Detection of Salmonella and Yersinia Infections in Aborted Fetuses from Northwest Iran Using Molecular (PCR) and Pathological Studies

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Abstract

Salmonella and *Yersinia* are members of the order Enterobacterales, which are known for being zoonotic pathogens. This study was conducted to detect *Salmonella* and *Yersinia* infections in aborted fetuses of sheep and goats from northwest Iran (East Azerbaijan province). To this end, a total of 62 aborted fetuses and 288 vaginal swabs were gathered from sheep and goat flocks. At first, bacterial cultures were performed on vaginal swab samples from adults and different tissues and abomasal contents from the fetuses. Then, the tissue samples were further analyzed through histopathology and molecular studies using conventional (Polymerase Chain Reaction) PCR with specific primers. Furthermore, the formalin-fixed tissue specimens were routinely processed for histopathological analysis. The invA target of *Salmonella* and the ail target (consistent with pathogenic *Y. enterocolitica*) were identified in 14.51% and 12.9% of the fetuses examined, respectively, by PCR, with two fetuses (3.22%) tested positive for both agents. Bacteriological analysis showed only one vaginal swab sample positive for Salmonella. Additionally, pathological

lesions in *Yersinia*-positive fetuses included multifocal necrotic hepatitis, interstitial pneumonia, multifocal myocarditis, and diffuse enteritis associated with inflammatory cell infiltration. In *Salmonella*-positive fetuses, the primary histopathological findings consisted of focal to multifocal necrotic and suppurative hepatitis in the liver, characterized by paratyphoid nodules, and suppurative bronchopneumonia distinguished by the presence of neutrophils. In conclusion, the present results indicate that *Salmonella* and *Yersinia* can be detected in the aborted fetus of sheep and goats and highlight the need for more consideration of their impact on economic loss and public health.

Keywords

Abortion, Salmonella, Yersinia, ail gene, PCR, Public health

Abbreviations: PCR: Polymerase Chain Reaction/ H&E: Hematoxylin and Eosin/ CI: Confidence Interval/ NTS: Non-Typhoidal Salmonella

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Introduction

Salmonella, as a genus of bacteria, is a member of the Enterobacteriaceae family (order Enterobacterales) [1], which mainly causes abortion in sheep [2]. Different species of *Salmonella* bacteria, such as *S. abortusovis*, *S. Arizona*, *S. Dublin*, and *S. Typhimurium* can cause abortion in sheep and goats [3, 4]. Some serovars can also cause gastrointestinal inflammation, often accompanied by additional symptoms such as uterine inflammation, anorexia, and wool loss [5]. Infections caused by Gram-negative bacteria such as *Salmonella* can release endotoxins, which lead to prostaglandin production, often resulting in abortion. Considering that, this pathogen poses a significant risk to the sheep population and can lead to considerable economic damage in sheep-

farming regions. Abortion is a major economic concern linked to salmonellosis in sheep. [6-8]. Salmonellosis is common among small ruminants and can manifest in clinical or subclinical forms. The infected animals with no symptoms, can still spread the bacteria through their feces, which plays an important epidemiological role in spreading the disease. Outbreaks are usually triggered by stress or other external factors, leading to abortion in sheep, particularly in naïve flocks, where 30-50% of pregnant ewes may abort in the third trimester, especially in southern Europe [6, 9] and Asia like Iran [10]. In addition to abortion, the disease can also cause epididymal-orchitis, arthritis, respiratory problems, meningitis, and stillbirths [9]. Nevertheless, sheep and goats generally maintain high fertility compared to other domesticated animals [11]. Beyond its significant economic impact on sheep farming, Salmonella is a major zoonotic pathogen. It is estimated to cause approximately 93.8 million cases of gastroenteritis and 155,000 deaths worldwide each year, with the large majority of infections (around 85%) linked to the consumption of contaminated food [12]. Human infection occurs mainly through animal-derived products such as meat and milk. The increasing prevalence of antimicrobial-resistant strains has further elevated the public health concern associated with this bacterium [13].

The genus *Yersinia* belongs to the family Yersiniaceae (order Enterobacterales) [14], which includes 18 different species [15] and is known for being a zoonotic pathogen transmitted through food [16]. In 1992, a specific strain of *Yersinia enterocolitica* was experimentally demonstrated for the first time as a factor contributing to placentitis and abortion in pregnant ewes [17]. Some *Yersinia* species are particularly infectious to humans and small ruminants [18]. These infections typically spreading via the fecal-oral route through contaminated food or water. Remarkably, *Yersinia* can endure and even multiply at low temperatures, such as 4°C, allowing them to persist outside a host for extended periods [19]. *Yersinia* represents very versatile organisms since they

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can survive in such diverse media as soil, plants, insects, and warm-blooded animals, including rodents, pigs, and humans [20]. This infection causes significant economic losses in sheep breeding due to abortion, neonatal death, and placentitis [21]. Other *Yersinia* species have also caused abortions in various animal species. Of particular note, however, is that *Yersinia* constitutes a significant cause of abortion and stillbirth in sheep, resulting in inflammatory responses in the placenta, liver, kidney, and spleen [22]. In goats, similar to other ruminants, this pathogen has serious effects on the health by causing enteritis, reduced milk yields, lymph node swelling, abortions, death of newborns, udder infections, bacteremia, and ocular lesions. In spite of all such impacts, only a few studies examined this organism in sheep and goats, focusing primarily on single incidences or small outbreaks. The comprehensive epidemiological investigation on large sheep and goat populations is lacking [23].

In this regard, not much information was found in this region about these infections and their role in abortion. Therefore, the objective of this research was to conduct a molecular (conventional PCR) and pathological examination of *Salmonella* and *Yersinia* infections in sheep and goat herds located in the East Azerbaijan area of northwest Iran.

Results

Molecular findings

The present results of the molecular study concerning the age categories are displayed in Table 1. In brief, the genomes of *Salmonella* and *Yersinia* were identified in 14.51% and 12.90% of the fetuses examined, respectively. Also, two out of 62 aborted fetuses (3.22%) tested positive for both infections. No positive samples were detected in the 5-6 month-old groups for either infection. Also, higher infection rates were found in the 2-3 month-old groups for both *Salmonella* and

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Yersinia infections, although there were no significant differences among the four age categories ($P > 0.05$).

Table 1. Infection rates based on PCR results for detection of *Salmonella* and *Yersinia* in the aborted fetuses (N = 62).

Infection / Age groups	Salmonella	Yersinia
60-90 days old (about 2-3 m) 5/62 (8.06%)	1/5 (20%) (95% CI: 0.2±0.35)	1/5 (20%) (95% CI: 0.2±0.35)
90-120 days old (about 3-4 m) 14/62 (22.58%)	1/14 (7.14%) (95% CI: 0.07±0.132)	1/14 (7.14%) (95% CI: 0.07±0.132)
120-150 days old (about 4-5 m) 36/62 (58.06%)	7/36 (19.44%) (95% CI: 0.194±0.128)	6/36 (16.66%) (95% CI: 0.16±0.124)
150-155 days old (Over 5 m) 7/62 (11.29%)	0/7 (0%) (95% CI: 0)	0/7 (0%) (95% CI: 0)
Total	9/62 (14.51%) (95% CI: 0.145±0.088)	8/62 (12.9%) (95% CI: 0.129±0.082)

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Pathological findings

At necropsy, macroscopic pathological lesions were identified in the organs that were examined. In fetuses testing positive for *Yersinia* via PCR, hemorrhagic fluid was noted in the abdominal and thoracic cavities, accompanied by pale round foci in the liver (Figures 1A and B). Besides, microscopic analyses (Figures 1C–F) revealed pathological lesions across various tissues. In *Yersinia*-positive fetuses, findings included multifocal necrotic hepatitis, interstitial pneumonia with pulmonary vascular hyperemia, multifocal myocarditis with associated cardiac vascular hyperemia, and diffuse enteritis characterized by significant mononuclear cell infiltration. In fetuses testing positive for *Salmonella* via PCR, the primary histopathological lesions comprised focal to multifocal necrotic and suppurative hepatitis in the liver, marked by paratyphoid nodules,

and suppurative bronchopneumonia with neutrophil infiltration and pulmonary vascular hyperemia in the lungs (Figures 1G–H).

Discussion

A notable finding of this study was the marked discrepancy between the bacteriological and molecular results for *Salmonella* detection. While PCR identified *Salmonella* DNA in 14.51% of fetuses, traditional culture methods isolated the bacterium from only one vaginal swab. This discordance is not unexpected and can be attributed to several factors. First, the direct culture method used for vaginal swabs and fetal organs, even with a selenite cysteine enrichment step, is inherently less sensitive than PCR. The bacteria may be present in low numbers, be non-viable due to autolysis in fetal tissues, or exist in a viable but non-culturable state, particularly in samples that may have been exposed to oxygen or antimicrobial substances. PCR, on the other hand, detects DNA from both live and dead cells, offering superior sensitivity. Second, the PCR assay targets a specific genetic sequence (invA) and can detect the pathogen even when overgrown by competing microbiota in culture. The single culture-positive result likely represents an infection with a high bacterial load where organisms were particularly robust.

The current study showed a 12.90% occurrence of *Yersinia* infection and 14.51% occurrence of *Salmonella* infection, both associated with marked pathological lesions in aborted fetuses of small ruminants in the East Azerbaijan province of Iran. Since different serotypes of *Salmonella* are common and can cause different rates of infection across regions, studies conducted in this field have shown different results [10]. For instance, PCR studies in different provinces of Iran reported Salmonella presence in abortion cases: 19.6% in Shiraz [24], 1.3% in Sistan [25], 8% in Lorestan [26], and 44.4% [27] and 23.68% [28] in two studies from Chahar Mahal Bakhtiari province. In northwestern Romania, abortion rates ranged from 13.92% to 16.66% during an outbreak in 2020-

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2021, resulting in economic losses due to increased mortality and decreased lamb viability. However, in two molecular studies in Zanjan province, no *Salmonella* agent was found [29, 30]. Also, recent findings in other countries have emphasized the significant impact of salmonellosis on sheep abortion and reproductive failure. In a flock outbreak in northwestern Romania, abortion rates were recorded between 13.92% and 16.66% during 2020–2021, causing economic losses due to increased mortality as well as decreased lamb viability [7]. In Dalmatia, Croatia, an outbreak from 2003-2004 showed abortion rates between 22% and 38% in five affected flocks, with Salmonella isolated from vaginal smears and fetuses. Another study focused on an outbreak of abortions in sheep caused by *Salmonella* in Dalmatia (south Croatia) during 2003-2004 using PCR, showed abortion rates between 22% and 38% in five affected flocks, with *Salmonella* pathogen isolated from 13 vaginal smears and two fetuses [31]. Similarly, another study conducted in Latium and Tuscany of Italy from 2015 to 2018 using qPCR, found that 16% of 148 positive tissues and swab samples were linked to *Salmonella* species [2]. Moreover, emerging evidence shows varying rates of non-typhoidal *Salmonella* (NTS) in sheep and goats across different regions of Ethiopia [32]. In this regard, one study reported an overall prevalence of 6.19% in the feces of eastern Hararghe [33] and 3.3% in Jimma [34]. Other studies also indicated *Salmonella* isolation rates of 2.1% [35] and 4.8% [36] in fecal samples obtained from apparently healthy slaughtered sheep in Debrezeit and central Ethiopia, respectively. A further investigation found that the prevalence of healthy slaughtered sheep and goats at an abattoir enterprise in Addis Ababa was 1.04% [37]. These findings highlight the regional differences in *Salmonella* prevalence among livestock in Ethiopia. In another study conducted in the Van region of Turkey, *Salmonella* species were identified in 14.2% of sheep abortion samples through RT-PCR [38]. These findings underscore Salmonella's role as a significant pathogen associated with abortion, highlighting the need for improved control

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measures and epidemiological research in the global sheep industry. These pieces of evidence indicate the role of *Salmonella* as an important bacterial pathogen associated with abortion, warranting special attention for improved control measures and epidemiological research in the sheep industry worldwide. The higher infection rates in the present study may be linked to the abortion history and traditional housing conditions of the affected animals. From the human health perspective, the detection of Salmonella in aborted fetuses of small ruminants has clear zoonotic implications. Human salmonellosis remains a major global public health threat, with NTS responsible for millions of cases of gastroenteritis annually and invasive disease causing substantial mortality, particularly in vulnerable populations. Transmission occurs primarily through contaminated animal products. Recent multi-country outbreaks illustrate the rapid international spread of these pathogens [12, 13].

It is believed that abortions caused by *Salmonella* are often precipitated by stressful situations like transport, dietary change, or feeding spoiled feed, particularly observed in the present study during autumn. Specifically, the transition from outdoor to indoor housing and a shift from pasture-based to hand-feeding diets were observed. In this condition, the organism first localizes in the intestine of the dam, leading to a second bacteremia that infects placentome. Excessive replication of the organism leads to nearly total destruction of the fetal villi and severe placentitis, which may result in abortion, occasionally occurring without the invasion of the fetus [9, 39]. A previous study highlighted necrosuppurative placentitis and pneumonia, with less common necrosuppurative hepatitis, splenitis, and nephritis in the affected fetuses as the predominant pathological changes [22]. In this regard, evidence of moderate to light accumulation of neutrophils in the fetal lungs was reported, along with multifocal suppurative hepatitis [9, 39]. Similar lesions were observed in the present study. However, the severity of the pathological lesions was notable. Fetal tissues may

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occasionally yield negative results on culture, aligning with the present findings. Almost pure cultures can be acquired from vaginal discharge samples collected immediately prior to abortion. The results of the current study indicated a 12.9% incidence of *Yersinia* infection in aborted fetuses. In this regard, in a previous experimental study, the infected ewes had a fever and severe placental inflammation, with fetal outcome directly depending on the pregnancy stages. In more detail, early in gestation, these animals either died with dead fetuses or carried dead fetuses [21]. The dead fetuses showed typical hepatic lesions, consistent with the findings in the present study. Subsequent various studies found *Yersinia* in aborted sheep fetuses in the UK, Australia, Sweden, Italy, and the Netherlands [40-42]. In Australia, a review of 529 abortions and stillbirth cases from 2000 to 2018 reported eight cases of sheep abortions linked to *Yersinia* species [42]. In the Netherlands, during five consecutive lambing seasons from 2006 to 2011, Dutch Animal Health Service investigated 453 abortion case referrals, analyzing infectious agents in 48% of sheep and 34% of goat cases. They identified that *Yersinia* was one of the primary infectious agents responsible for abortion in sheep [43]. The results of laboratory investigations during the lambing season of 2015 to 2016, based on pathology records and samples from aborted lambs and goats, detected *Yersinia* in swabs from the fetal oropharynx, and fetal lung punctures of two embryos via PCR [40]. In Iran, there have been no reports of *Yersinia* isolation from sheep and goat abortions. However, a study conducted in Shahrekord isolated *Yersinia* from small ruminant milk, through analyzing 100 samples and finding 9 (9%) positive for the pathogen using the culture method in a CIN environment and PCR methods [44]. The 12.9% occurrence of Yersinia infection in aborted fetuses further emphasizes the zoonotic potential of this pathogen. Pathogenic Yersinia species share key virulence mechanisms and are capable of causing both intestinal and extraintestinal disease in humans. The presence of Yersinia DNA together with marked pathological lesions in

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fetal tissues indicates active infection in the flock and raises concern about possible transmission to humans through contaminated animal products or environmental exposure [45].

The pathogenesis of abortion caused by *Yersinia* probably involves the invasion of the intestinal epithelium, temporary bacteremia, and localization within the caruncle, resulting in villus infarction and chorion invasion. Documented gross lesions are derived from restricted observations that tend to be comparable in aborted fetuses and stillborns from both sheep and goats [39]. Herein, hemorrhagic fluid was found in the abdominal and thoracic cavities associated with pale pinpoint foci in the liver. Histologically, there are commonly pathological lesions in the placenta and conjunctiva [39]. Several previous evidences have presented marked microscopic lesions in some tissues, which are consistent with the present observations. In more detail, the lungs contain a few mononuclear cells in the airways, wherease hepatic lesions consist of focal areas of necrosis infiltrated by mononuclear cells. Similar foci are visible on microscopic examination of the myocardium. The intestinal lamina propria and submucosa are heavily populated with plasma cells and mononuclear cells [22, 23, 39]. The current pathological findings align with those of others.

Conclusion

Herein, the present results demonstrate that *Salmonella* and *Yersinia* can find in the aborted fetus of the sheep and goats and highlight the need for more consideration of their impact on economic loss and public health. Regarding the economic losses caused by abortion, treatment costs, and more importantly, the zoonotic nature of these diseases, it is crucial to implement control and prevention measures for public health. In the present study, species identification was not performed; this could be addressed in future studies to compare the roles of different species in the occurrence of these infections.

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Materials and Methods

Ethical approval

All applicable international, national, and institutional regulations regarding the care and use of animals (animal welfare was considered to collect the vaginal swabs without harmful action), were adhered to, including the protocol sanctioned by the Animal Research Ethics Committee of the University of Tabriz (ID: IR.TABRIZU.REC.1403.049). Aborted fetuses were obtained after naturally occurring abortions; owner consent was obtained for sample collection.

Study area

The current research was conducted across seven cities in the East-Azerbaijan province of northwest Iran, which include Tabriz, Charuymaq, Khoda Afrin, Jolfa, Heris, Bostan Abad, and Mianeh. This investigation provides insights into Salmonella and Yersinia infections as part of a broader study examining both infectious and non-infectious factors contributing to abortion in small ruminants within the East Azerbaijan province of northwest Iran. To achieve this, from November 2023 to February 2024, a total of 62 aborted fetuses and 288 vaginal swabs from adult animals with recent abortion histories were gathered from sheep and goat herds in the specified areas. All herds were maintained under traditional conditions. All samples were sourced from herds maintained under traditional farming practices.

Sample collection

Initially, the age of the aborted fetuses was calculated using the formula $(X + 17) \times 1/2$, where X represents the size of the fetus in centimeters (crown- rump length). A systematic necropsy was conducted, and the pathological lesions were documented. Subsequently, 50 mg of abomasal content was placed in a 2 mL microtube and preserved at -70 °C for subsequent molecular analyses.

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Furthermore, tissue samples from various organs, such as the liver, lung, heart, and small intestine, were collected and immersed in a 10% formalin solution for histopathological examination.

Isolation and identification of Salmonella

Vaginal swabs (n = 288), fetal parenchymal organs (n = 62 each for liver, spleen, and lung), and fetal gastric contents were directly incubated on blood agar and Xylose- Lysine- Deoxycholate (XLD) agar for 72 h at 35 ˚C in aerobic conditions. Subsequently, vaginal swabs and organ samples were placed in 10 mL selenite cysteine broth and incubated for 16 h at 35˚C under aerobic conditions, before being transferred to XLD agar. Colonies that grew were re-inoculated to obtain pure cultures, which were identified through catalase and oxidase tests. Typical colonies were selected for further testing using standard biochemical methods. Full identification of suspected *Salmonella* isolates was completed by comparing the morphological and biochemical results with established standard [31]. To monitor for environmental and cross-contamination, sterile swabs moistened with sterile saline were exposed to the sampling environment and processed identically to the clinical samples. Additionally, sterile broth media without any inoculated sample were incubated alongside test samples as negative controls during each enrichment and plating step.

Culture of Yersinia

Fresh samples of the lung, placenta, and abomasal fluid collected during the postmortem examination were sent for routine microbiological analysis. The samples were incubated on 5% sheep blood agar and MacConkey agar at 22˚C for up to five days. Colonies resembling *Y. enterocolitica* reference strains were examined using Gram-stained smears. Gram-negative bacilli exhibiting Yersinia-like characteristics were sub-cultured onto nutrient agar and incubated aerobically overnight for additional testing. Organisms were tentatively identified as *Y. enterocolitica* or similar bacteria if they were oxidase negative yet produced catalase, urease, and

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ornithine decarboxylase, and tested positive for acid while being negative for H2S on triple sugar iron agar slopes (TSI) [46].

Pathological study

The tissue samples were preserved in a 10% neutral buffered formalin solution for a minimum of 48 hours. They were then processed routinely using a DS2080/H tissue processor (Didsabz, Iran), embedded in paraffin, cut into 5 µm thick sections, and stained with common hematoxylin and eosin (H&E). Finally, the sections were studied under a light microscope (Olympus, CH-30, Japan), and the observed lesions were recorded.

Molecular studies (DNA extraction and PCR assay)

The genomes of Salmonella sp. and Yersinia were identified using conventional PCR as presented in Table 2. The specificity of the PCR assays was confirmed by testing the primer sets against *Chlamydia abortus,* which is commonly found in ovine reproductive samples, and yielded no amplification product. All PCR runs included a no-template control (nuclease-free water) to rule out reagent contamination and a positive control (DNA from reference strains) to ensure assay performance.

Table 2. Characteristics of the primers and PCR reaction conditions used in this study.

Bacteria	Sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	Cycles	References
Salmonella sp.	F: GCCGAAGATGAGTGTGTCCAGTT R: CCGTGTTCTTACCCACCGTATC	152	62	35	[27]
Yersinia	F: TTAATGTGTACGCTGGGAGTG R: GGAGTATTCATATGAAGCGTC	425	55	40	[47]

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Statistical analyses

The Chi-Square test was employed to assess the correlations between infections and age categories (four categories, comprising 2-3, 3-4, 4-5, and over 5 month-old fetuses). Differences were deemed significant at $P < 0.05$. The analyses were conducted using IBM SPSS Statistics version 22 software, and a 95% confidence interval (CI) was also evaluated.

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References

1. Schwieters, A., & Ahmer, B. M. (2024). Identification of new SdiA regulon members of Escherichia coli, Enterobacter cloacae, and Salmonella enterica serovars Typhimurium and Typhi. *Microbiology Spectrum, 12*(12), e01929-24. https://doi.org/10.1128/spectrum.01929-24

2. Santori, D., Fanelli, R., Di Donato, F., Dottarelli, S., Barone, A., & Sezzi, E. (2024). Biomolecular survey on the main infectious causes of abortion in sheep in the Italian regions of Latium and Tuscany. *Open veterinary journal, 14*(6), 1447–1452. https://doi.org/10.5455/OVJ.2024.v14.i6.12

3. Didkowska, A., Żmuda, P., Liberska, M., & Anusz, K. (2022). Current state of knowledge regarding bacterially-induced abortion in sheep. *Annals of Agricultural and Environmental Medicine, 29*(3), 321-325. https://doi.org/10.26444/aaem/146605

4. Noakes, D. E., Parkinson, T. J., & England, G. C. W. (2019). *Veterinary reproduction and obstetrics* (10th ed.). Elsevier. https://cir.nii.ac.jp/crid/1970867909881667647

5. Uzzau, S., Leori, G. S., Petruzzi, V., Watson, P. R., Schianchi, G., Bacciu, D., Mazzarello, V., Wallis, T. S., & Rubino, S. (2001). Salmonella enterica serovar-host specificity does not correlate

with the magnitude of intestinal invasion in sheep. *Infection and Immunity, 69*(5), 3092–3099. https://doi.org/10.1128/iai.69.5.3092-3099.2001

6. {Amagliani, 2021 #282}Amagliani, G., La Guardia, M. E., Dominici, S., Brandi, G., & Omiccioli, E. (2021). Salmonella Abortusovis: An Epidemiologically Relevant Pathogen. *Current Microbiology*, *79*(1), 3. https://doi.org/10.1007/s00284-021-02689-1

7. Pall, E., Olah, D., Chira, A., Duca, G., Spînu, M., Șandru, C. D., & Vasiu, A. (2022). Ethio-epidemiological analysis of an abortigenic outbreak of salmonellosis in sheep. *Scientific Works. Series C, Veterinary Medicine, 68*(1). https://doi.org/10.15835/swscvm.22.1.11445

8. Esmaeili, H., Shakeri, A. P., Rad, Z. N., Arani, E. B., Villanueva-Saz, S., Ruiz, H., & Lacasta, D. (2022). Causes of abortion in Iranian sheep flocks and associated risk factors. *Veterinary Research Communications*, *46*(4), 1227-1238. https://doi.org/10.1007/s11259-022-09986-5

9. Belloy, L., Decrausaz, L., Boujon, P., Hächler, H., & Waldvogel, A. S. (2009). Diagnosis by culture and PCR of Salmonella Abortusovis infection under clinical conditions in aborting sheep in Switzerland. *Veterinary Microbiology*, *138*(3), 373-377. https://doi.org/https://doi.org/10.1016/j.vetmic.2009.03.026

10. Behzadi Shahrbabak, M. J. (2019). A review on infectious agents of sheep and goats abortion in Iran. *New Findings in Veterinary Microbiology, 1*(2), 102-113. https://doi.org/10.35066/J040.2018.780

11. Edmondson, M. A., Roberts, J. F., Baird, A. N., & Bychawski, S. (2012). Theriogenology of sheep and goats. In D. G. Pugh & A. N. Baird (Eds.), *Sheep and goat medicine* (2nd ed., pp. 150–230). W.B. Saunders. https://doi.org/10.1016/C2009-0-60474-8

Uncorrected proof

12. Lamichhane, B., Mawad, A. M. M., Saleh, M., Kelley, W. G., Harrington, P. J., Lovestad, C.W., et al. (2024). Salmonellosis: An Overview of Epidemiology, Pathogenesis, and Innovative Approaches to Mitigate the Antimicrobial Resistant Infections. *Antibiotics,* 13(1), 76.

13. Teklemariam, A. D., Al-Hindi, R. R., Albiheyri, R. S., Alharbi, M. G., Alghamdi, M. A., Filimban, A. A. R., et al. (2023). Human Salmonellosis: A Continuous Global Threat in the Farm-to-Fork Food Safety Continuum. *Foods (Basel, Switzerland)*, 12(9), 1756.

14. Mekonnen, Y. (2024). A review on yersiniosis. *World Journal of Veterinary Science, 4*(1), Article 1022. https://doi.org/10.18280/wjvs.040122

15. Dheyab, Z. S. (2022). Clinically important Yersinia: minireview. *Journal of Medical Research and Health Sciences, 5*(10), 2295-2306. https://doi.org/10.52845/JMRHS/2022-5-10-3

16. Kaasch, A., Dinter, J., Goeser, T., Plum, G., & Seifert, H. (2012). Yersinia pseudotuberculosis bloodstream infection and septic arthritis: case report and review of the literature. *Infection*, 40(2), 185-190. https://doi.org/10.1007/s15010-011-0160-2

17. Corbel, M. J., Ellis, B., Richardson, C., & Bradley, R. (1992). Experimental Yersinia enterocolitica placentitis in sheep. *British Veterinary Journal, 148*(4), 339-349. https://doi.org/10.1016/0007-1935(92)90086-G

18. Rivas, L., Strydom, H., Paine, S., Wang, J., & Wright, J. (2021). Yersiniosis in New Zealand. *Pathogens, 10*(2), 191. https://doi.org/10.3390/pathogens10020191

19. Bergman M, Chafel R, Mecsas J. Yersinia. Pathogenesis of bacterial infections in animals. 2010:309-23.

Uncorrected proof

20. Ochoa TJ, O'Ryan M. 148 - Yersinia Species. In: Long SS, editor. Principles and Practice of Pediatric Infectious Diseases (Sixth Edition). Philadelphia: Elsevier; 2023. p. 864-9.e2.

21. Karbe, E., & Erickson, E. D. (1984). Ovine abortion and stillbirth due to purulent placentitis caused by Yersinia pseudotuberculosis. *Veterinary Pathology, 21*(6), 601-606. https://doi.org/10.1177/030098588402100610

22. Costa, L., Cristo, T., Conti, C., Silva, M., Melo, I., Krasilchik, S., et al. (2021). Sepsis due to Yersinia enterocolitica in an aborted equine fetus: case report. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 73*, 417-422. https://doi.org/10.1590/1678-4162-12077

23. Giannitti, F., Barr, B. C., Brito, B. P., Uzal, F. A., Villanueva, M., & Anderson, M. (2014). Yersinia pseudotuberculosis infections in goats and other animals diagnosed at the California Animal Health and Food Safety Laboratory System: 1990–2012. *Journal of Veterinary Diagnostic Investigation, 26*(1), 88-95. https://doi.org/10.1177/1040638713516624

24. Firouzi, R. (2006). Bacteriological study of abortion in ewes of Shiraz area. *Journal of Veterinary Research, 61*(1), 15-17. https://doi.org/10.22067/veterinary.v61i1.23248

25. Mahdavi Roshan, H., Saadati, D., & Najimi, M. (2018). Molecular detection of Brucella melitensis, Coxiella burnetii and Salmonella abortusovis in aborted fetuses of Baluchi sheep in Sistan region, south-eastern Iran. *Iranian Journal of Veterinary Research, 19*(2), 128-132. https://doi.org/10.22099/ijvr.2018.4858

26. Malakshahe K. Investigating of bacterial agents in abortion of sheep in Lorestan province by PCR method: Veterinary Faculty. Shahrekord University. 2017.

Uncorrected proof

27. Sharifzadeh, A., Doosti, A., & Khaksar, K. (2008). A multiplex PCR for the detection of Brucella spp. and Salmonella abortusovis from aborted ovine fetus. *Research Journal of Biological Sciences, 3*(1), 109-111.

28. Hashemi S, Mahzounieh M, Yek Taneh F, Sheykhi N, editors. Evaluation of the Prevalence of salmonella abortion in sheep of Chaharmahal and Bakhtiari province. The 2nd National Congress Of Veterinary Laboratory Sciences; 2012.

29. Saleh, M., Harkinezhad, M. T., Marefat, A., & Salmani, V. (2013). An outbreak of abortion in Afshari sheep with probable involvement of Campylobacter fetus. *Iranian Journal of Veterinary Medicine, 7*(1), 51-56. https://doi.org/10.22059/ijvm.2013.32023

30. Saleh, M., Harkinezhad, M. T., Marefat, A., & Salmani, V. (2014). Detection of some bacterial causes of abortion in Afshari sheep using Real Time PCR detection and sensitivity assessment of Campylobacter primers. *Journal of Agricultural Biotechnology, 6*(3), 107-120. https://doi.org/10.22103/jab.2014.1330

31. Habrun, B., Listes, E., Spicic, S., Cvetnic, Z., Lukacevic, D., Jemersic, L., Lojkic, M., & Kompes, G. (2006). An outbreak of Salmonella Abortusovis abortions in sheep in south Croatia. *Journal of Veterinary Medicine, Series B: Infectious Diseases and Veterinary Public Health, 53*(6), 286-290. https://doi.org/10.1111/j.1439-0450.2006.00959.x

32. Gojam A, Tulu D. Infectious causes of abortion and its associated risk factor in sheep and goat in Ethiopia. *Iranian Journal of Veterinary Science and Technology,* 2020; 4:7-12.

33. Hailu, T., & Kebede, B. (2016). Isolation of non-typhoidal Salmonella from sheep faeces in Eastern Hararghe, Ethiopia. *Applied Microbiology: Open Access, 2*(3), 1000120. https://doi.org/10.4172/2471-9315.1000120

Uncorrected proof

34. Anbessa Dabassa AD, Ketema Bacha KB. The prevalence and antibiogram of Salmonella and Shigella isolated from abattoir, Jimma town, South West Ethiopia. 2012.

35. Woldemariam, E., Molla, B., Alemayehu, D., & Muckle, A. (2005). Prevalence and distribution of Salmonella in apparently healthy slaughtered sheep and goats in Debre Zeit, Ethiopia. *Small Ruminant Research, 58*(1), 19-24. https://doi.org/10.1016/j.smallrumres.2004.08.008

36. Molla, W., Molla, B., Alemayehu, D., Muckle, A., Cole, L., & Wilkie, E. (2006). Occurrence and antimicrobial resistance of Salmonella serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. *Tropical Animal Health and Production, 38*(6), 455-462. https://doi.org/10.1007/s11250-006-4325-4

37. Bedaso Kebede Kassaye, B. K. K., Dinsefa Jemal Hassen, D. J. H., Kifle Alemu Leja, K. A. L., & Biniam Tsegaye, B. T. (2015). Study on prevalence and distribution of Salmonella isolates from apparently healthy sheep and goats slaughtered at Addis Ababa Abattoir enterprise, Ethiopia. *International Journal of Livestock Production, 6*(7), 143-148. https://doi.org/10.4172/2157-7579.1000268

38. Gülaydın, Ö., Öztürk, C., Ekin, İ. H., İlhan, Z., & İlhan, F. (2023). Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods. *Acta Veterinaria Brno, 92*(1), 69-77. https://doi.org/10.2754/avb202392010069

39. Jubb K, Kennedy P, Palmer N, Maxie M. Jubb. Kennedy, and Palmer's Pathology of Domestic Animals: Female Genital System 6th ed: Elsevier. 2016.

Uncorrected proof

40. Brom, R. v. d., Santman-Berends, I., Dijkman, R., Vellema, P., Dijkman, R., & Engelen, E. v. (2021). An accessible diagnostic toolbox to detect bacterial causes of ovine and caprine abortion. *Pathogens, 10*(9), 1147. https://doi.org/10.3390/pathogens10091147

41. Momtaz, H., Rahimian, M. D. Dehkordi, F.S. (2013). Identification and characterization of Yersinia enterocolitica isolated from raw chicken meat based on molecular and biological techniques. *Journal of Applied Poultry Research*, 22(1), pp.137-145. https://doi.org/10.3382/japr.2012-00549

42. Clune, T., Beetson, S., Besier, S., Knowles, G., Paskin, R., Rawlin, G., et al. (2021). Ovine abortion and stillbirth investigations in Australia. *Australian Veterinary Journal, 99*(3), 72-78. https://doi.org/10.1111/avj.13040

43. van den Brom, R., Lievaart-Peterson, K., Luttikholt, S., Peperkamp, K., Wouda, W., & Vellema, P. (2012). Abortion in small ruminants in the Netherlands between 2006 and 2011. *Tijdschrift voor Diergeneeskunde, 137*(7), 450-457. Retrieved from https://pubmed.ncbi.nlm.nih.gov/22900421/

44. Elaheh, A. Identification and analysis of pathogenic genes of Yersinia enterocolitica isolated from sheep and goat milk in Shahrekord. The World of Microbes. 2016;10:256-62.

45. Seabaugh, J. A., & Anderson, D. M. (2024). Pathogenicity and virulence of Yersinia. *Virulence*, 15(1).

46. Paz, M., Ceolin Lamego, E., Santos, I., Henker, L., Lorenzett, M., Lopes, B., et al. (2025). Abortion due to Yersinia enterocolitica infection in an ewe in Southern Brazil. *Ciência Rural, 55*(2), e20230442. https://doi.org/10.1590/0103-8478cr20230442

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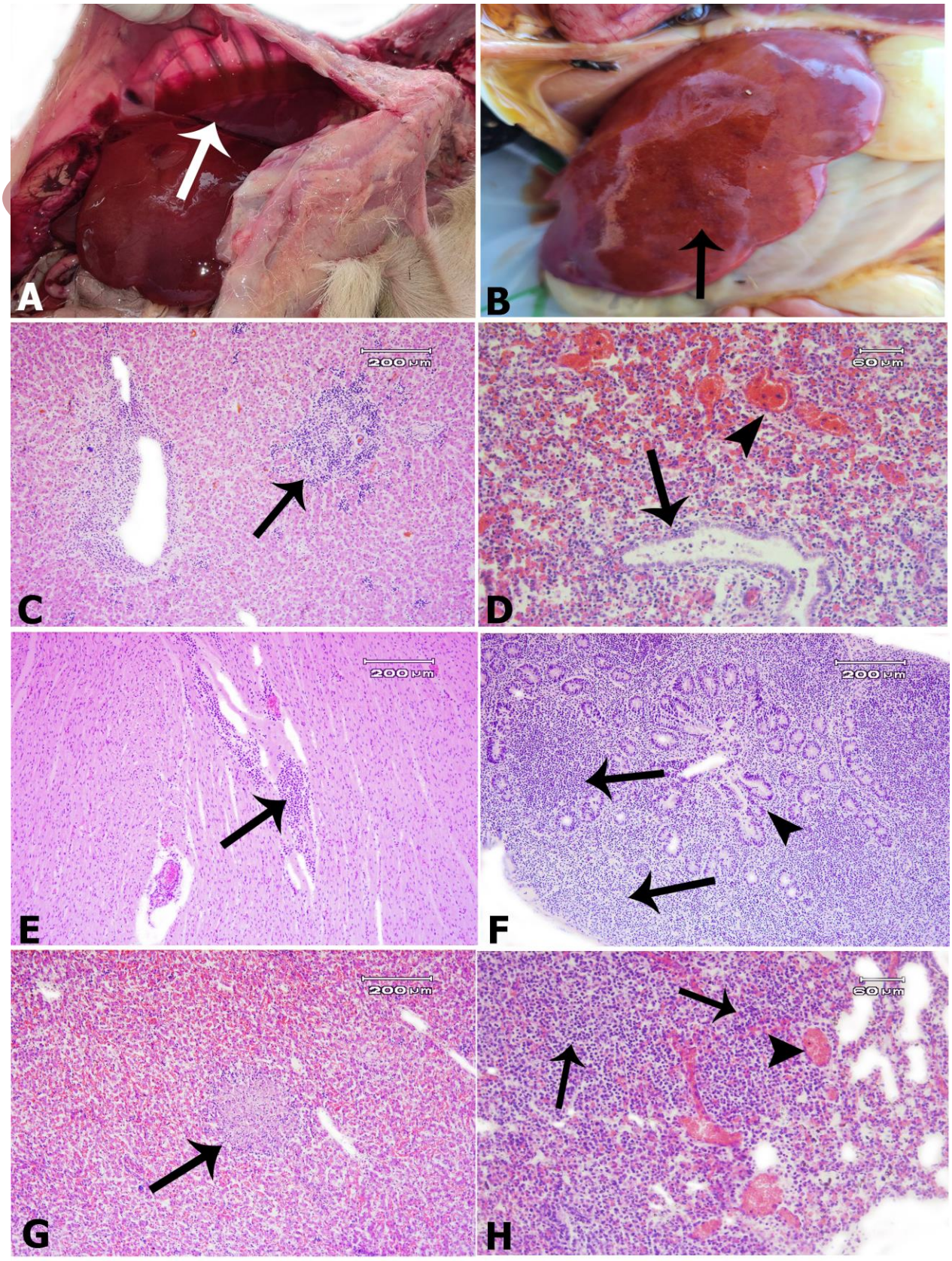
47. Wannet, W.J.B., Reessink, M., Bruningd, H.A., Maas, H.M. (2001) Detection of pathogenic Yersinia enterocolitica by a rapid and sensitive duplex PCR assay. *Journal of Clinical Microbiology*, 39 (12): 4483-4486.

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Figure legends

Figure 1. A-F: The aborted fetuses naturally infected by *Yersinia* A: hemorrhagic fluid (arrow) in the thoracic cavity in *Yersinia*-positive fetus. B: Pale round pinpoint foci in the liver (arrow). C: The focal necrosis associated with inflammatory cell infiltration (arrow) in the liver. D: Interstitial pneumonia is associated with the presence of mononuclear cells in the airways (arrow) and vascular hyperemia (arrowhead). E: focal myocarditis associated with mononuclear cell infiltration (arrow). F: diffuse enteritis associated with mononuclear cell infiltration (arrows) and intestinal gland necrosis (arrowhead). G: focal necrosis characterized by paratyphoid nodule (arrow) in the liver. H: Interstitial pneumonia associated with the presence of neutrophils (arrow) and vascular hyperemia (arrowhead) H&E.

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A
B
200 µm
C
60 µm
D
200 µm
E
200 µm
F
200 µm
G
60 µm
H

عنوان مقاله: شناسایی عفونت‌های سالمونلا و یرسینیا در جنین‌های سقط‌شده شمال غرب ایران با استفاده از مطالعات مولکولی و پاتولوژیک

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خلاصه فارسی: *سالمونلا* و *یرسینیا* دو جنس از خانواده انتروباکتریاسه هستند که به‌عنوان پاتوژن‌های مشترک بین انسان و دام شناخته می‌شوند. این مطالعه با هدف شناسایی عفونت‌های *سالمونلا* و *یرسینیا* در جنین‌های سقط‌شده گوسفند و بز در شمال‌غرب ایران (استان آذربایجان شرقی) انجام شد. برای این منظور، ۶۲ جنین سقط‌شده و ۲۸۸ نمونه سواب واژینال از گله‌های گوسفند و بز جمع‌آوری شد. ابتدا، کشت باکتریایی روی نمونه‌های سواب واژینال از بالغین و بافت‌های مختلف و محتویات شیردان جنین‌ها انجام گرفت. سپس، نمونه‌های بافتی با استفاده از هیستوپاتولوژی و مطالعات مولکولی با روش PCR معمولی و پرایمرهای اختصاصی مورد مطالعه و بررسی قرار گرفتند. همچنین، نمونه‌های بافتی تثبیت‌شده با فرمالین برای بررسی‌های هیستوپاتولوژیک به‌صورت معمول پردازش شدند. ژنوم‌های *سالمونلا* و *یرسینیا* به‌ترتیب در ۱۴،۵۱٪ و ۱۲،۹٪ از جنین‌های بررسی‌شده با PCR شناسایی شدند، و دو جنین (۳،۲۲٪) برای هر دو عفونت مثبت بودند. مطالعات باکتری‌شناسی تنها یک نمونه سواب واژینال مثبت برای *سالمونلا* را نشان داد. همچنین، ضایعات پاتولوژیک در جنین‌های مثبت برای *یرسینیا* شامل هپاتیت نکروتیک چندکانونی، پنومونی بینابینی، میوکاردیت چندکانونی و انتریت منتشر همراه با نفوذ قابل‌توجه سلول‌های تک‌هسته‌ای بود. در جنین‌های مثبت برای *سالمونلا*، ضایعات هیستوپاتولوژیک اصلی شامل هپاتیت نکروتیک و چرکی کانونی تا چندکانونی در کبد با ندول‌های پاراتیفوئیدی و برونکوپنومونی چرکی با حضور نوتروفیل‌ها بود. در نتیجه، با توجه به یافته‌های این مطالعه، نرخ بالای عفونت‌های *سالمونلا* و *یرسینیا* در جنین‌های سقط‌شده لزوم توجه جدی به تأثیر آن‌ها بر خسارات اقتصادی و سلامت عمومی را برجسته می‌کند.

واژگان کلیدی: نشخوارکنندگان کوچک، سقط جنین، *سالمونلا*، *یرسینیا*، سلامت عمومی

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