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Original Article

Brucella as the main cause of abortion among livestock in Armenia

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Abstract

Introduction: Abortion in livestock can have a significant impact on animal husbandry, as well as raise public health concerns when caused by zoonotic pathogens. Thus, the involvement of bacterial (e.g., *Brucella* spp. and *Enterobacteriaceae*) and fungal infections in livestock abortions in Armenia was explored.

Methodology: From 2018 to 2022, 168 aborted foetal tissues from cattle and small ruminants in Armenia were tested for fungal and *Enterobacteriaceae* infections by culture. The API 20E biochemical test was performed on bacteria-positive samples. Culture-negative samples were further tested by qPCR to detect Brucella DNA. In all qPCR-positive aborted foetuses, maternal blood samples (n = 129) were collected ≥ 30 days post-abortion for serological diagnosis.

Results: Overall, 33 foetal samples were positive by culture: 28 for Aspergillus spp. and 5 for Salmonella spp. Brucella DNA was detected in 129 out of 135 culture-negative samples; in addition, anti-Brucella antibodies were found in 124 maternal blood samples. A total of 6 (3.5%) samples were classified as indeterminate by any assay.

Conclusions: Our results suggest that Brucella is the major cause of abortions in cattle and small ruminants in Armenia, while other bacterial and fungal infections were involved in less than 20% of cases. Based on these findings, it is recommended to test all samples first by serology and qPCR to detect Brucella infections. For Brucella-negative samples, additional methods can be used to detect other abortifacient agents. This protocol will be useful for laboratories that operate at Biosafety Level 2 and are unable to isolate this bacterium.

Key words: *Brucella*; Armenia; abortions; livestock; fungal infection; *Enterobacteriaceae*.

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Introduction

Abortions in livestock can result in significant economic losses due to reduced meat and milk production, which includes the loss of potential offspring that would contribute to the overall herd size. The economic hardships are compounded by the additional costs for veterinary services, treatment, and re-breeding of the animals [1]. These issues are amplified when the cases of abortions are infectious, since in this case, the abortions can be extensive in a herd [2,3]. Moreover, some abortifacient agents such as *Brucella* spp., *Chlamydia abortus*, and *Coxiella burnetii* are zoonotic, which increases the public health importance of these concerns, requiring a balance in managing and controlling the infectious agents to protect both animals and humans [4]. Viruses, parasites, fungi, and bacteria can cause abortions among animals, including *C. abortus*, *C. burnetii*, *Toxoplasma gondii*, *Salmonella enterica*, *Campylobacter* spp., *Brucella* spp., and pestivirus, the most common affecting livestock [5-9]. The distribution, prevalence, and role of these and other infectious agents in causing abortion depend on various factors, including the epidemiological situation, herd management practices, herd size, and surveillance and control strategies [10-13].

Brucellosis was first identified in the 1850s in Malta [14] and described as a disease with a series of fevers. It has since expanded to have a distribution with more than 500,000 human cases per year worldwide [15]. More recent statistical risk modeling and analyses suggest that underreporting and misdiagnosis could raise this estimate to as high as 2 million cases per year [16]. The Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (WOAH) consider brucellosis to be one of the most widespread zoonoses worldwide [17]. In livestock, brucellosis primarily affects sexually mature animals and results in late-trimester abortions, weak calves, and infertility characterized by inflammation of the placenta and epididymitis. Diseased animals excrete the pathogen through uterine discharge, vaginal discharge, and milk [18]. These bacteria can also spread within the herd through ingestion of contaminated material [19]. Brucellosis can be viewed as an animal disease in which

humans are the accidental host. However, in countries such as Armenia, where brucellosis is endemic, the incidence of brucellosis in high-risk subpopulations may exceed 10%. The disease in humans occurs through ingestion or inhalation of the pathogen, which can occur by direct contact through skin abrasions and contact with infected animals (particularly aborted foetuses, foetal membranes, and vaginal secretions) and consumption of unprocessed milk products [20-22].

Although bovine brucellosis has been eradicated in many high-income countries in Europe as well as in Australia, Canada, Israel, Japan, and New Zealand, it is still widespread geographically, and uncontrolled cases are reported in areas of Africa, the Mediterranean, the Middle East, and Central Asia, where the disease is endemic. In the Caucasus Region, the disease is endemic in Armenia, Azerbaijan, Turkey, and Georgia [23-26]. In many low-income countries, the prevalence of brucellosis in humans and animals is increasing due to a lack of education and awareness concerning the disease and implementation of disease prevention practices [27].

Considering the economic and public health importance of brucellosis in Armenia, animal surveillance and control measures are implemented with support from the governmental budget. The required activities are carried out in accordance with the instructions and regulations under the order 418-N "Instruction for brucellosis control and surveillance measures," approved by the Head of the Food Safety Inspection Body (FSIB) in 2013 [28]. Control measures are based on a test-and-slaughter approach and are conducted twice a year among sexually mature cattle and small ruminants (e.g., sheep and goats). Diagnosis is carried out by serological methods in regional laboratories using the Rose Bengal test (RBT). Positive samples are then sent to the Reference Laboratory for Especially Dangerous Pathogens (RLEDP) for final confirmation. In this study, we investigated the role of *Brucella* spp. and other bacterial and fungal abortifacient agents as causes of abortions in cattle and small ruminants in Armenia.

Methodology

Study area and sample collection

Since this was the first study conducted in Armenia investigating the causes of abortion in livestock, previously published research identifying brucellosis, fungal infections, and *Enterobacteriaceae* as common infectious agents [5,7,9] was reviewed to inform the

Figure 1. Distribution of reported cases of abortions in cattle and small ruminants in 10 Armenian regions (Marzes) from 2018-2022.

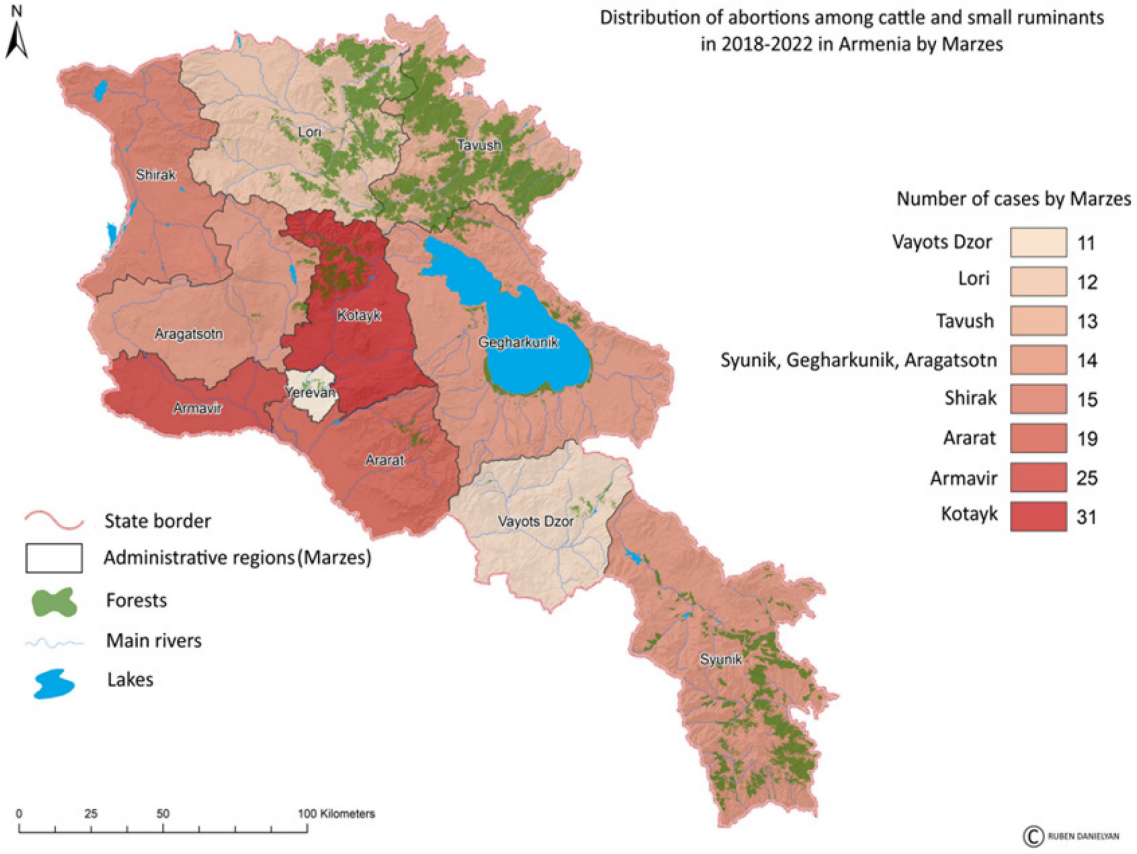


Table 1. Sample collection by year.

Year	Cattle		Small Ruminants		Total Samples	
	Aborted Fetus	Maternal Blood	Aborted Fetus	Maternal Blood	Aborted Fetus	Maternal Blood
2018	19	13	22	19	41	32
2019	24	19	13	9	37	28
2020	10	8	21	18	31	26
2021	15	10	23	17	38	27
2022	9	5	12	11	21	16
Total	**77**	**55**	**91**	**74**	**168**	**129**

initial testing strategy.

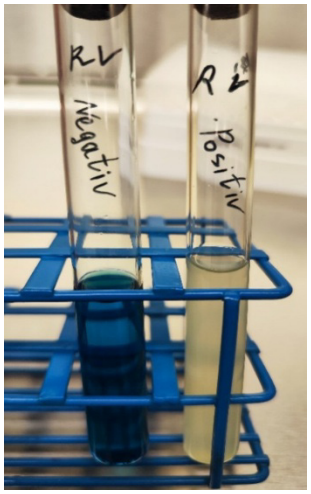
The study was conducted from 2018 to 2022 at the RLEDP of the Food Safety Inspection Body (FSIB) of the Republic of Armenia. RLEDP is the only laboratory in the country responsible for the final diagnosis of infectious diseases in animals that received samples from all 10 regions of Armenia during this time frame (Figure 1). Accordingly, 168 aborted foetus samples (77 from cattle and 91 from small ruminants) and 129 maternal blood samples (55 cattle and 74 small ruminants) were received and analysed (Table 1). The sample collection process was as follows. Owners of ill animals contacted community veterinarians, who work in collaboration with inspectors from the FSIB (the state-approved organization in the veterinary field) to collect field samples from the animals. FSIB inspectors and community veterinarians followed strict biosafety and biosecurity standard operating procedures (SOPs) to collect samples before transporting them to the RLEDP Biosafety Level 2 (BSL-2) laboratory, typically within 24 hours of the discovery of the aborted foetus. Inspectors either transported the entire foetus or performed a necropsy and transported individual organs to the Department of Pathology and Histology at RLEDP. Upon arrival, the foetus or organs were processed in biosafety cabinets by laboratory staff, following strict biosafety and biosecurity SOPs to protect personnel and prevent cross-contamination. Tissue samples of 4–5 grams (including spleen, liver, and/or lymph nodes, as well as foetal stomach contents) were separated and provided to the Department of Microbiology and Parasitology for further testing. Maternal blood samples were collected in the field using vacutainer tubes and transported to RLEDP. In the laboratory, blood samples were centrifuged at 1,500 × g for 10 minutes. Serum was aliquoted and stored at 2–8°C for short-term use or at –20°C for long-term storage, depending on the testing schedule.

All individual samples from the 168 aborted foetuses were analysed according to the following algorithm. Firstly, detection of bacterial (*Enterobacteriaceae*) and fungal infections was performed by traditional culture methods; biochemical tests were performed in those samples that produced colonies typical of *Salmonella* spp. or *Enterobacteriaceae*. In all cases where the samples were negative for *Salmonella* or fungi, samples were tested by qPCR for detecting *Brucella* DNA; no *Brucella* culture or isolation could be performed in the RLEDP laboratory since its biosafety level is BSL-2. If *Brucella* DNA was detected in an aborted foetus, maternal blood samples were collected 30-40 days after the abortion to be tested for detecting anti-*Brucella* antibodies using serological methods (RBT, enzyme-linked immunosorbent assay -ELISA-, and fluorescence polarization assay -FPA-). Armenia follows a specific diagnostic algorithm for the diagnosis of brucellosis among livestock, which includes both screening and confirmatory testing. According to this algorithm, an animal is considered brucellosis-positive when at least two out of three serological tests yield positive results.

Microbiological testing

All tissue samples from aborted foetuses were examined separately (e.g., some cases provided multiple samples for each foetus). Before plating on agar, the samples were homogenized using a Glas-Col tissue homogenizer (Glas-Col, LLC, Terre Haute, USA) at 4,000 rpm for 1 minute. Then samples were transferred to plastic tubes.

Figure 2. Results on Rappaport-Vassiliadis Soya broth enrichment media.



Samples were cultured on MacConkey agar at 37 °C for 24 hours [29] for identifying Gram-negative enteric species. In parallel, for the selective isolation and differentiation of *Salmonella* spp., samples were first enriched in Rappaport-Vassiliadis Soy broth at 41 °C for 24-72 hours. If *Enterobacteriaceae* are present, the blue colour of the medium becomes turbid (Figure 2). Subsequently, samples were re-cultured on three selective media (Xylose Lysine Deoxycholate -XLD- agar, Hektoen Enteric -HE- agar, and Brilliant Green agar) at 37°C for 24 hours [30]. On XLD, typical *Salmonella* colonies have clear (transparent) borders and a black centre, with a reddish-pink band visible in the surrounding medium (Figure 3). On HE agar, *Salmonella* spp. produce transparent green or blue-green colonies with or without black centers, and may appear as almost completely black colonies (Figure 4). On Brilliant Green agar, typical *Salmonella* colonies appear as pinkish-white or red colonies surrounded by a red halo in the medium (Figure 5).

Finally, samples were cultured in Lysine Decarboxylase Broth, Urea Broth, and on Triple Sugar Iron (TSI) agar to confirm typical *Salmonella* colonies [30]. In the case of a positive reaction for *Salmonella* spp. the color of the Lysine Decarboxylase Broth remains unchanged (Figure 6). Urea broth can be used to determine the urea activity of *Enterobacteriaceae* microorganisms. Positive urease tubes turn the phenol indicator a deep violet-red color, while negative urease tubes show no color change and remain yellow (Figure 7). When using TSI agar, the presence of bubbles or splits in the medium indicates gas formation. If present, the black butt represents the production of H_2S. In the case of fermentation of *Salmonella typhimurium* on TSI agar, a red slope, a yellow butt, and gas production are observed. If the sample is *Salmonella enterica*, a red slope, yellow butt with blackening of the butt, and gas production are present..

Figure 3. Result on XLD selective media.

Figure 4. Result on Hektoen Enteric selective media.

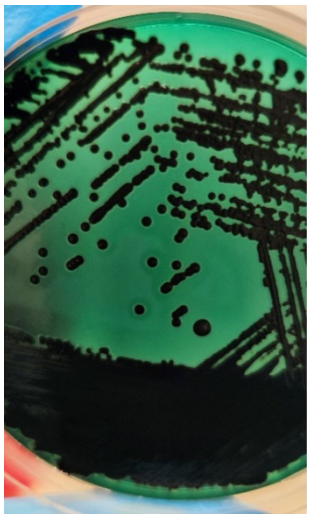
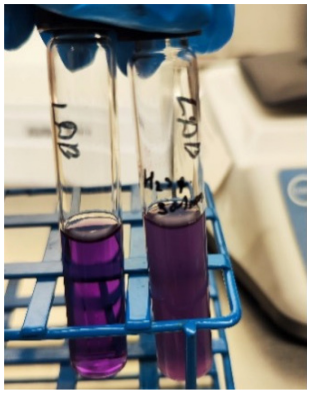


Figure 5. Result on Brilliant Green selective and differential media.

Figure 6. Result on Lysine Decarboxylase Broth media.



API 20E biochemical test (Biomerieux, Inc., Marcy-I'Etoile, France) was used for bacterial identification. Sample preparation for API 20E for *Salmonella* and other *Enterobacteriaceae* species was carried out according to the manufacturer's instructions. Briefly, two suspected colonies from XLD and Hektoen Enteric (1 suspected colony from each media) agar were transferred to non-selective Columbia agar, then incubated at 36 ± 1°C for 18-22 hours. A single colony is then picked and placed in sterile distilled water to create a suspension that is aliquoted into each well of the API 20E strip. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of certain reagents, depending on the test. The reactions are read according to the API Reading Scale by color and then identified using the API catalogue or online resource. These results are then combined with the TSI agar-based results to suggest the potential presence of *S. typhimurium*; however, true confirmation should be performed using serological or molecular methods.

The examination of fungi was conducted by culturing on Sabouraud or Czapeck Dox agars (depending on the availability), followed by incubation at 25°C for 5 days. Other aspects of vegetative morphology commonly used for identification purposes were colony color, diffusible pigments, metabolites, and mycelial structures. On Sabouraud agar, *Aspergillus* colonies are round, with deep grooves running from the centre of the colony to the periphery. *Aspergillus* colonies vary widely in size, color, and germination rate. Different species of *Aspergillus* can vary in color, surface, and conidial heads on Czapeck Dox agar, which supports the growth of most saprophytic Aspergilli. Fungal cultures positive for *Aspergillus* produced black woolly colonies (Figure 8). Petri dishes containing typical fungal growth were also examined microscopically by magnification powers of 40x and 100x, with typical *Aspergillus* spp. identified (Figure 9).

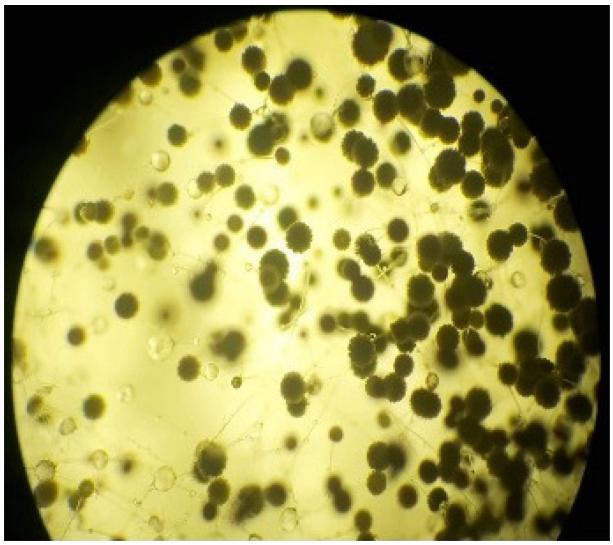
Figure 8. Colonies on Czapeck Dox agar agar.

Figure 7. Result on Urea broth media.

qPCR for detecting Brucella DNA

DNA extraction was performed from homogenized tissue samples using the ID Gene Mag Fast Extraction Kit (Innovative Diagnostics, Grabels, France) following the manufacturer's specifications. For DNA detection, the ID Gene *Brucella* spp triplex assay (Innovative Diagnostics, Grabels, France) was used following the manufacturer's instructions. This qPCR assay allows detecting *Brucella abortus, B. melitensis,* and *B. suis*, but does not differentiate the species. A positive *Brucella* DNA control was included with an expected cycle threshold (Ct) value of 28. Samples with

Figure 9. Microscopic characters of Aspergillus isolates (40x).



Ct values between 24-32 were considered positive.

Serological detection of anti-Brucella antibodies

The RBT was performed in duplicate with a validated antigen (IDvet Rose Bengal Antigen, Grabels, France) according to the manufacturer's instructions. Briefly, the serum sample and antigen were mixed in equal volumes of 30 µL, shaken for 4 minutes, and observed for the presence of agglutination.

FPA was performed in duplicate using the Brucella FPA test kit (Ellie LLC, USA) according to the manufacturer's instructions. Blank readings of all samples and controls were taken using the FPA device (Brucella FPA®, Diachemix, LLC, USA), and the millipolarization (mP) units were recorded, followed by a final test reading to determine the change in mP values compared to the negative control. The ΔmP values of ≤ 10 were considered negative, values between 10 to 20 were considered suspect, and values > 20 were considered positive.

For ELISA, the ID Screen Brucellosis Serum Indirect Multi-species test (IDvet, France) was used for the detection of antibodies against *Brucella abortus*, *Brucella melitensis,* and *Brucella suis,* according to the manufacturer's instructions.

Results

Among the 168 foetal tissue samples evaluated by the microbiological tests, 28 (16.6%) samples were identified as *Aspergillus* species (Table 2). This fungal species was associated with 11.7% (9/77) of cattle abortions and 20.9% (19/91) of small ruminant abortions. The percentage of positive aborted foetuses varied by year, ranging from 12.2 to 21.1 (Table 2). *Aspergillus* species was identified in tissues from all 10 regions of Armenia (Table 2). Additionally, *Salmonella* spp. was found in 5 (3.0%) out of the 168 foetus samples (Table 2). According to the API 20E biochemical test results combined with the TSI agar testing, four samples were preliminarily identified as *S. typhimurium*, with the last case only identified as *Salmonella* sp. It should be noted that accurate identification of *S. typhimurium* requires confirmation through serological or molecular techniques. *Salmonella*-positive samples included three cases from cattle (3.9%) and two cases (2.2%) from small ruminants. This bacterium was sporadically detected across all years except 2020 and was found in 50% of the studied regions (Table 2).

Samples negative by microbiological testing (n = 135) were tested by PCR for brucellosis. Of these, 129 samples (95.5%) were positive, with 63 cases in cattle and 66 in small ruminants. *Brucella*-positive cases from aborted foetuses were recorded in all years - with positivity rates consistently above 93% - and across all regions. The lowest number of cases was detected in the Tavush Region, while the highest was found in the Kotayk Region. (Table 2). The follow-up serological testing of 129 maternal blood samples identified 124 (96.1%) samples as positive for anti-*Brucella* antibodies. Positive animals were again distributed across all regions and years (Table 2), with 51 out of 55 cattle samples (92.7%) and 73 out of 74 small ruminant samples (98.6%) confirmed as Brucella-positive by serology. A total of 6 out of 168 cases (3.6%) remained undiagnosed during the study.

Table 2. Number of samples tested and positive results by year.

	Aborted Fetus			**Maternal Blood Sample**
	***Aspergillus* spp.+/n (%)**	***Salmonella* spp.+/n (%)**	**PCR for Brucellosis +/n (%)**	**Serology for Brucellosis +/n (%)**
Year				
2018	5/41 (12.2)	2/41 (4.9)	32/34 (94.1)	30/32 (93.7)
2019	7/37 (18.9)	1/37 (2.7)	28/29 (96.6)	27/28 (96.4)
2020	5/31 (16.1)	0/31 (0)	26/26 (100)	26/26 (100)
2021	8/38 (21.1)	1/38 (2.6)	27/29 (93.1)	25/27 (92.6)
2022	3/21 (14.3)	1/21 (4.8)	16/17 (94.1)	16/16 (100)
Total	**28/168 (16.7)**	**5/168 (3)**	**129/135 (95.5)**	**124/129 (96.1)**
Region*				
Kotayk	3	0	27	26
Armavir	2	1	20	20
Ararat	2	1	16	16
Gegharkunik	2	0	12	12
Aragatsotn	1	2	11	11
Shirak	2	0	10	10
Syunik	4	0	10	9
Vayots Dzor	2	0	9	9
Lori	4	0	8	8
Tavush	6	1	6	3

* No data on the total number of samples from each region was available.

Discussion

This study presents novel data, as it is the first epidemiological investigation of certain bacterial and fungal pathogens affecting domestic ruminants in Armenia. Our results indicate that between 2018 and 2022, brucellosis was the leading cause of abortion in cattle and small ruminants in Armenia, with the highest prevalence observed in 2020. Although the vast majority of abortions in Armenia were caused by brucellosis, other investigations report *C. burnetii, C. abortus, T. gondii*, and *Campylobacter* spp. as the leading causes of infectious abortions [1,3,4,6,8,10-12,31].

It is important to note that several factors during the study period may have affected the accurate identification of all brucellosis cases and potential dual infections. For instance, in 2020, veterinary services were disrupted due to the COVID-19 pandemic, and further complications arose from the war in Nagorno-Karabakh. These events led to increased uncontrolled movement of animals and hindered the implementation of comprehensive surveillance and control measures. Many veterinarians and FSIB inspectors were unable to perform their duties due to COVID-19 restrictions. Additionally, several staff members, including FSIB inspectors, were reassigned to support COVID-19 testing and response efforts, limiting their capacity to carry out routine veterinary responsibilities.

The highest numbers of abortion cases related to *Brucella* infection were detected in the Kotayk, Armavir, and Ararat Regions, likely due to geographic and demographic factors. Kotayk serves as a major crossroads for regular animal movement during the pasture season, increasing the risk of disease transmission. Additionally, these three regions have large populations of the Yezidi national minority, whose primary occupation is animal husbandry. Many of the small ruminants they raise are not registered or included in official surveillance programs, which complicates disease control efforts and limits the scope of epidemiological studies.

One of the limitations of this study was the lack of certain diagnostic tests in the laboratory, which prevented the identification of the causative agents in a small number of abortion cases. Specifically, diagnostic tools for both bacterial and viral pathogens have yet to be implemented, which may have helped identify the remaining 3.6% of undiagnosed cases. Furthermore, tests for detecting potential mixed infections were not performed, and this limitation should be addressed in future studies. Given the high percentage of Brucella-positive samples observed in this study, the diagnostic algorithm should be revised to ensure that all samples are systematically tested for *Brucella* infection. Therefore, the percentage of abortions caused by *Brucella* in Armenia is most likely underestimated, as it is probable that some samples testing positive for other bacterial and fungal infections were also co-infected with *Brucella*. The limitations regarding diagnostic capacity have been presented to the relevant state authorities to encourage the implementation and provision of additional diagnostic kits and reagents. These enhancements are essential to strengthen the diagnostic capabilities of the RLEDP and improve the accuracy of future surveillance efforts.

Armenia's current brucellosis surveillance and control strategy follows a test-and-slaughter policy conducted twice yearly for sexually mature cattle and small ruminants, once at the beginning of the pasture season (March - April), and again at its end (October - November). However, samples are also submitted by local veterinarians and FSIB inspectors outside of these designated periods for the diagnosis of infectious animal diseases. Abortion cases reported to veterinarians and FSIB inspectors underscore the need for expanded diagnostic testing beyond the routine schedule. One limitation is that not all abortions are reported, and not all foetal samples are submitted for laboratory diagnostic testing. As a result, the data presented here may not fully reflect the overall number of abortions or their underlying causes. Based on our experience working with farmers and veterinarians in Armenia, there is a prevailing stereotype that brucellosis is the sole cause of abortions. This perception may lead to the concealment or underreporting of abortion cases by farmers seeking to avoid financial losses. Nevertheless, the additional cases that are reported allow for year-round diagnostic testing, highlighting shortcomings in the current test-and-slaughter strategy. Another concern is the extended intervals between planned testing periods (May - September and December - February), which may contribute to gaps in disease detection and control. Furthermore, the absence of a national animal identification system in Armenia presents a significant challenge, as it leaves an unknown number of animals unaccounted for, potentially evading annual testing and continuing to harbor and spread infectious diseases.

In addition to *Brucella*, the identification of *Aspergillus* spp. and *Salmonella* spp. in aborted foetal samples in this study warrants further attention and shows that more work is needed to include testing for multiple pathogens in aborted foetuses from Armenia. Although not commonly reported as leading causes of

abortion in livestock, both pathogens have been implicated in sporadic abortion events globally. *Aspergillus* spp., particularly *A. fumigatus*, have been associated with bovine and ovine abortions in several countries, including the United States, India, and parts of Europe, where fungal placentitis and mycotic abortions have been documented [31–33]. *Salmonella* spp., especially *S. typhimurium* and *Salmonella dublin*, are also recognized causes of abortion in cattle and small ruminants in countries such as the UK, the USA, and Canada [34–36]. Both pathogens also carry significant zoonotic risks. Farmers, veterinarians, and laboratory staff may be exposed during the handling of infected aborted materials or contaminated tissues, especially in the absence of proper protective measures. *Salmonella* can be transmitted through direct contact or ingestion of contaminated animal products, leading to gastrointestinal illness or systemic infection in humans. Likewise, *Aspergillus* spp. can cause opportunistic infections, particularly in immunocompromised individuals, and inhalation of fungal spores poses a respiratory health risk [37-39]. These findings reinforce the importance of incorporating biosafety protocols and personal protective equipment (PPE) during sampling, handling, and laboratory procedures, and highlight the need to educate veterinary personnel and farmers about the zoonotic implications of these pathogens.

Conclusions

Our results revealed that *Brucella* spp. is responsible for the majority of abortion cases in domestic ruminants from Armenia, although *Aspergillus* spp. and *Salmonella* spp. were also identified in a limited number of cases. Further studies for unravelling the role of viruses in abortions of domestic ruminants are needed. Given the high prevalence of Brucella in aborted fetuses and the limitations of our BSL-2 laboratory, which prevents us from isolating Brucella, we recommend that all abortion cases be screened for brucellosis using serological methods and qPCR. Samples testing negative for Brucella should then be examined using microbiological techniques to identify other bacterial and fungal pathogens. This approach would optimize diagnostic efficiency while minimizing the risks associated with handling Brucella, which requires BSL-3 containment.

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Conflict of interest

No conflict of interest is declared.

References

1. Alzuguren O, Domínguez L, Chacón G, Benito AA, Mencía-Ares O (2023) Infectious abortions in small domestic ruminants in the Iberian Peninsula: Optimization of sampling procedures for molecular diagnostics. Front Vet Sci 10: 1152289. doi: 10.3389/fvets.2023.1152289.
2. Entrican G, Wattegedera S, Rocchi M, Wheelhouse N (2009) Pregnancy, indoleamine 2,3-dioxygenase (IDO) and chlamydial abortion: an unresolved paradox. Vet Microbiol 135: 98-102. doi: 10.1016/j.vetmic.2008.09.028.
3. Vidić B, Savić-Jevđenić S, Grgić Ž, Bugarski D, Maljković M (2007) Infectious abortion in sheep. Biotechnol. Anim. Husb 23: 383-389.
4. Alemayehu G, Mamo G, Alemu B, Desta H, Tadesse B, Benti T, Bahiru A, Yimana M, Wieland B (2021) Causes and flock level risk factors of sheep and goat abortion in three agroecology zones in Ethiopia. Front Vet Sci 8: 615310. doi: 10.3389/fvets.2021.615310.
5. Amagliani G, La Guardia ME, Dominici S, Brandi G, Omiccioli E (2021) Salmonella Abortusovis: An epidemiologically relevant pathogen. Curr. Microbiol. 79: 3533. doi: 10.1007/s00284-021-02689-1.
6. Berri M, Rekiki A, Boumedine KS, Rodolakis A (2009) Simultaneous differential detection of Chlamydophila abortus, Chlamydophila pecorum and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. BMC Microbiol 9: 130. doi: 10.1186/1471-2180-9-130.
7. Borel N, Frey CF, Gottstein B, Hilbe M, Pospischil A, Franzoso FD, Waldvogel A (2014) Laboratory diagnosis of ruminant abortion in Europe. Vet J 200: 218-229. doi: 10.1016/j.tvjl.2014.03.015.
8. Fernández-Escobar M, Calero-Bernal R, Benavides J, Regidor-Cerrillo J, Guerrero-Molina MC, Gutiérrez-Expósito D, Collantes-Fernández E, Ortega-Mora LM (2020) Isolation and genetic characterization of *Toxoplasma gondii* in Spanish sheep flocks. Parasit Vectors 13: 396. doi: 10.1186/s13071-020-04275-z.

9. Rossetti CA, Maurizio E, Rossi UA (2022) Comparative review of brucellosis in small domestic ruminants. Front Vet Sci 9: 887671. doi: 10.3389/fvets.2022.887671.
10. Esmaeili H, Bolourchi M, Mokhber-Dezfouli MR, Khaltabadi Farahani R, Teimourpour A (2021) Detection of *Chlamydia a*bortus and risk factors for infection in small ruminants in Iran. Small Ruminant Research 197: 106339. doi: 10.1016/j.smallrumres.2021.106339.
11. Nayeri T, Sarvi S, Moosazadeh M, Daryani A (2021) Global prevalence of *Toxoplasma gondii* infection in the aborted fetuses and ruminants that had an abortion: A systematic review and meta-analysis. Vet Parasitol 290: 109370. doi: 10.1016/j.vetpar.2021.109370.
12. Van Engelen E, Luttikholt S, Peperkamp K, Vellema P, Van den Brom R (2014) Small ruminant abortions in The Netherlands during lambing season 2012-2013. Vet Rec 174: 506/ doi: 10.1136/vr.102244.
13. Zendoia II, Barandika JF, Hurtado A, López CM, Alonso E, Beraza X, Ocabo B, García-Pérez AL (2021) Analysis of environmental dust in goat and sheep farms to assess *Coxiella burnetii* infection in a Q fever endemic area: Geographical distribution, relationship with human cases and genotypes. Zoonoses and Public Health 68: 666-676. doi: 10.1111/zph.12871.
14. Wyatt HV (2013) Lessons from the history of brucellosis. Rev Sci Tech 32: 17-25. doi: 10.20506/rst.32.1.2181.
15. Rubach MP, Halliday JE, Cleaveland S, Crump JA (2013) Brucellosis in low-income and middle-income countries. Curr Opin Infect Dis 26: 404-412. doi: 10.1097/QCO.0b013e3283638104.
16. Laine CG, Johnson VE, Scott HM, Arenas-Gamboa AM (2023) Global estimate of human brucellosis incidence. Emerg Infect Dis 29: 1789-1797. doi:10.3201/eid2909.230052.
17. Corbel MJ (2006) Brucellosis in humans and animals. World Health Organization. Available https://iris.who.int/handle/10665/43597. Assessed: 11 March 2024.
18. Głowacka P, Żakowska D, Naylor K, Niemcewicz M, Bielawska-Drózd A (2018) Brucella - virulence factors, pathogenesis and treatment. Pol J Microbiol 67: 151-161. doi: 10.21307/pjm-2018-029.
19. Chen S, Zhang H, Liu X, Wang W, Hou S, Li T, Zhao S, Yang Z, Li C (2014) Increasing threat of brucellosis to low-risk persons in urban settings, China. Emerg Infect Dis 20: 126-130. doi: 10.3201/eid2001.130324.
20. Dhanashekar R, Akkinepalli S, Nellutla A (2012) Milk-borne infections. An analysis of their potential effect on the milk industry. Germs 2: 101-109. doi: 10.11599/germs.2012.1020.
21. Khurana SK, Sehrawat A, Tiwari R, Prasad M, Gulati B, Shabbir MZ, Chhabra R, Karthik K, Patel SK, Pathak M, Iqbal Yatoo,M, Gupta VK, Dhama K, Sah R, Chaicumpa W (2021) Bovine brucellosis - a comprehensive review. Vet Q 41: 61-88. doi: 10.1080/01652176.2020.1868616.
22. Moreno E (2014) Retrospective and prospective perspectives on zoonotic brucellosis. Front Microbiol 5. doi: 10.3389/fmicb.2014.00213.
23. Akhvlediani T, Bautista CT, Garuchava N, Sanodze L, Kokaia N, Malania L, Chitadze N, Sidamonidze K, Rivard RG, Hepburn MJ, Nikolich MP, Imnadze P, Trapaidze N (2017) Epidemiological and clinical features of brucellosis in the country of Georgia. PLoS One 12: e0170376. doi: 10.1371/journal.pone.0170376.
24. Khatibi M, Abdulaliyev G, Azimov A, Ismailova R, Ibrahimov S, Shikhiyev M, Agalarov D, Seyidov T, Omarov A, Suleymanova C, Zeynalova S, Abdullayev R, Hajiyeva A, Jackson R (2021) Working towards development of a sustainable brucellosis control programme, the Azerbaijan example. Res Vet Sci 137: 252-261. doi: 10.1016/j.rvsc.2021.05.014.
25. Porphyre T, Jackson R, Sauter-Louis C, Ward D, Baghyan G, Stepanyan E (2010) Mapping brucellosis risk in communities in the Republic of Armenia. Geospat Health 5: 103-118. doi: 10.4081/gh.2010.191.
26. Yumuk Z, O'Callaghan D (2012) Brucellosis in Turkey - an overview. Int J Infect Dis 16: e228-235. doi: 10.1016/j.ijid.2011.12.011.
27. Zhen Q, Lu Y, Yuan X, Qiu Y, Xu J, Li W, Ke Y, Yu Y, Huang L, Wang Y, Chen Z (2013) Asymptomatic brucellosis infection in humans: implications for diagnosis and prevention. Clin Microbiol Infect 19: E395-397. doi: 10.1111/1469-0691.12250.
28. FAO (2013) Order No.418-N of the Chief of State Veterinary Service validating the regulation on brucellosis control and prevention. Available: https://www.ecolex.org/fr/details/legislation/order-no-418-n-of-the-chief-of-state-veterinary-service-validating-the-regulation-on-brucellosis-control-and-prevention-lex-faoc185067/. Accessed: 19 June 2024.
29. International Organization for Standardization (2017) Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae*. Part 1: Detection and enumeration by MPN technique with pre-enrichment. ISO 21528-1:2017(E). Available https://www.iso.org/standard/55228.html. Accessed: 11 August 2024.
30. International Organization for Standardization (2017) Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp. ISO 6579-1:2017. Available https://www.iso.org/standard/56712.html. Assessed: June 19 2024.
31. Caswell JL, Williams KJ (2016) Fungal and algal diseases of the respiratory system. In: Maxie MG (ed.), Jubb, Kennedy & Palmer's Pathology of domestic animals, 6th ed., Vol. 2, Elsevier: 533–560.
32. Krishnamoorthy P, Raj GD, Nachimuthu K, Kumanan K (2007) Mycotic abortion in dairy cattle due to Aspergillus fumigatus. Mycopathologia 164: 245–247, doi:10.1007/s11046-007-9047-7.
33. Wouda W, Peperkamp NHMT, Roumen MPHJM (1997) Incidents of abortion in cattle associated with mycotic placentitis. Veterinary Quarterly 19: 94–98. doi: 10.1080/01652176.1997.9694766.
34. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesús J, Platt DJ, Olsen JE (2000) Host adapted serotypes of Salmonella enterica. Epidemiol Infect 125: 229–255. doi: 10.1017/S0950268899004379.
35. Wray C, Davies RH (2000) Salmonella in domestic animals. CABI Publishing, Wallingford, UK, 429-446 pp.
36. Smith BP, Reina-Guerra M, House JK, Döpfer D, Smith NH (2020) Salmonella Dublin: an emerging infectious disease for the dairy industry. J Vet Interm Med 34: 1673–1683. doi:10.1111/jvim.15817.
37. Latgé JP, Chamilos G (2020) *Aspergillus fumigatus* and aspergillosis in humans: pathogenicity, clinical manifestations,

and treatment. CSH Perspect Med 10: a038430. doi: 10.1101/cshperspect.a038430.

38. Klich MA (2009) Environmental and occupational exposure to *Aspergillus fumigatus*. Med Mycol 47: S107–S112. doi: 10.1080/13693780802708031.

39. Seyedmousavi S, Guillot J, Arné P, de Hoog GS, Mouton JW, Melchers WJ, Verweij PE (2015) Aspergillus and aspergillosis in wild and domestic animals: a global health concern with parallels to human disease. Med Mycol 53: 765-797. doi:10.1093/mmy/myv067.