and to analyze this biological network. Cytoscape software is a powerful instrument for drawing and analyzing networks [13]. It is a platform that can be employed to run the ClusterVis plugin. In this step, the gene co-expression network data obtained from the string site were imported into the Cytoscape software version 3.8.2. Next, five performance modules were obtained using the ClusterVis plugin and the Algorithm: FAG-EC parameters and setting the algorithm parameters to DefinitionWay: Strong and OutputThreshold: 10. The list of cluster genes is provided in the supplementary file.

The GO section contains information about BP, CC, and MF. The BP refers to a biological target that comprises a gene or gene product. The procedure is performed through one or several regular sets of MFs. The CC denotes the location and structure in the cell in which the gene product is active. The MFs are defined as the biochemical activity (involving a particular binding of ligands or structures) of a gene product [16].

In this step, the list of functional cluster genes, which were obtained from the previous step, was entered into the DAVID site. Several categories of functional information were obtained after providing the requested information and selecting the species Felis catus as the test species. The GO and pathway sections were among the objectives of our study. In the GO section, the findings of all BP, CC, and MF categories were presented in different evaluations for each category. Moreover, we loaded and stored data for all categories and clusters to select more detailed data. All data were arranged according to the p-value and the pathways of each of the five clusters were separately loaded for reviewing and reporting purposes. The PATHWAY database is a collection of diagrams drawn manually. They are called KEGG reference pathway diagrams (maps), each of which is related to a known network of functional importance. The KEGG site was used to gain access to the pathway database [16–19].

The DGIdb is a web resource that collects and presents information on gene-drug interactions from papers, databases, and other web-based sources [20]. It lacks information on animal medications and exclusively includes human medications. Nonetheless, this study could suggest suitable drug candidates for further progressive studies. The set of all involved genes was entered into the clusters in this database. All the predicted drugs for these genes

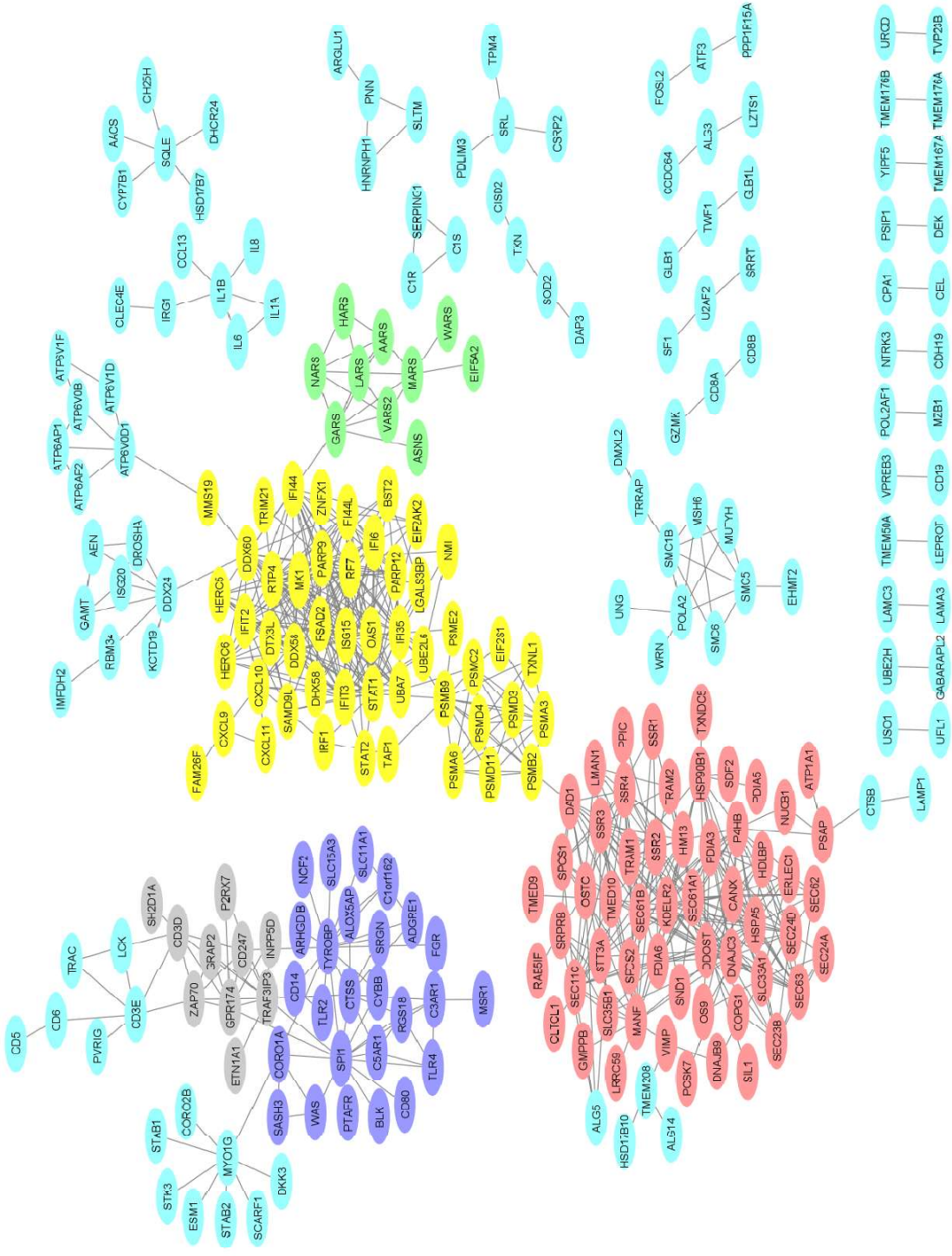

Figure 1.
Gene co-expression network for the primary gene list. In this network, there are 263 nodes (genes) and 698 edges (co-expression). Edges represent the interactions between nodes. Genes are shown in different colors according to their clusters. Cluster 1 is represented in pink, Cluster 2 in yellow, Cluster 3 in green, Cluster 4 in blue, and Cluster 5 in gray. Genes that are not assigned to any clusters are shown in light blue.

were extracted, and the medications were examined based on their effects on the involved genes and their participation in the main pathways.

GO and Pathway Enrichment Analysis:

Pathway analysis identified key pathways for each module based on p-value and gene participation:

- Module 1: Proteasome, Influenza A, Measles, Hepatitis C, and Herpes simplex infection.
- Module 2: Protein processing in the endoplasmic reticulum (26/56 genes, p-value 1.81e-34) and protein export.
- Module 3: Aminoacyl-tRNA biosynthesis.
- Module 4: Phagosome and Tuberculosis.
- Module 5: T-cell receptor signaling pathway.

Complete pathway details and participating genes are in the supplementary files.

Functional Interpretation of GO Co-expression Networks in FIP:

GO analysis using DAVID categorized information into BP, CC, and MFs for all five clusters reported with p-values < 0.

Key BPs included:

- Module 1: Response to virus, defense response, and proteolysis.
- Module 2: Intracellular protein transport and protein targeting to the membrane.
- Module 3: tRNA aminoacylation and amino acid activation.
- Module 4: Regulation of cytokine production and immune response.
- Module 5: Positive regulation of leukocyte-mediated immunity.

Detailed BP, CC, and MF data are provided in the supplementary files to manage complexity and maintain study focus.

This study employed a bioinformatics approach to unravel the genes and pathways involved in FIP, proposing repurposable drugs. Expression-modified genes from healthy and FIP-infected groups were sourced from NCBI [11]. A gene co-expression network was constructed and analyzed, revealing five gene clusters. Drugs targeting these genes were identified from the DGIdb human drug database.

Proposed Candidate Drugs

Genes from identified modules underwent analysis using the DGIdb database to pinpoint potential drugs visualized via Cytoscape (Figure 2). Notably, BORTEZOMIB and CARFILZOMIB (linked to nine genes), OPROZOMIB and IXAZOMIB CITRATE (eight genes), and MARIZOMIB (four genes) emerged as proteasome inhibitors [21–25]. Other medications with significant genetic associations included BCG VACCINE, IC14, NELFINAVIR, and RITONAVIR. Comprehensive drug predictions are detailed in the supplementary files.

The major pathways obtained using bioinformatics databases involved proteasome, protein processing in the endoplasmic reticulum, protein export, aminoacyl-tRNA biosynthesis, phagosome, tuberculosis, and the T-cell receptor signaling pathway. The proteasome pathway, crucial for cellular regulation and quality control (p-value: 4.76E-12), emerged prominently.

Proteasome inhibitors, such as BORTEZOMIB [23], CARFILZOMIB [22], OPROZOMIB [22], IXAZOMIB CITRATE [24], and MARIZOMIB [25], originally used in cancer therapy [26], were highlighted as potential treatments for FIP. Their role in regulating key cellular proteins via the ubiquitin-proteasome system suggests antiviral potential, crucial for various stages of coronavirus infection and potentially reducing antiviral resistance [27, 28]. The study recommends these drugs as robust therapeutic options for FIP.

The tuberculosis pathway (*p*-value: 5.86E-04) shares similarities with FIP, involving expression-modified genes CD14, TLR4, and TLR2 [11, 29, 30], also in the Toll-like receptor signaling pathway (p-value: 0.001873) [31]. One of the drugs targeting the CD14 gene is IC14 [32], which can serve as a potential FIP treatment by inhibiting its activity, as seen in studies on SARS-CoV-2 [33].

In addition to the tuberculosis pathway, the Toll-like receptor signaling and phagosome pathways (p-value: 3.92E-07) also share common genes with tuberculosis (i.e., LTR4). The drugs suggested on the tuberculosis pathway can be effective on the two mentioned pathways.

NELFINAVIR, a protease inhibitor, showed to be promising in reducing HIV viral load and increasing CD4+ cells [34], demonstrating antiviral effects on FIP in vitro [1] and potential with Galanthus nivalis agglutinin for FIP [35] and SARS-CoV-2 inhibition [36].

RITONAVIR, another HIV protease inhibitor [37], was tested with LOPINAVIR for SARS-CoV-2 but did not reduce mortality [38]. GC376 [39] and GS-441524 [40] showed promise in FIP treatment, though concerns about resistance and tissue distribution remain. Effective treatments for FIP are crucial due to the toxicity of FIPV inhibitory doses and the absence of approved therapies [41, 42].

REMDESIVIR)GS-5734(, a nucleoside analog with broad antiviral activity, including FIPV [42], MERS CoV, and SARS CoV [43], has FDA approval for SARS-CoV-2 treatment [44]. XRAPHCONN®, containing GS-441524, shows promise in FIP treat-

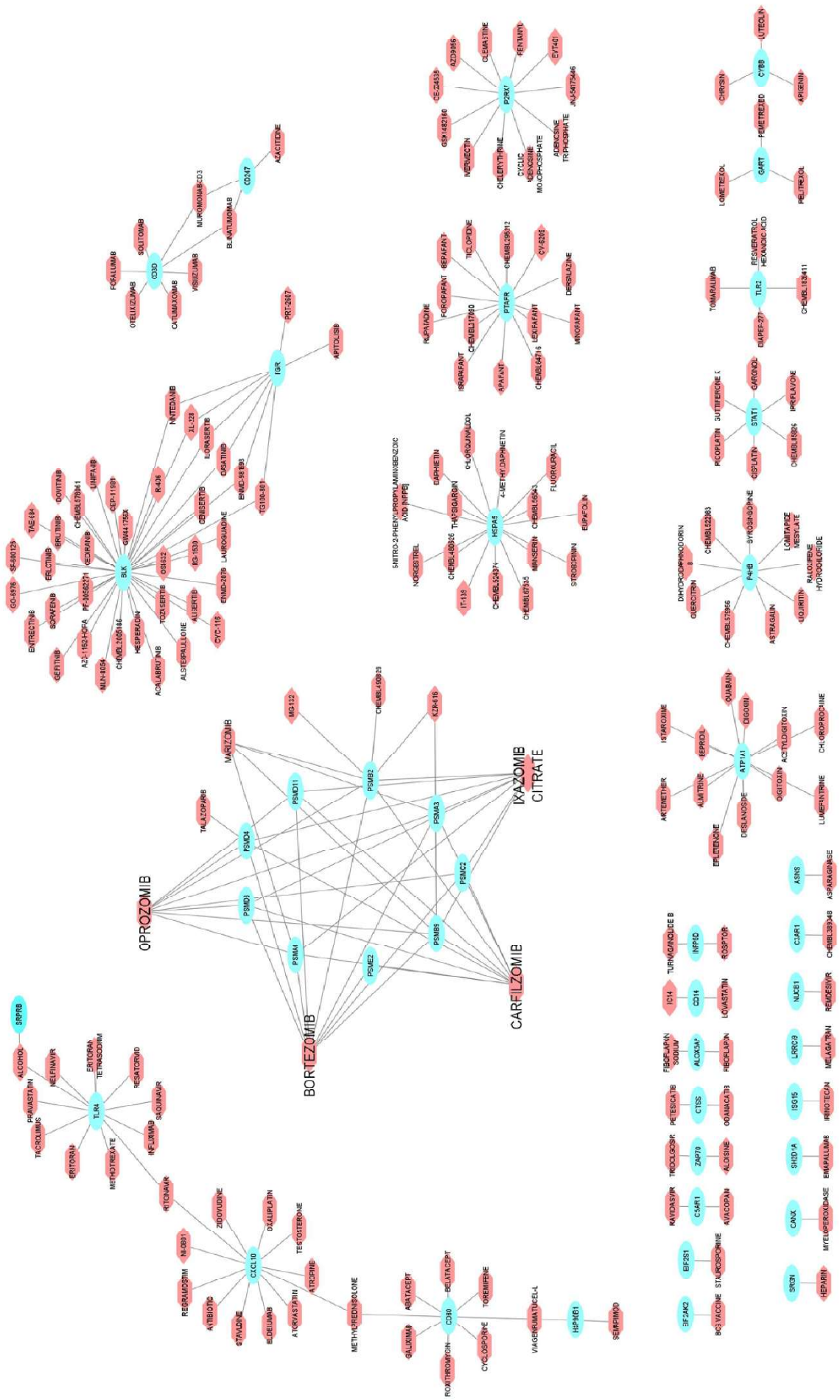

Figure 2.
Drug-Gene interaction network. Circle and hexagon nodes indicate genes and drugs, respectively.

ment but lacks widespread approval despite being easier to synthesize than REMDESIVIR [5, 45].

The development of medicines for treating FIP and other animal coronaviruses can prevent animal deaths and future virus spread. However, this study's computational approach and limited experimental resources could not validate the drugs or confirm the analyzed pathways in vitro and in vivo. Experimental validation and clinical trials are necessary to ensure

Authors' Contributions

M.A.A and H.M.G designed the study. M.A.A, J.SH and H.M.G performed the analyses. M.A.A and H.M.G reconstructed the networks. J.SH, A.R and H.M.G interpreted the results. M.A.A and H.M.G. wrote draft of the manuscript. All authors reviewed the manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

Reference

1. Olsen M, Cook SE, Huang V, Pedersen N, Murphy BG. Perspectives: potential therapeutic options for SARS-CoV-2 patients based on feline infectious peritonitis strategies: central nervous system invasion and drug coverage. Int J Antimicrob Agents. 2020 Jun;55(6):105964. Doi: 10.1016/j.ijantimicag.2020.105964.

2. Lin L, Yao D, Wu L, Fan F, Liu Y, Zhoul Z. Molecular epidemiology of type I and II feline coronavirus from cats with suspected feline infectious peritonitis in China between 2019 and 2021. Archives of Virology 2022; 167: 189–194.Doi: 10.3201/eid1807.120143.

3. Pedersen NC. An update on feline infectious peritonitis: Virology and immunopathogenesis. Veterinary Journal. 2014; 201(2): 123–132. Doi: 10.1016/j.tvjl.2014.04.017.

4. de Groot RJ, Baker SC, Baric R, et al. Coronaviridae - Positive Sense RNA Viruses - Positive Sense RNA Viruses (2011) - ICTV. Comité Internacional de Taxonomía de Virus (ICTV), https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae (2020, accessed 4 December 2021).

5. AAddie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, et al. Feline infectious peritonitis. ABCD guidelines on prevention and management. J Feline Med Surg. 2009 ;11(7):594-604. doi: 10.1016/j.jfms.2009.05.008. 6.

6. Tuanthap S, Chiteafea N, Rattanasrisomporn J, Choowongkomon K. Comparative sequence analysis of the accessory and nucleocapsid genes of feline coronavirus strains isolated from cats diagnosed with effusive feline infectious peritonitis. Arch Virol. 2021;166(10):2779-2787. Doi: 10.1007/s00705-021-05188-7

7. elten S, Hartmann K. Diagnosis of Feline Infectious Peritonitis: A Review of the Current Literature. Viruses. 2019 Nov 15;11(11):1068. Doi: 10.3390/v11111068.

8. Takano T, Hohdatsu T, Hashida Y, Kaneko Y, Tanabe M, Koyama H. A "possible" involvement of TNF-alpha in apoptosis induction in peripheral blood lymphocytes of cats with feline infectious peritonitis. Vet Microbiol. 2007 Jan 31;119(2-4):121-31. Doi: 10.1016/j.vetmic.2006.08.033.

9. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 2022;50(W1):W216-W221. Doi: 10.1093/nar/gkac194.

10. Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Statistical Applications in Genetics and Molecular Biology; 4. Epub ahead of print 2005. Doi: 10.2202/1544-6115.1128.

11. Malbon AJ, Russo G, Burgener C, Barker EN, Meli ML, Tasker S, Kipar A. The Effect of Natural Feline Coronavirus Infection on the Host Immune Response: A Whole-Transcriptome Analysis of the Mesenteric Lymph Nodes in Cats with and without Feline Infectious Peritonitis. Pathogens. 2020 Jun 29;9(7):524. Doi: 10.3390/pathogens9070524.

12. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49(D1):D605-D612. Doi: 10.1093/nar/gkaa1074. Erratum in: Nucleic Acids Res. 2021;49(18):10800. Doi: 10.1093/nar/gkab835.

13. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov;13(11):2498-504. Doi: 10.1101/gr.1239303.

14. Wang J, Zhong J, Chen G, Li M, Wu FX, Pan Y. ClusterViz: A Cytoscape APP for Cluster Analysis of Biological Network. IEEE/ACM Trans Comput Biol Bioinform. 2015 ;12(4):815-22. Doi: 10.1109/TCBB.2014.2361348.

16. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000 ;25(1):25-9. Doi: 10.1038/75556

17. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019;28(11):1947-1951. Doi: 10.1002/pro.3715.

18. Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 2021;49(D1):D545-D551. Doi: 10.1093/nar/gkaa970.

19. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research 2000; 28: 27–30.

20. Freshour SL, Kiwala S, Cotto KC, Coffman AC, McMichael JF, Song JJ, et al. Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsource efforts. Nucleic Acids Res. 2021;49(D1):D1144-D1151. Doi: 10.1093/nar/gkaa1084.

21. Hari P, Matous JV, Voorhees PM, Shain KH, Obreja M, Frye J, et al. Oprozomib in patients with newly diagnosed multiple myeloma. Blood Cancer J. 2019;9(9):66. Doi: 10.1038/s41408-019-0232-6.

22. Kortuem KM, Stewart AK. Carfilzomib. Blood. 2013;121(6):893-7. Doi: 10.1182/blood-2012-10-459883.

23. Mujtaba T, Dou QP. Advances in the understanding of mechanisms and therapeutic use of bortezomib. Discov Med. 2011 ;12(67):471-80.

24. Offidani M, Corvatta L, Caraffa P, Gentili S, Maracci L, Leoni P. An evidence-based review of ixazomib citrate and its potential in the treatment of newly diagnosed multiple myeloma. Onco Targets Ther. 2014;7:1793-800. Doi: 10.2147/OTT.S49187.

25. Potts BC, Albitar MX, Anderson KC, Baritaki S, Berkers C, Bonavida B, et al. Marizomib, a proteasome inhibitor for all seasons: preclinical profile and a framework for clinical trials. Curr Cancer Drug Targets. 2011;11(3):254-84. Doi: 10.2174/156800911794519716.

26. Adams J. The development of proteasome inhibitors as anticancer drugs. Cancer Cell. 2004;5(5):417-21. oi: 10.1016/s1535-6108(04)00120-5.

27. Schneider SM, Lee BH, Nicola AV. Viral entry and the ubiquitin-proteasome system. Cell Microbiol. 2021;23(2):e13276. Doi: 10.1111/cmi.13276.

28 Raaben M, Posthuma CC, Verheije MH, te Lintelo EG, Kikkert M, Drijfhout JW, et al. The ubiquitin-proteasome system plays an important role during various stages of the coronavirus infection cycle. J Virol. 2010 ;84(15):7869-79. Doi: 10.1128/JVI.00485-10.

29. Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA, et al. MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and Mycobacterium tuberculosis. PLoS Pathog. 2009;5(6):e1000474. Doi: 10.1371/journal.ppat.1000474.

30. Cubillos-Angulo JM, Fernandes CD, Araújo DN, Carmo CA, Arriaga MB, Andrade BB. The influence of single nucleotide polymorphisms of NOD2 or CD14 on the risk of Mycobacterium tuberculosis diseases: a systematic review. Syst Rev. 2021;10(1):174. Doi: 10.1186/s13643-021-01729-y.

31. Won Y, Yang JI, Park S, Chun JS. Lipopolysaccharide Binding Protein and CD14, Cofactors of Toll-like Receptors, Are Essential for Low-Grade Inflammation-Induced Exacerbation of Cartilage Damage in Mouse Models of Posttraumatic Osteoarthritis. Arthritis Rheumatol. 2021;73(8):1451-1460. Doi: 10.1002/art.41679.

32 Henderson RD, Agosti JM, McCombe PA, Thorpe K, Heggie S, Heshmat S, et al. Phase 1b dose-escalation, safety, and pharmacokinetic study of IC14, a monoclonal antibody against CD14, for the treatment of amyotrophic lateral sclerosis. Medicine (Baltimore). 2021;100(42):e27421. Doi: 10.1097/MD.0000000000027421.

33. Messner CB, Demichev V, Wendisch D, Michalick L, White M, Freiwald A, Tet al.Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. Cell Syst. 2020 ;11(1):11-24.e4. Doi: 10.1016/j.cels.2020.05.012.

34. Bardsley-Elliot A, Plosker GL. Nelfinavir: an update on its use in HIV infection. Drugs. 2000;59(3):581-620. Doi: 10.2165/00003495-200059030-00014.

35. Hsieh LE, Lin CN, Su BL, Jan TR, Chen CM, Wang CH, et al. Synergistic antiviral effect of Galanthus nivalis agglutinin and nelfinavir against feline coronavirus. Antiviral Res. 2010 ;88(1):25-30. Doi: 10.1016/j.antiviral.2010.06.010.

36. Ohashi H, Watashi K, Saso W, Shionoya K, Iwanami S, Hirokawa T, et al. Potential anti-COVID-19 agents, cepharanthine and nelfinavir, and their usage for combination treatment. iScience. 2021;24(4):102367. Doi: 10.1016/j.isci.2021.102367.

37. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet. 1998 Oct;35(4):275-91. Doi: 10.2165/00003088-199835040-00002. Erratum in: Clin Pharmacokinet 1998;35(6):473.

38. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020;382(19):1787-1799. Doi: 10.1056/NEJMoa2001282.

39. Pedersen NC, Kim Y, Liu H, Galasiti Kankanamalage AC, Eckstrand C, Groutas WC, et al. Efficacy of a 3C-like protease inhibitor in treating various forms of acquired feline infectious peritonitis. J Feline Med Surg. 2018;20(4):378-392. Doi: 10.1177/1098612X17729626.

40. Murphy BG, Perron M, Murakami E, Bauer K, Park Y, Eckstrand C, et al. The nucleoside analog GS-441524 strongly inhibits feline infectious peritonitis (FIP) virus in tissue culture and experimental cat infection studies. Vet Microbiol. 2018;219:226-233. Doi: 10.1016/j.vetmic.2018.04.026.

41. Richman DD. Antiviral drug resistance. Antiviral Res. 2006 ;71(2-3):117-21. Doi: 10.1016/j.antiviral.2006.03.004.

42. Delaplace M, Huet H, Gambino A, Le Poder S. Feline Coronavirus Antivirals: A Review. Pathogens. 2021;10(9):1150.

Doi: 10.3390/pathogens10091150.

43. McFee RB. COVID-19: Therapeutics and interventions currently under consideration. Dis Mon. 2020;66(9):101058. Doi: 10.1016/j.disamonth.2020.101058.

44. Amirian ES, Levy JK. Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. One Health 2020; 9: 100128. Doi:10.1016/j.onehlt.2020.100128.

45. Yan VC, Muller FL. Advantages of the Parent Nucleoside GS-441524 over Remdesivir for Covid-19 Treatment. ACS Med Chem Lett. 2020;11(7):1361-1366. Doi: 10.1021/acsmedchemlett.0c00316.

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Online supplemental material

Supplementary Excel file. The complete information of all modules separately for BP, CC, MF, and pathways, as well as gene-drug network data, are provided in this supplementary Excel file.

Visual abstract. Visual abstract of the research process

Table 1.
The details of the gene co-expression modules.

module no.	NO.	List of genes
co-expression-module-1	49	EIF2AK2, PSMD11, NMI, TXNL1, PSMB2, PSME2, PSMA6, PSMD3, PSMD4, FAM26F, CXCL11, STAT1, IFIT3, IFIT2, IRF1, UBA7, CXCL9, TRIM21, RSAD2, DHX58, CXCL10, TAP1, SAMD9L, PARP12, ISG15, STAT2, MX1, IRF7, IFI35, RTP4, LGALS3BP, OAS1, HERC6, BST2, IFI44L, HERC5, IFI44, MMS19, DTX3L, PARP9, DDX58, ZNFX1, DDX60, PSMA3, UBE2L6, PSMC2, EIF2S1, IFI6, PSMB9
co-expression-module-2	56	SRPRB, TMED10, LRRC59, TRAM2, SSR3, P4HB, MANF, KDELR2, SPCS1, SEC62, SSR2, PPIC, SLC33A1, PCSK7, SSR4, DNAJC3, OS9, DAD1, HSP90B1, ERLEC1, SLC35B1, SEC61B, SDF2, HSPA5, TXNDC5, VIMP, HDLBP, DNAJB9, SEC61A1, SEC24D, PDIA5, PDIA3, SEC63, DDOST, CANX, LMAN1, TRAM1, SND1, SSR1, HM13, SIL1, SEC24A, SEC23B, COPG1, NUCB1, STT3A, CLTCL1, GMPPB, RAB5IF, PSAP, ATP1A1, PDIA6, TMED9, SPCS2, OSTC, SEC11C
co-expression-module-3	10	ASNS, MARS, HARS, AARS, WARS, EIF5A2, VARS2, GARS, NARS, LARS
co-expression-module-4	26	WAS, SASH3, TLR2, CD14, CORO1A, NCF2, TYROBP, SPI1, BLK, CTSS, SLC11A1, ADGRE1, CYBB, SLC15A3, CD80, PTAFR, FGR, C5AR1, ARHGDIB, TLR4, SRGN, MSR1, RGS18, C1orf162, ALOX5AP, C3AR1
co-expression-module-5	10	CD247, TRAF3IP3, BTN1A1, GRAP2, SH2D1A, P2RX7, GPR174, INPP5D, CD3D, ZAP70

Table 2.
The Significant Biological Pathways for all Five Clusters.

Cluster no.	PATHWAY	*p*-Value
co-expression-module-1	Proteasome	4.76E-12
co-expression-module-2	Influenza A	1.06E-08
co-expression-module-3	Measles	7.51E-07
co-expression-module-4	Hepatitis C	8.76E-07
co-expression-module-5	Herpes simplex infection	6.34E-06

Supplemental material Cont.

Table 3.
The candidate drugs.

Drug	NO.	Gene
BORTEZOMIB	9	PSMD11, PSMB2, PSME2, PSMA6, PSMD3, PSMD4, PSMA3, PSMC2,PSMB9
CARFILZOMIB	9	PSMD11, PSMB2, PSME2, PSMA6, PSMD3, PSMD4, PSMA3, PSMC2,PSMB9
OPROZOMIB	8	PSMD11, PSMB2, PSMA6, PSMD3, PSMD4, PSMA3, PSMC2, PSMB9
IXAZOMIB CITRATE	8	PSMD11, PSMB2, PSMA6, PSMD3, PSMD4, PSMA3, PSMC2, PSMB9
MARIZOMIB	4	PSMB2, PSMA6, PSMA3, PSMB9
RITONAVIR	2	CXCL10, TLR4
NELFINAVIR	1	TLR4
IC14	1	CD14
BCG VACCINE	1	EIF2AK2

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Abstract in Persian

باکتری های منتقله از غذا در ایران: بررسی سیستماتیک ۲۳ ساله غذاهای پرخطر

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چکیده

بیماری های منتقله از غذا به دلیل شیوع بالا، میزان مرگ و میر و زیان های اقتصادی، یک نگرانی مهم بهداشت عمومی جهانی هستند. این مطالعه با هدف بررسی سیستماتیک عوامل بیماری زا و شیوع مواد غذایی شناسایی شده در ایران طی ۲۳ سال گذشته، با هدف ارائه مروری بر ارزیابی ریسک و رویکردهای پیشگیرانه در سراسر کشور انجام شد. با استفاده از کلیدواژه‌های مناسب و جستجو در پایگاه‌های اطلاعاتی اصلی مانند Google Scholar، PubMed، Scopus، ScienceDirect و پایگاه دانش علمی ایرانیان، در ابتدا ۴۷۴۰ مقاله یافتیم. در نهایت ۳۲۸ مقاله برای ارزیابی درج شد. در بین این مقالات، انتشارات مربوط به سالمونلا، استافیلوکوکوس اورئوس و لیستریا بیشترین تعداد را داشتند. گوشت طیور به‌عنوان منبع اصلی پاتوژن‌های اصلی غذا در ایران از جمله سالمونلا (۲۴/۸۳ درصد)، لیستریا مونوسیتوژنز (۳۰/۷۷ درصد)، انتروکولیتیکا (۱۸/۱۳ درصد) و کمپیلوباکتر (۳۶/۶ درصد) ظاهر شد. با توجه به شیوع بالای باکتری های منتقله از غذا در غذاهای ایرانی، ارائه اقدامات کنترلی موثر برای کاهش خطر و بار بیماری های ناشی از غذا ضروری است. به طور خاص، گوشت طیور که خطر بالایی برای بروز بیماری‌های منتقله از طریق غذا در ایران دارد، باید در معرض ارزیابی بیشتر خطرات و اجرای اقدامات کنترلی در کل زنجیره غذایی قرار گیرد.

واژگان کلیدی

باکتری های منتقله از غذا، سالمونلا، استافیلوکوکوس اورئوس، لیستریا، غذا، شیوع

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اثرات سیتوتوکسیک نانوذرات دی اکسید تیتانیوم بر رده سلولی سرطانی MCF-7

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چکیده

سرطان یکی از شایع ترین بیماری ها در سراسر جهان بوده و بسیاری از افراد از انواع مختلف سرطان رنج می برند. امروزه نانوذرات TiO_2کاربردهای درمانی گسترده ای دارند. مطالعه حاضر به منظور بررسی سمیت سلولی TiO_2 بر رده سلولی سرطان پستان انجام شد. سلول های سرطانی MCF-7 و رده سلولی فیبروبلاست HFF کشت داده شدند و پس از تأثیر غلظت‌های ۲۰۰، ۱۰۰، ۵۰، ۲۵ میکروگرم بر میلی‌لیتر نانوذرات دی اکسید تیتانیوم، میزان بقای سلول‌ها با استفاده از روش HFF طی ۴۸ ساعت و ۷۲ ساعت اندازه‌گیری و IC50 تعیین شد. تیمار سلول‌های MCF-7 و HFF با غلظت‌های مختلف TiO_2 نشان داد که نانوذرات دی اکسید تیتانیوم در غلظت‌های ۲۰۰ میکروگرم بر میلی‌لیتر بیشترین سمیت سلولی را نشان می‌دهند. نتایج فلوسایتومتری نیز آپوپتوز را در سلول های MCF-7 و HFF تایید کرد. نتایج میکروسکوپ نوری نشان داد که نانوذرات دی اکسید تیتانیوم می توانند در دوز ۲۰۰ میکروگرم بر میلی لیتر در یک دوره درمان ۴۸ و ۷۲ ساعت در رده های سلولی MCF-7 و HFFباعث ایجاد سمیت وابسته به غلظت شوند. نتایج تصویربرداری میکروسکوپ الکترونی از سلول‌های سالم و سرطانی پستان تیمار شده با غلظت ۲۰۰ میکروگرم بر میلی‌لیتر نانوذرات دی اکسید تیتانیوم در مدت ۷۲ ساعت نشان دهنده پارگی غشای میتوکندری و نشت ماتریکس به سیتوپلاسم و تورم شبکه آندوپلاسمی خشن است. با توجه به نتایج به‌دست‌آمده، نانوذرات TiO_2 را می‌توان به عنوان کاندیدای دارویی آینده‌نگر برای اهداف دارویی توصیه کرد، اگرچه مطالعات بیشتری در این زمینه مورد نیاز است.

واژگان کلیدی

MTT، TEM سرطان پستان، فلوسیتومتری، سنجش میکرونوکلئوس، سنجش

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اثرات ضد دیابتی و حفاظتی عصاره اتانولی آنغوزه بر بیضه موش های صحرایی دیابتی شده با استرپتوزوتوسین: یک مطالعه هیستوپاتولوژیکی.

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چکیده

دیابت یکی از شایع ترین بیماری های متابولیک در سراسر جهان است که همه اندام ها از جمله دستگاه تناسلی را درگیر می کند. امروزه بسیاری از محققین از گیاهان دارویی به جای داروهای شیمیایی برای کاهش عوارض آنها استفاده می کنند. آنغوزه یکی از گیاهان دارویی است که سال هاست برای درمان بسیاری از بیماری ها به طور سنتی مورد استفاده قرار می گیرد. مطالعه حاضر به بررسی اثرات ضد دیابتی و محافظتی آنغوزه بر بیضه موش های صحرایی نر دیابتی شده با استرپتوزوتوسین پرداخته است. بررسی هیستومورفولوژی موش‌های دیابتی تیمار شده با عصاره آنغوزه بهبود قابل توجهی در بیضه‌ها نشان می‌دهد. مطالعات بافت شناسی نشان می دهد که درمان با عصاره آنغوزه به طور قابل توجهی تعداد اسپرم را در لوله های اسپرم ساز افزایش می دهد و فیبروز را کاهش می دهد. مطالعه ما اثرات بهبود بخش آنغوزه بر پارامترهای هیستومورفومتریک و بیوشیمیایی در دیابت و آسیب بیضه ناشی از آن را تایید می کند که تا حدی به وجود ترکیبات فعال زیستی و آنتی اکسیدان ها در آنغوزه نسبت داده می شود.

واژگان کلیدی

آنغوزه، موش صحرایی، دیابت، سیستم تولید مثلی، اسپرماتوژنز، گیاهان دارویی

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اثرات پیشگیرانه سیلیمارین بر مسمومیت ناشی از دیکلوفناک در کبوتر اهلی

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چکیده

این مطالعه به بررسی اثرات سیلیمارین بر سمیت حاد کبد و کلیه ناشی از دیکلوفناک در کبوترهای اهلی (Columba livia) پرداخت. استفاده از داروهای ضدالتهابی غیراستروئیدی (NSAID) منجر به عوارض جانبی دارویی مانند خونریزی های قلبی عروقی و گوارشی و همچنین عوارض کلیوی می شود. طیف وسیعی از خواص دارویی سیلیمارین حجم قابل توجهی از تحقیقات با هدف درک اثربخشی آن در درمان فرآیندهای مختلف بیماری را توضیح می دهد. ۱۵ کبوتر به طور تصادفی در ۳ گروه (۱، ۲ و ۳) قرار گرفتند. کبوترهای گروه ۱ به عنوان گروه کنترل منفی عمل کردند و به آنها آب لوله کشی به عنوان دارونما داده شد. گروه های ۲ و ۳ در ابتدای آزمایش (ساعت صفر) تا ۲۴ ساعت دیکلوفناک ۱۵ میلی گرم بر کیلوگرم PO q12h تجویز کردند. کبوترهای گروه ۳ با سیلیمارین ۳۵ میلی گرم بر کیلوگرم، از ۱۲ ساعت پس از قرار گرفتن در معرض دیکلوفناک، با سیلیمارین به مدت ۲ روز تحت درمان قرار گرفتند. خون از کبوترها در ساعت های ۰، ۱۲، ۲۴ و ۴۸ آزمایش برای آنالیزهای بیوشیمی سرم جمع آوری شد. نتایج نشان داد که تیمار کبوتر با سیلیمارین باعث کاهش سطح سرمی AST، ALT، اسید اوریک و اوره و افزایش نسبتاً آلبومین و پروتئین کل شد. مشاهدات بالینی نشان دهنده وجود علائم مسمومیت بود که شامل از دست دادن اشتها، اسهال و بی حالی بود. این علائم در گروه سیلیمارین سریع‌تر بهبود یافت. می توان نتیجه گرفت که سیلیمارین آسیب حاد کبد و کلیه ناشی از دیکلوفناک را در کبوتر کاهش می‌دهد.

واژگان کلیدی

دیکلوفناک، کبوتر اهلی، بیوشیمی سرم، سیلیمارین

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اثر سرکۀ سیب و ویتامین روی پارامترهای هماتولوژیکی و ایمنوگلوبولین تام در گوسفند: مطالعه پایلوت

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چکیده

زمینه مطالعه: سرکۀ سیب یک ترکیب تخمیری است که دارای اسید استیک، فلاونوئیدها، ترکیبات فنولیک، اسیدهای ارگانیک، مواد معدنی و ویتامین هاست. ویتامین در تکامل سیستم ایمنی دخیل است و در پاسخ های ایمنی سلولی و روندهای ایمنی هومورال نقش دارد.ما این مطالعه را با هدف بررسی اثر سرکۀ سیب و ویتامین A روی پارامترهای هماتولوژیکی و ایمنوگلوبولین تام در بره-های قزل انجام دادیم. ۱۰ راس برۀ قزل مورد مطالعه قرار گرفتند. این بره ها به طور تصادفی به سه گروه تقسیم شدند (کنترل، گروه تحت تجویز سرکۀ سیب، و گروه تحت تجویز ویتامین A). پارامترهای هماتولوژیکی به وسیلۀ هماتولوژی روتین مشخص شدند. غلظت ایمنوگلوبولین G تام به وسیلۀ روش ایمنواسی توربی دی متریک اندازه گیری شد.گلوبول های سفید، لنفوسیت ها و ایمنوگلوبولین G به صورت معنی داری پس از تجویزز سیب سرکه افزایش یافتند ($p < 0.05$). ایمنوگلوبولین G و لنفوسیت ها در بره های تحت تجویز خوراکی سرکۀ سیب نسبت به گروه کنترل به صورت معنی-داری بالاتر بودند ($p < 0.05$). گلوبول های سفید، نوتروفیل ها و ایمنوگلوبولین G پس از تجویز ویتامین A به صورت معنی داری افزایش یافتند ($p < 0.05$). نوتروفیل ها و ایمنوگلوبولین G در بره های تحت تجویز ویتامین A نسبت به بره های گروه کنترل به صورت معنی داری بالاتر بودند ($p < 0.05$).تجویز ویتامین A و سرکۀ سیب در بره های ایمن است. آن ها همچنین سیستم ایمنی را تقویت می کنند.

واژگان کلیدی

ویتامین A ، ایمنوگلوبولین G، بره، پارامترهای هماتولوژیکی، سرکه سیب

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ارزیابی ایمنی متقاطع واکسن سونیکه تری والان کلی باسیلوز پرندگان برعلیه سویه O26 اشریشیا کلی بیماریزای پرندگان در جوجه های گوشتی

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چکیده

کلی باسیلوز در صنعت طیور جهان شیوع دارد. بین سویه های مختلف اشریشیا کلی بیماریزای پرندگان ایمنی متقاطع وجود ندارد. اگر واکسنی بتواند ایمنی متقاطع ایجاد کند، آن واکسن نقش کلیدی در پیشگیری از کلی باسیلوز خواهد داشت. در این مطالعه، واکسن سونیکه تری والان کلی باسیلوز که محتوی سه سروتیپ O1:K1، O2:K1، O78:K80 و آلوم بعنوان ادجوانت می باشد، برای ارزیابی ایمنی متقاطع علیه سویه O26 استفاده شد. نودو شش قطعه جوجه گوشتی بصورت تصادفی به چهار گروه تقسیم گردید. گروه A، گروه واکسینه که با O78 چالش داده شد. گروه B، گروه غیرواکسینه که با O78 چالش داده شد. گروه C، گروه واکسینه که با O26 چالش داده شد. گروه D، گروه غیرواکسینه که با O26 چالش داده شد. در ۱۴ روزگی جوجه های گروه A و C یک دز واکسن، و جوجه های گروه B و D نرمال سالین به روش زیرجلدی دریافت کردند. در ۳۵ روزگی همه گروهها چالش داده شدند. نشانه های بالینی و کالبدگشایی، جداسازی باکتری، وزن گیری، مصرف غذا، ضریب تبدیل غذایی، و تیتر علیه آنتی ژن های پیکری سه سویه موجود در واکسن، O78 و O26 تعیین و یاداشت شد. نتایج بعد از چالش نشان داد در میزان مصرف غذا و ضریب تبدیل غذایی بین گروه C و گروه D اختلاف معنی داری وجود ندارد ($p > 0.05$)، اما میزان رشد و تیتر آنتی بادی علیه آنتی ژن های پیکری در گروه C بطور معنی داری بالاتر از گروه D می باشد ($p < 0.05$). در سن ۴۹-۴۲ روزگی، میزان رشد، ضریب تبدیل غذایی، و تیتر علیه آنتی ژن های پیکری در گروه A در مقایسه با گروه B، بطور معنی داری بالاتر است ($p < 0.05$). به نظر می رسد واکسن مذکور ایمنی هومولوگ و هترولوگ به ترتیب علیه O78 و O26 ایجاد می کند.

واژگان کلیدی

کلی سپتی سمی، طیور، پیشگیری، هترولوگ، هومولوگ

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شناسایی و مطالعه ژن‌های تأثیرگذار در بیماری پریتونیت عفونی گربه و هدف‌گذاری مجدد داروها با رویکرد زیست سامانه‌ای

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۳ گروه زیست‌شناسی، واحد تبریز، دانشگاه آزاد اسلامی، تبریز، ایران.

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چکیده

پریتونیت عفونی گربه (FIP) یک بیماری عفونی سیستمیک گربه‌ها با منشأ ویروسی از خانواده کرونا ویروس است. عدم وجود علائم واضح ویروس قبل از شروع فرم بالینی بیماری و همچنین نبود تست‌های تشخیصی آسان و ارزان که بتواند وجود قطعی ویروس را تأیید کند از دیگر مشکلات کنترل و پیشگیری از انتشار ویروس است. از طرفی نیز ای بیماری هنوز پروتکل دارویی و درمانی تأیید شده‌ای نداشته است. در این مقاله در ابتدا شبکه هم بیانی ژن توسط پایگاه داده String و نرم‌افزار Cytoscape بازسازی و ماژول بندی شد. مسیرها و ژن آنتولوژی ماژول‌ها توسط پایگاه داده DAVID و KEGG به دست آمد. از مهم‌ترین مسیرهای احتمالی به‌دست‌آمده می‌توان به این مسیرها اشاره کرد:-Proteasome،Protein processing in endoplasmic retic ulum، Protein export، Aminoacyl-tRNA biosynthesis، Phagosome، Tuberculosis و T cell receptor signaling pathway. در بخش دیگر پژوهش از استراتژی بازسازی شبکه ژن-دارو به‌منظور شناسایی داروی بالقوه استفاده‌شده است که با بهره‌گیری از پایگاه داده DGIdb و با استفاده نرم‌افزار Cytoscape شبکه ژن-دارو ترسیم شد که داروهای BORTEZOMIB، CARFILZOMIB، OPROZOMIB، IXAZOMIB CITRATE، MARIZOMIB، BCG VACCINE، IC14، NELF-INAVIR، RITONAVIR و... از موارد پیشنهادی ما برای این بیماری معرفی‌شده است. اگرچه استراتژی محاسباتی ما داروهای کاندید قابل‌استفاده مجدد را در برابر بیماری FIP پیش‌بینی می‌کند. با این‌حال، کارآزمایی‌های تجربی دقیق‌تر و همچنین تجزیه و تحلیل بالینی عملکرد دارو، سمیت دارو، و اعتبارسنجی تجربی برای دستیابی به یک پروتکل درمانی دقیق و بهبودیافته ضروری است.

واژگان کلیدی

پریتونیت عفونی گربه، شبکه هم بیانی ژن، شبکه‌های زیستی، بیوانفورماتیک، خانواده کروناویروس

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Author Index

Author index

Guide for authors

SCOPE

Iranian journal of Veterinary Science and Technology (IJVST) publishes important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, large and small animal medicine, poultry diseases, diseases of equine species, and aquaculture. Articles can comprise research findings in basic sciences, as well as applied veterinary findings and experimental studies and their impact on diagnosis, treatment, and prevention of diseases. IJVST publishes four kinds of manuscripts: Research Article, Review Article, Short Communication, and Case Report.

Guide for Authors

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3. The submitted manuscript should be accompanied by a written statement signed by the corresponding author on behalf of all the authors that its publication has been approved by all co-authors, stating that the whole manuscript or a part of it has not been published.
4. Ethics: Authors must state that the protocol for the research project has been approved by the Ethics Committee of the institution within which the work was undertaken. Authors are responsible for animal welfare and all statements made in their work.

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PREPARATION OF MANUSCRIPT

Manuscripts should be written in English, with Abstract in both English and Persian (where applicable), typewritten in MS Word program, double-spaced, in 12-point "Times New Roman" font on A4 paper size. Authors are requested to reserve margins of 2.5 cm all around the pages. Manuscript should also have line numbers. All pages of the manuscripts should also be enumerated.

Research Articles should contain Title page, Abstract, Keywords, List of Abbreviations, Introduction, Results, Discussion, Materials and methods, References, and Figure legends. Tables and figures should be appended as individual files.

Review Articles should contain Title page, Abstract, Keywords, List of Abbreviations, Introduction, appropriate sections dependeing to the subject, Conclusions and future directions. Tables and figures should be appended as individual files. The review article should provide an update on recent advances in a particular field. Authors wishing to submit review articles should contact the Editor with an outline of the proposed paper prior to submission.

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FFull Title Page should include title (concise and informative), author(s) (including the complete name, department affiliation, and institution), running head (condensed title) (≤ 50 characters, including spaces), name and address of the authors to whom correspondence and reprint requests should be addressed, Acknowledgements, Author contributions, and Conflict of interest.

Guide for Authors

Acknowledgements: Personal acknowledgement, sources of financial support, contributions and helps of other researchers and everything that does not justify authorship should be mentioned in this section, if required.

Author contributions: Authors are required to include a statement to specify the contributions of each author. The statement describes the tasks of individual authors referred to by their initials. Listed below is an example of author contributions statement:

Conceived and designed the experiments: HD, SS. Performed the experiments: SS. Analyzed the data: HD, SS, MMM, ARB. Research space

and equipment: HD, MMM, ARB. Contributed reagents/materials/analysis tools: HD. wrote the paper: SS, HD.

Conflict of interest: All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no conflicts of interest then please state 'The authors declare that there is no conflict of interest'. This form can be downloaded from the IJVST website.

Abstract

Abstract (in English and Persian) no more than 250 words should contain the purpose of the study, findings and the conclusion made on the basis of the findings. Authors who are not native Persian speakers may submit their manuscript with an abstract in English only. Abbreviations and reference citations may not be used in the abstracts.

Keywords

Forindexing purposes, each submitted manuscript should include three to seven keywords, following the abstract and preferably chosen from the Medical Subject Headings (MESH). Keywords should express the precise content of the manuscript.

Introduction

Introduction should be as concise as possible, and clearly explain the main objective and hypothesis of the investigation.

Results

Results indicate the results of an original research in a clear and logical sequence. Do not repeat data that are already covered in tables and illustrations. In manuscripts describing more than one animal, all animals should be assigned a case number.

Discussion

Discussion should include the answer to the question proposed in the introduction and empha-

size the new and important aspects of the study and the conclusions that follow from them. It could include the implication, application, or speculation of the findings and their limitations, relate the observations to other relevant studies, and links the conclusions with the goals of the study. Recommendations, when appropriate, may be included.

Materials and methods

Materials and methods should be described in sufficient details to allow other researchers to reproduce the results. Specify any statistical computer programs used .The methods of data collection and use of statistical analysis will be checked by the referees and if necessary, a statistician. Drugs and therapeutic agents, reagents, softwares and equipments should be given in the format: name (trade name, manufacturer name, city, country), e.g. Statview 5 (SAS Institute, Inc., Cary, NC, USA).

Animals: All animal experiments should comply with the ARRIVE (https://arriveguidelines.org/) guidelines and the authors should clearly indicate in the manuscript the ethical code of the study.

Gene names: The standard gene names, as provided by HGNC (HUGO Gene Nomenclature Committee) should be used. Gene names must be italicized. If the case of mammalian species and if gene names refer to rodent species, they must be upper case; if they refer to non-rodent species they must be written in capitals. If they refer to other species, they must written lower case. Protein names are written in capitals and are not italicized. As an example:

Mouse beta actin gene: Actb

Bovine beta actin gene: ACTB

Chicken beta actin gene: actb

Beta actin protein: ACTB

Quantitative PCR: If the quantitative PCR method has been used, the related section in Materials and Methods and Results must be written following the reference:

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009 Apr;55(4):611-22.

Protocol for DNA/RNA extraction, including quantification and determination of purity.

Reverse transcription (if used): amount of RNA, concentration of all reagents: primers concentration (either random primers or oligonucleotides), reverse transcriptase and master mix components.

qPCR: sequence of forward and reverse primers, probes, amplicon size, accession number of Genebank; thermocycler parameters (i.e. denaturation, annealing and extension steps, number of cycles, melting curves); validation of PCR products; non-template controls for reverse transcription and

qPCR should be included in all reactions; and

Data analysis: details for the quantitative or relative analysis.

Use of antibodies: Authors must show that the antibodies are validated and their specificity sis confirmed.

References

Must be up-to-dated and limited to those that are necessary. Lists of references should be given in numerical order in the text, and in the reference list. Please use Vancouver style. To download the Vancouver Style follow the link in the IJVST website which could be used in the Endnote software.

Use of Italics

Gene symbols, Latin terms (i.e. in vivo, in vitro, ex vivo, in utero, in situ, and etc.) and species scientific names (using the binomial nomenclature), should be typed in italics, while the first letter of the genus name must be capitalized (i.e. Homo sapiens).

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diagrams

For the use of bar diagrams the following publication should be consulted:
Weissgerber TL, Milic NM, Winham SJ, Garovic VD. Beyond bar and line graphs: time for a new data presentation paradigm. PLoS Biol. 2015 Apr22;13(4):e1002128. The bar diagrams should be provided in color and in a well-designed and professional format. Please do not use different shades of gray. The axes of diagrams should have titles and units. Also, the source file of the image (Excel etc.) should

be provided for typesetting.Illustrations should be numbered as cited in the sequential order in the text, with a legend at the end of the manuscript. Color photographs are accepted at no extra charge. The editors and publisher reserve the right to reject illustrations or figures based upon poor quality of submitted materials.

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-Authors may be requested to provide the original data from their study for editorial review and should be ready to make the data publicly available if feasible.
-The corresponding author is responsible for ensuring that all co-authors have approved the manuscript prior to submission.
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-Authors are required to identify in their manuscript if their work involves chemicals, procedures, or equipment that have any inherent unusual hazards.
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- If the decision is 'Needs Revision,' authors are expected to respond systematically and promptly to the reviewers' comments, addressing point by point, and revising their manuscript accordingly. The revised manuscript should then be submitted to the journal within the given deadline.
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- Reviewers are expected to provide insightful comments that assist the editors in making a decision about whether or not to publish the submitted manuscript.

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- Reviewers are obligated to treat the manuscripts they receive for peer review as confidential and must not use any information obtained through this process for personal gain.

-Reviewers are expected to provide technical, professional, and objective comments on the manuscripts they are invited to review.

-Reviewers are expected to avoid personal biases in their comments and judgments, and express their views clearly with supporting arguments that assist the author in improving the manuscript.

-Reviewers are expected to identify any relevant published work that has not been cited by the authors. If a statement has been previously reported elsewhere, it should be accompanied by the appropriate citation.

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The process has been explained in the section "Peer Review Process".

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-The editors ought to uphold the anonymity of both reviewers and authors.

-The editors should disclose any potential conflicts of interest and make efforts to avoid them. If such circumstances arise, they are expected to delegate the handling of the manuscript to another member of the editorial board.

-The editors, particularly the Editor-in-Chief, should demonstrate a willingness to investigate cases of plagiarism and fraudulent data. When ethical concerns are raised about a submitted manuscript or published paper, the editors will take appropriate measures in response. Any reported incidents of unethical publishing behavior will be thoroughly examined, even if they come to light years after publication.

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-The editors must not share any information about submitted manuscripts with anyone until they are published, as appropriate.

-The Editor-in-Chief and members of the editorial board will not use unpublished materials disclosed in a submitted paper for their own research purposes without obtaining explicit written consent from the author.

-Editors are expected to give fair consideration to all manuscripts submitted for publication, eval-

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"Ferdowsi University of Mashhad press (FUM)" is promising to ensure that the decision on manuscript submissions is only made based on professional judgment and will not be affected by any commercial interests.

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Plagiarism:

Plagiarism is intentionally using someone else's ideas or other original material as if they are one's own. Copying even one sentence from someone else's manuscript, or even one of your own that has previously been published, without proper citation is considered by the JAM as plagiarism. All manuscripts under review or published with JAM are subject to screening using plagiarism prevention software (e.g. iThenticate). Thus, plagiarism is a serious violation of publication ethics.

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Duplicate Publication:

Duplicate publication occurs when two or more articles, without full cross referencing, share essentially the same hypotheses, data, discussion points, and conclusions.

Redundant Publications:

Redundant publications involve the inappropriate division of study outcomes into several articles,

most often consequent to the desire to plump academic vitae.

Data Fabrication:

Data fabrication means the researcher did not really carry out the study, but made up data or results and had recorded or reported the fabricated information. Data falsification means the researcher did the experiment, but manipulated, changed, or omitted data or results from the research findings.

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Citation Manipulation implies excessive citations in the submitted manuscript that do not contribute to the scholarly content of the article and have been included solely for the purpose of increasing citations to a given author's work, or to articles published in a particular journal. This leads to misrepresenting the importance of the specific work and journal in which it appears and is thus a form of scientific misconduct.

Improper Author Contribution or Attribution:

All listed authors must have made a significant scientific contribution to the research in the manuscript and approved all its claims. Do not forget to list everyone who made a significant scientific contribution, including students and laboratory technicians.

Handling Misconduct Cases

The Editorial board of IJVST takes the necessary measures to examine the incoming papers on their originality, reliability of contained information and correct use of citations.

-If any of the unethical publishing behavior is detected by the Journal Editorial board or by one of the reviewers, the first action is to inform the Editor-in-chief by supplying copies of the relevant material and a draft letter to the corresponding author asking for an explanation in a nonjudgmental manner.

- If the infraction is less severe, the Editor, upon the advice of the Committee on Publication Ethics, sends the author a letter of reprimand and reminds the JAM publication policies; if the manuscript has been published, the Editor may request the author to publish an apology in the journal to correct the record.

- If the author's explanation is unacceptable and it seems that serious unethical conduct has taken place, the matter is referred to the Publication Committee via Editorial board. After deliberation, the Committee will decide whether the case is sufficiently serious to warrant a ban on future submissions.

Post-Publication Discussions and Corrections

This journal allows debate post publication on journal's site, through "Send comment about this article" section to the editor up to one month before final publication. Our mechanisms for correcting, revising or retracting articles after publication depends on the content of the received comment and if the sent comments are useful and applicable for readers/authors, they will be showed under reference section of the articles pages.

PEER REVIEW PROCESS

Iranian Journal of Veterinary Science and Technology peer reviews all submitted manuscripts with contents within the scope of the journal.

Initial assessment

The submitted manuscript will be subjected to a primary review by the editor or a member of the editorial board for suitability and relevance of the findings to the scope of the journal and quality of the science presented in the paper (sufficient originality, having a message that is important to the general field of Veterinary Medicine, quality of data, novelty, English language, and overall manuscript quality) within two weeks. If the paper is evaluated to be relevant to the scope of the journal and having enough scientific rigor and novelty, it will be sent for the next stage. Otherwise, those manuscripts which are evaluated as not-appropriate in the initial review will be rejected at this stage.

Initial screen

The initial screen will be performed by the editorial office for the structure and format of the manuscript.

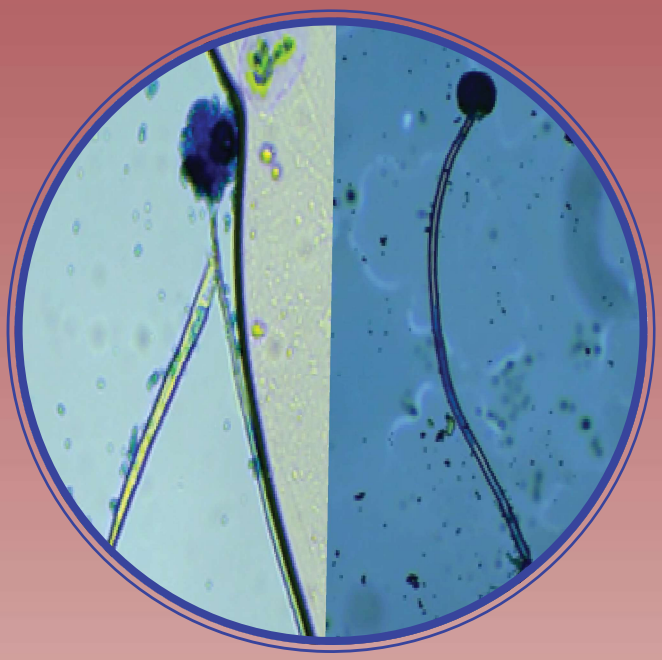
Peer review (double-blind)

The manuscripts which are found to be appropriate after the initial screen will be sent for external review by experts in the related field. We have prepared a checklist for reviewers that summarizes their evaluation of the manuscript. The items in this checklist are:

1. TITLE is clear and adequate
2. ABSTRACT clearly presents objects, methods, and results.
3. INTRODUCTION well-structured and provides a rationale for the experiments described.
4. MATERIALS AND METHODS are sufficiently explained and is detailed enough to be reproduced.
5. RESULTS are clearly presented and supported by figures and tables.
6. DISCUSSION properly interprets the results and places the results into a larger research context, and contains all important references.
7. Conclusions are logically derived from the data presented.
8. English Language/style/grammar is clear, correct, and unambiguous.
9. Figures and tables are of good quality and well-designed and clearly illustrate the results of the study.
10. References are appropriate.
11. Regarding this article are you concerned about any issues relating to author misconduct such as plagiarism and unethical behavior.
12. Comments on the importance of the article.

Final Decision

Based on the reviewers' recommendations a final decision is made by the editor and if needed the help of a member of the editorial board (depending on the field of study). Decisions will include accept, minor revision, major revision with and without re-review, and reject. We aim to reach a final decision on each manuscript as soon as their review results are available.



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