



Seroprevalence and Risk Factors for Infection with Bovine Respiratory Syncytial Virus, Bovine Parainfluenza Virus-3, and Bovine Adenovirus-3 in Dairy Cattle Farms of Fars Province, Southern Iran

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ABSTRACT

The seroprevalences of BRSV, BPIV-3, and BAV-3 were studied in the dairy cattle of Fars Province in the south of Iran and their associated risk factors were determined. Serum samples ($n = 420$) were collected from 36 cattle herds in the northern, central, and southern regions of the study area. Commercial enzyme-linked immunosorbent assay kits were used to detect antibodies against these viruses. The Chi-square test and logistic regression were used to identify potential risk factors. Antibodies were estimated 100% for all the studied viruses at the herd level and 76.43%, 76.90%, and 92.62% at the animal level for BRSV, BPIV-3, and BAV-3, respectively. In logistic regression analysis, age for all the viruses, season for BPIV-3 and BAV-3, and region and farming type for BAV-3 were significantly related to seroprevalence at the animal level. A significant association of dual infections with studied viruses was identified. The present study demonstrated that BRSV, BPIV-3, and BAV-3 are very prevalent in the dairy herds of southern Iran and highlighted the necessity to establish a control program.

Keywords

Seroprevalence, Bovine respiratory syncytial virus, Bovine parainfluenza virus-3, Bovine adenovirus-3

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Abbreviations

BRSV: Bovine respiratory syncytial virus
BPIV-3: Bovine parainfluenza virus-3
BAV-3: Bovine adenovirus-3
BRD: Bovine respiratory disease

CI: Confidence interval
OD: Optical density
PP: Percent positivity

Introduction

BRD, a global health problem causing severe economic losses due to body weight decline, yield loss, veterinary costs, medical fees, and animal mortality in cattle herds worldwide [1]. They may cause BRD alone or in mixed infections with other viruses or bacterial species, including *Mannheimia haemolytica*, *Mycoplasma bovis*, *Pasteurella multocida*, and *Histophilus somni*. They are the most well-known organisms that damage the respiratory tract of cattle and create opportunities for bacteria to colonize the lungs. Viruses are usually the first pathogens to intervene, while bacteria act as secondary invaders, aggravating the condition of formerly diseased animals [2].

BRSV is classified in the Pneumovirus genus and Pneumovirinae subfamily within the Paramyxoviridae family [3]. It is enveloped and contains a negative sense, single-stranded RNA genome encoding 11 proteins. Cattle are the natural hosts of BRSV, and a seroprevalence of 30%-70% has been detected. BRSV mostly affects young animals under one year old and calves often show severe clinical symptoms, such as fever, cough, loss of appetite, increased respiratory rate, and nasal discharge. BRSV infection is associated with high morbidity (60%-80%), and mortality can reach 20% in some outbreaks [4]. Previous studies identified environmental and climatic stressors, herd type, size and density, age group, purchasing new animals, and coinfection with bovine viral diarrhea virus as the main risk factors associated with BRSV infection [5-9].

BRSV and BPIV-3 are two closely related viruses often involved in BRD outbreaks [10]. BPIV-3 is a non-segmented, single-stranded, negative-sense, and enveloped RNA virus. It belongs to the Respirivirus genus of the subfamily Paramyxovirinae, family Paramyxoviridae, and spreads primarily by large droplet transmission [11].

Aerosols and fomites contaminated with nasal discharge can transmit BPIV-3 from animal to animal. The morbidity and mortality of BPIV-3 infections are low, and generally remain subclinical but may present with symptoms, such as reluctance to eat, cough, discharge from the nose, other respiratory signs, fever, lacrimation, and conjunctivitis [12, 13]. Several studies determined the seroprevalence of BPIV-3 infection in Isfahan (84.4%), Qazvin (95.2%), and Kerman (100%) Provinces of Iran and other countries (85.6% and 43% in Turkey and Mexico, respectively) which indicated broad virus dissemination [5, 6, 14-16].

BAV-3 belongs to the Mastadenovirus genus of the family Adenoviridae, which are non-enveloped double-stranded DNA viruses [17]. BAV-3 is considered one of the most important respiratory pathogens in cattle, especially newborn calves. Although BAV-3

infection usually occurs in a subclinical form, clinical symptoms, including fever, dyspnea, as well as nasal and conjunctival discharge have been described in severe infections, especially associated with immunosuppressive factors as crowding or co-infection with other viral or bacterial agents [18]. Data on the serological detection of BAV-3 are rare in Iran and limited to Isfahan and Kerman Provinces which reported 55.6% and 100% seroprevalence [14, 15].

Fars Province ranks first in the south of Iran in terms of cow population with 0.4 million cattle (mostly crossbreeds) and supplies the country with milk and meat. However, there is no official viral respiratory disease control program on dairy cattle farms, and limited knowledge is available on the prevalence of BRSV, BPIV-3, and BAV-3 infection in Fars Province. In order to set up a favored control program, it is important to know the prevalence and potential risk factors of infection. Therefore, the current research aimed to determine the prevalence of antibodies and risk factors of BRSV, BPIV-3, and BAV-3 infections in Fars Province, Iran.

Result

Only apparently healthy cattle were sampled, and finally, 432 blood samples were taken. The lack of kits caused 12 samples to be removed randomly. Finally, 140 specimens from each region (a total of 420 samples) were used in the test, which was considered to be approximately 10% higher than the calculated number. Seroprevalence at the herd level was estimated at 100% for all the studied viruses. Out of 420 animals, 321 (76.43%, 95% CI: 72.17%-80.24%), 323 (76.90%, 95% CI: 72.64%-80.68%), and 389 (92.62%, 95% CI: 89.71%-94.75%) were positive serologically for BRSV, BPIV-3, and BAV-3, respectively. Geographic region, gender, and age significantly affected ($p < 0.05$) BRSV serostatus, and then they were used in logistic regression analysis (Table 1). All the studied risk factors significantly affected the seroprevalence of BPIV-3 at the animal level. The variable "breeding type" was not included in the multivariable logistic regression analysis of BAV-3 due to the lack of statistically significant differences in the univariable model. In logistic regression analysis, age for all the viruses, season for BPIV-3 and BAV-3, and region and farming type for BAV-3 were significantly related to seroprevalence at the animal level (Table 2).

More than half of the sera (66.67%) had antibodies against all three viruses, and 6% of the sera were free of antibodies. The status of co-infection is shown in Table 3. A significant association of co-infections with BRSV and BPIV-3 ($\varphi = 0.494$, $p < 0.001$), BRSV and BAV-3 ($\varphi = 0.337$, $p < 0.001$), and BPIV-3 and

Table 1.
Univariate
seroposit

Virus
Factor
Season
warm
Cold
Geographic region
Central
Northern
Southern
Gender
Male
Female
Age
Calf
Adult
Farming type
Industrial
Traditional
Breeding type
Artificial
Mating
† Seroprevalence

able analysis of associated variables for Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza virus-3 (BPIV-3), and bovine adenovirus-3 (BAV-3) tivity in animal-level.

es	BRSV				BPIV- 3				BAV-3						
	Number		Serop† (%)	χ ^{2*}	P-value	Number		Serop† (%)	χ ^{2*}	P-value	Number		Serop† (%)	χ ^{2*}	P-value
	T	P			T	P					T	P			
				1.07	0.30				16.42	0.001				4.21	0.04
	210	165	78.60			210	179	85.2			210	200	95.2		
	210	156	74.30			210	144	68.6			210	189	90.0		
phic				29.50	0.001				9.06	0.011				23.89	0.001
	140	85	60.7			140	98	70.0			140	118	84.3		
rn	140	115	82.1			140	119	85.0			140	132	94.3		
rn	140	121	86.4			140	106	75.7			140	139	99.3		
				31.67	0.001				17.03	0.001				7.45	0.006
	38	15	39.5			38	19	50.0			38	31	81.6		
	382	306	80.1			382	304	79.6			382	358	93.7		
				118.25	0.000				33.14	0.000				44.88	0.000
	80	24	30.0			80	42	52.5			80	60	75.0		
	340	297	87.4			340	281	82.6			340	329	96.8		
g				1.92	0.17				4.38	0.04				9.72	0.002
ial	212	156	73.6			212	154	72.6			212	188	88.7		
onal	208	165	79.3			208	169	81.3			208	201	96.6		
g				1.08	0.298				8.69	0.003				2.65	0.103
al	219	189	86.3			219	191	87.2			219	210	95.9		
	113	102	90.3			113	84	74.3			113	112	99.1		

valence *Chi-square T: Tested P: Positive

Table 2. Logistic regression analysis of associated factors for Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza virus-3 (BPIV-3), and bovine adenovirus-3 (BAV-3) seropositivity in animal-level.

Viruses	BRSV				BPIV-3			BAV-3						
	Factor	Class	OR†	95% CI††	P-value	OR	95% CI†	P-value	OR	95% CI†	P-value			
season		warm				3.15	1.87	5.31	0.000	2.54	1.06	6.07	0.036	
		Cold				1			1					
Region		Central	1.014	0.50	2.07	0.21	1			1				
		Northern	0.633	0.31	1.29	0.97	1.65	0.82	3.31	0.164	2.84	1.05	7.66	0.039
		Southern	1				0.63	0.33	1.21	0.169	13.27	1.64	107.48	0.015
Sex		Male	1.01	0.40	2.59	0.98	1			1				
		Female	1				1.72	0.73	4.07	0.219	0.582	0.19	1.82	0.352
Age		Calf	1				1			1				
		Adult	13.44	6.89	26.23	0.000	4.39	2.28	8.43	0.000	6.07	2.43	15.15	0.000
Farming type		Industrial				1				1				
		Traditional					1.61	0.98	2.65	0.062	3.08	1.23	7.72	0.016
Breeding type		Artificial				1								
		Mating					1.86	0.61	5.66	0.273				

† Confidence interval, ††Odds ratio

Table 3.

The rates of dual infections of Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza virus-3 (BPIV-3), and bovine adenovirus-3 (BAV-3)

BRSV and BPIV-3		BRSV and BAV-3		BPIV-3 and BAV-3	
BRSV(+)&BPIV-3(+)	67.62%	BRSV(+)&BAV-3(+)	74.52%	BPIV-3(+)&BAV-3(+)	75.48%
BRSV(+)&BPIV-3(-)	8.81%	BRSV(+)&BAV-3(-)	1.90%	BPIV-3(+)&BAV-3(-)	1.43%
BRSV(-)&BPIV-3(+)	9.29%	BRSV(-)&BAV-3(+)	18.10%	BPIV-3(-)&BAV-3(+)	17.14%
BRSV(-)&BPIV-3(-)	14.29%	BRSV(-)&BAV-3(-)	5.48%	BPIV-3(-)&BAV-3(-)	5.95%
Phi value	0.494	Phi value	0.337	Phi value	0.385
p-value	0.001	p-value	0.001	p-value	0.001

BAV-3 ($\phi = 0.385$, $p < 0.001$) has been identified in cattle.

Discussion

Antibodies against BRSV, BPIV-3, and BAV-3 were found in all herds in this study. Vaccination against BRSV, BPIV-3, and BAV-3 was not practiced in the herds of Fars Province. Therefore, the presence of antibodies indicates exposure to these viruses. Limited knowledge is available on the herd-level prevalence of BRSV, BPIV-3, and BAV-3 infections in Iran. BRSV, BPIV-3, and BAV-3 were found in all the herds representing a seroprevalence of 100% at the herd level in Fars Province, and these rates are similar to the findings in Kerman Province, Iran [15]. The results from the Aegean Region in Turkey, São Paulo State in Brazil, and Northern Italy also demonstrated that antibodies to BRSV were detected in 100% of studied dairy herds [7, 8, 20]. The high herd prevalence of BRSV was also reported in dual-purpose cattle herds in some Latin American countries (91.3% and 93.2% in Ecuador and Mexico, respectively) [21, 22]. Poor biosecurity measures, such as failure to quarantine newly purchased animals, inability to diagnose subclinical BRSV cases, and the lack of vaccination programs against respiratory diseases may play a role in the high seroprevalence rate of BRSV at the herd level [7]. In endemic areas, observing biosecurity can protect herds from invading viral infections and reduce the morbidity rate [20].

The prevalence of BRSV (76.43%) in our study was considered high at the animal level, com-

pared to the other report (51.1%) from the central region of Iran [14]. High individual seroprevalence of BRSV in the present study is also consistent with 80.48% and 79.5% of individual seroprevalences observed in Brazil and Ecuador, respectively [7, 22]. The animal-level seroprevalence of BRSV was reported 69.1% in Italy and 52.2% in Mexico [8, 21]. Some explanations for these variabilities are the differences in the number of samples, time of sample collection, route of antibody detection, housing and management, and inadequate knowledge of the disease [20]. In this study, a Chi-square analysis of the variables showed that region, gender, and age significantly affected the prevalence of antibodies to BRSV. The probability of BRSV infection in adults increased significantly ($p < 0.05$) by a factor of 13.44 compared to calves. The high risk of BRSV associated with age is in agreement with other reports. This association was explained by the longer exposure to the pathogen, decreased maternal antibodies, and reinfection with BRSV throughout life for older animals [20, 22]. A study in eastern and southeastern Poland confirmed the presence of BRSV infections in young cattle under 12 months of age in 60% of the dairy and beef herds examined, which was similar to other parts of Poland and Europe [23].

The reports from various countries have shown a great variation in BPIV-3 seroprevalence. In Iran, the seroprevalence of BPIV-3 was reported 100% at the herd level in Kerman and Qazvin Provinces, and 84.4%, 90%, and 95.5% at the animal level in Isfahan, Khorasan Razavi, and Qazvin Provinces, respectively [14-16, 24]. Others reported

a lower prevalence of antibodies against BPIV-3 in Saudi Arabia (67.6%), Turkey (56.2%), western Kenya (20.1%), and Grenada (13.4%) [25-28]. Despite a high seroprevalence, BPIV-3 has been identified less frequently in livestock farms. This is probably due to the lack of clinical cases, the similarity of symptoms to other respiratory diseases, and the lack of diagnostic kits. It has been hypothesized that small ruminants, particularly goats with high BPIV-3 prevalence, may act as reservoirs or vectors in the transmission of BRDC to cattle [25]. The most common respiratory virus in our study was BAV-3, with a seroprevalence of 92.62%, which was in agreement with the findings in north-western Turkey, which reported a seroprevalence of 92.3% for BAV-3 [5]. Other reports from Iran showed that although the herd level seroprevalence of BAV-3 in Fars Province (100%) is similar to that in Kerman Province, the animal level prevalence of the virus is higher than that reported in Isfahan province (55.6%) [14, 15]. The prevalence of BAV-3 was 61.9% in serum samples from calves showing respiratory disorders symptoms [24]. Although preliminary Chi-square tests showed associations ($p < 0.05$) between the presence of antibodies to BPIV-3 and all the studied factors, season and age were significant in logistic regression analysis. Older animals had 4.39- and 6.07-fold greater odds of seropositivity for BPIV-3 and BAV-3 than calves, respectively. This higher seropositivity probably results from the fact that older animals were exposed to the active substance longer than younger animals [6, 27]. The prevalence of BPIV-3 and BAV-3 was higher in the warm seasons than cold seasons in the present study. Immunosuppressive stress is induced by various factors, such as dehydration, and high temperature may contribute to a difference between seroprevalence in seasons. A higher seroprevalence of BAV-3 in the southern region than in the northern region of the study area was manifested by risk factors analysis, which may be due to a higher temperature in the southern region compared to the northern region. Regarding the type of husbandry, the static analysis confirmed that cattle in traditional farms were 3.08 times more likely to be seropositive for BAV-3 than industrial farms. The likely causes for this difference

have been ascribed to variations in herd hygiene, diet, and management system. Poor diet, early weaning, dehydration, low or high temperatures, inadequate rest, and transportation can trigger immunosuppressive stress [29]. A lower prevalence of BAV-3 in industrial farms can be due to many factors, for instance, the control of environmental factors, the establishment of biosecurity measures, and good management practices [14].

A significant association of dual infection with BRSV, BPIV-3, and BAV-3 was shown in the current study. The frequencies of mixed infection in the present study were higher than those in Isfahan Province. They reported 3.7% triple virus infection and 10% and 18.9% dual infections of BRSV plus BPIV-3 and BPIV-3 plus BAV-3, respectively [14].

In summary, this study demonstrated that BRSV, BPIV-3, and BAV-3 are very common in dairy cattle farms in the study area. Although the high seroprevalence found is not synonymous with disease, it represents a worrying epidemiological scenario as it is potentially important in the bovine respiratory disease complex. Therefore, a comprehensive epidemiological study on bovine respiratory viruses and other related bacterial species, including *Mycoplasma* spp, *Mannheimia/Pasteurella*, and *Haemophilus/Histophilus*, in Fars Province is proposed. Some preventive measures, such as quarantine, mass vaccination, and biosecurity, alongside raising farmer awareness of known risk factors, can help establish a control program on dairy farms.

Materials and Methods

Study location

This cross-sectional study with random cluster sampling was designed in Fars Province, southern Iran. This Province is located between latitude 27°03' to 31°40' N and longitude 50°36' to 55°035' E in an area of about 133000 km² with a mean annual rainfall of about 230 mm in the south of Iran and contains 29 counties. Fars Province is classified into three regions based on topographic features. The northern region surrounds an area of the north, northwest, and west of the Province with mild cold winters. Central region is characterized by a relatively temperate climate with rainfall in winter and a hot and dry climate in summer. Finally, the southern region, which extends from south to southeast area, is defined by very warm summers (Statistical Yearbook of Fars Province 2019). The counties of Fars Province were assigned into northern, central, and southern regions based on

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their geographical locations. Three industrial and three traditional dairy farms were randomly selected in each of these regions. Sampling was performed two times; one in the warm season (June-July) and another in the cold season (November-December) 2017.

Sample size

A total of 36 dairy farms were selected. Selected farms were visited, the purposes and details of the research project were described to the farm owners at the start of the study, and verbal consent was obtained. The target population was cattle herds, and the sampling unit was cattle. The formula below was used to calculate the sample size [19].

$$n = ((1.96)^2 \times EP \times (1-EP)) / d^2$$

Where n is the sample size, d represents precision of 0.05 at a 95% confidence level, and EP refers to an abbreviation for the expected prevalence, which was assumed to be 50% because the data on the seroprevalence of studied viruses are scarce in the south of Iran. Blood samples were taken from 12 cows on each farm, including six adult females, three adult males, and three calves under six months of age. Adult females were substituted if there were insufficient young or male animals. Generally, no vaccine was used against BPIV-3, BRSV, and BAV-3 in the study location.

Samples and antibodies evaluation

The sterile vacuum tubes without anticoagulant (VAC-UETTE®, Greiner Bio-One GmbH, Kremsmünster, Austria) were used for blood sample collection from the jugular vein. The tubes were labeled and immediately transported to the laboratory in a chilled state. Sera were collected after 10 min centrifugation at 3000 rpm and stored in a microtube at -20°C until analysis.

Antibodies against BRSV, BPIV-3, and BAV-3 were screened using an ELISA kit developed commercially by Bio-X Diagnostics (Rocheffort, Belgium). According to the kit

instructions, a dilution buffer was prepared, and the samples were diluted in a dilution plate. The kit's reference sera were also diluted in a tube. The samples and controls were poured into the wells of antigen-coated microtiteration plate and incubated at 21°C for 1 h. The plate was rinsed with washing solution, and after three rinses, the diluted conjugate solution was added to each well. The plate was washed again after another incubation, and the reaction was made visible by chromogen combination for 10 min. Next, 1 M phosphoric acid stopped the reaction in the last step, and the ODs were recorded at 450 nm. The following formula was used to calculate percent positivity:

$$PP = (OD_{corr} \text{ of sample}) / (OD_{corr} \text{ of positive control}) \times 100$$

OD_{corr} is an abbreviation for corrected optical density, which is equal to OD_{test} of antigen or positive control minus OD_{control}.

The sample was considered positive for BRSV, BPIV-3, and BAV-3 if PP was greater than 20%, 20%, and 10%, respectively. Herds were considered positive for herd prevalence calculation when at least two antibody-positive samples were detected.

Statistical analysis

All statistical analyses were performed using the SPSS software version 22. Descriptive data analysis was carried out to calculate the animal and herd level seroprevalences. Associations between outcomes (BRSV, BPIV-3, and BAV-3 serostatus) and geographic region, cattle specifications, and farm features at the animal level were investigated by the Chi-square test. Logistic regression was used to find the effects of potential risk factors on the seroprevalence outcomes. The strength of the association between outcome and variables was assessed using odds ratios and a 95% confidence interval. Phi and Cramer's V measures were used for the correla-

tion of the coexistence of antibodies to BRSV, BPIV-3, and BAV-3.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Majid Hashemi, Mehran Bakhshesh and Mohsen Manavian. The first draft of the manuscript was written by Majid Hashemi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare no conflict of interest.

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