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Descriptive epidemiology of brucellosis in small ruminants and swine in the highland zones of Armenia

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Abstract. Brucellosis is an important zoonotic disease that causes substantial economic losses in livestock production and poses a serious public health concern. Despite long-standing surveillance and control activities, brucellosis continues to affect livestock populations in several high-altitude regions of Armenia. This study aimed to describe the epidemiological characteristics of brucellosis in small ruminants and swine and to estimate the associated economic losses. During 2021–2025, sero-epidemiological monitoring was conducted in five high-altitude regions of Armenia: Aragatsotn, Gegharkunik, Kotayk, Shirak, and Syunik. A total of 50,000 small ruminants and 5,000 swine were examined. Serum samples were initially screened using the Rose Bengal Test (RBT), while RBT-positive or doubtful samples underwent further assessment by the Serum Agglutination Test (SAT) and enzyme-linked immunosorbent assay (ELISA). Epidemiological indicators, including seroprevalence, focus index, and unsafe area index, were calculated, and economic losses associated with forced slaughter and reproductive failure were estimated. Overall, 6,727 seropositive small ruminants (13.5%) and 20 seropositive swine (0.4%) were identified. The highest small-ruminant seroprevalence was recorded in Gegharkunik and Aragatsotn, whereas swine seropositivity was geographically restricted and highest in Kotayk. The sequential serological approach showed close agreement between RBT screening and subsequent SAT and ELISA findings. Total estimated economic damage amounted to 885.09 million Armenian drams (AMD), including losses associated with forced slaughter and reproductive failure. The findings demonstrate a substantial epidemiological and economic burden of brucellosis, particularly in small ruminants, and support strengthened risk-based surveillance, biosecurity, early detection, and coordinated One Health approaches to brucellosis control in high-risk livestock populations of Armenia.

Key words: brucellosis, small ruminants, pigs, epidemiology, high-altitude regions, zoonosis, serological diagnostics, Armenia

INTRODUCTION

Brucellosis is a chronic zoonotic disease of major veterinary and public health importance that causes reproductive disorders, production losses, and substantial economic damage in livestock systems. Among the causative agents, *Brucella melitensis* is primarily associated with sheep and goats and remains widely distributed in endemic livestock populations, particularly in the Mediterranean region, Asia, Africa, and parts of Central and South America (López et al., 2018; Li et al., 2023; Ameen et al., 2023). *Brucella suis* is primarily associated with swine, and previous investigations have documented the occurrence of swine brucellosis in Armenia (Sargsyan et al., 2022a). Despite long-standing control efforts, brucellosis remains one of the most economically important zoonotic infections and continues to constrain the sustainable development of livestock production systems (Sidorchuk et al., 2024).

Human-to-human transmission of brucellosis is exceedingly rare. Human infection generally occurs through direct or indirect contact with infected animals or contaminated biological materials, consumption of unpasteurized milk and other contaminated animal-origin products, and occupational exposure among livestock workers, veterinarians, slaughterhouse personnel, and laboratory staff (WHO et al., 2006; Smirnova et al., 2013; Kefaloudi et al., 2022). The zoonotic nature of the disease and the close epidemiological relationship between animal infection and human exposure make brucellosis particularly relevant within the One Health framework.

The genus *Brucella* comprises multiple species with distinct principal animal hosts and epidemiological characteristics. Species of major veterinary importance include *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, and *B. canis*, while additional *Brucella* species have been identified in wildlife, rodents, and marine mammals (WHO et al., 2006; Wattam et al., 2014; Kroese et al., 2018). *Brucella* spp. are facultative intracellular pathogens capable of surviving and replicating within host cells, particularly cells of the mononuclear phagocyte system. Infection may involve lymphoid tissues, reproductive organs, mammary glands, joints, and other organs, contributing to chronic and systemic disease manifestations (WHO et al., 2006; Wattam et al., 2014).

Infected animals constitute the principal source of environmental contamination and transmission within livestock populations. *Brucella* spp. may be excreted in large quantities through aborted fetuses, placental tissues, fetal fluids, vaginal secretions, and milk. Contaminated biological materials and the livestock environment may facilitate direct and indirect transmission, particularly during abortion and parturition. Transmission risk may be amplified in extensive and community-based livestock systems where animals belonging to different owners share grazing areas, watering points, or have frequent direct contact (WHO et al., 2006).

The epidemiology of brucellosis is strongly influenced by livestock production systems, animal movement, and opportunities for direct or indirect contact between susceptible and infected animals. Mixed-species husbandry and shared grazing environments may complicate surveillance and disease-control activities, particularly in community-based livestock systems (WHO et al., 2006; Sargsyan et al., 2024). Previous investigations in Armenia have documented brucellosis in both small-ruminant and swine populations; however, epidemiological information on swine remains comparatively limited (Sargsyan et al., 2022a; Sargsyan et al., 2024).

Diagnosis and surveillance of animal brucellosis rely on epidemiological information, clinical observations, and laboratory testing. Serological methods, including the Rose Bengal Test (RBT), Serum Agglutination Test (SAT), and enzyme-linked immunosorbent assay (ELISA), are widely used in surveillance and diagnostic algorithms, whereas molecular methods such as polymerase chain reaction (PCR) may provide additional information for the detection and differentiation of *Brucella* species (Popova et al., 2017; Bertelloni et al., 2018; Paul et al., 2020). The appropriate selection and sequential application of diagnostic methods are particularly important in large-scale epidemiological monitoring.

Despite long-standing surveillance and control activities, brucellosis continues to represent an important animal-health problem in Armenia. Previous community-level spatial analysis of national brucellosis control data demonstrated a widespread but geographically heterogeneous distribution of serological evidence of infection in Armenian livestock populations and identified the need for geographically targeted, risk-based surveillance and disease-control interventions (Porphyre et al., 2010). Earlier and more recent Armenian investigations have also documented continuing epidemiological concerns related to brucellosis in ruminant and swine populations (Egoyan et al., 2018; Sargsyan et al., 2022a; Sargsyan et al., 2024). However, updated integrated descriptive epidemiological information combining seropositivity, regional distribution, focus-level indicators, and direct economic losses in small ruminants and swine remains limited. This knowledge gap restricts the characterization of current brucellosis patterns in high-risk livestock populations and the development of more targeted surveillance and control priorities.

Accordingly, the present study addressed the following research questions: (1) what are the patterns of brucellosis seropositivity in small ruminants and swine in selected high-altitude regions of Armenia; (2) how do seroprevalence, focus index, and unsafe area index vary among the investigated regions and between the two animal groups; (3) what level of agreement is observed between RBT screening and subsequent SAT and ELISA findings within the sequential serological diagnostic approach; and (4) what are the estimated direct economic losses associated with forced slaughter and reproductive failure in the monitored livestock populations?

The scientific objective of this study was to provide an updated descriptive epidemiological assessment of brucellosis in small ruminants and swine in five high-altitude regions of Armenia and to characterize regional and species-specific patterns of seropositivity and epidemiological distribution. The practical objective was to generate evidence to support risk-based surveillance, veterinary decision-making, targeted disease-control measures, and coordinated One Health approaches for reducing the epidemiological and economic burden of brucellosis.

MATERIALS AND METHODS

Study area

The study was conducted in five administrative regions (marzes) of the Republic of Armenia: Aragatsotn, Gegharkunik, Kotayk, Shirak, and Syunik. Epidemiological investigations were carried out from 2021 to 2025. These regions include major highland livestock-producing areas characterized predominantly by extensive and community-

based livestock management, seasonal grazing, and movement of animals between settlements and pasture areas.

The five regions were included because of their epidemiological relevance to brucellosis surveillance and the presence of previously affected or epidemiologically suspected communities. The study therefore followed a risk-based monitoring approach rather than random population-based sampling. In the investigated areas, livestock production is predominantly organized at the household and community level, and large commercial farms are uncommon. Animals belonging to individual livestock owners are frequently managed within shared community-based grazing and production systems.

Study design and animal population

A repeated descriptive sero-epidemiological monitoring study was conducted from 2021 to 2025 to assess the occurrence and regional distribution of brucellosis seropositivity in small ruminants and swine in selected livestock populations. The epidemiological dataset comprised records for approximately 50,000 small ruminants and 5,000 swine examined during the five-year monitoring period. For the regional epidemiological assessment, approximately 10,000 small ruminants and 1,000 swine were represented from each of the five investigated regions.

Communities were included in the monitoring programme on the basis of previous occurrence of brucellosis or epidemiological suspicion. The study covered 470 communities across the five regions: 105 in Aragatsotn, 87 in Gegharkunik, 60 in Kotayk, 116 in Shirak, and 102 in Syunik. Because livestock production in the investigated areas is predominantly organized at household and community level, the epidemiological unit used for geographical assessment was the community rather than the individual commercial farm. Livestock owners in the selected communities cooperated with the monitoring activities, and no refusals to participate were reported.

The investigated small-ruminant population consisted predominantly of sheep, while goats represented approximately 0.1% of the small-ruminant population included in the monitoring dataset. The swine population consisted predominantly of animals raised under meat–fat production systems.

Sample collection and transportation

Blood samples were collected from the jugular vein using sterile single-use needles and vacuum blood collection tubes. Each sample was labelled with the animal identification information used within the monitoring records to ensure sample traceability. Samples were transported in insulated containers under refrigerated conditions to the Veterinary and Sanitary Expertise Research Center Laboratory of the Armenian National Agrarian University. Serum was separated by centrifugation and stored at +2 to +8 °C until serological examination.

Serological diagnostics

Serological examination was performed using a sequential diagnostic approach. Serum samples were initially screened using the Rose Bengal Test (RBT). Samples showing positive or doubtful reactions in the RBT were subjected to further examination using the Serum Agglutination Test (SAT) and enzyme-linked immunosorbent assay (ELISA).

The RBT was used as the primary rapid screening assay. For SAT, serum samples were serially diluted at 1:25, 1:50, 1:100, 1:200, and 1:400, and agglutination reactions were evaluated semi-quantitatively from +1 to +4. ELISA was performed using a commercial diagnostic kit (BRUT0050, Nova Test), and optical density was measured photometrically at 450/620 nm in accordance with the manufacturer's instructions. Serological procedures and interpretation followed the diagnostic approach applied within the national brucellosis monitoring framework and established methodological guidance (Popova et al., 2017; Sargsyan et al., 2022b).

Molecular characterization of *Brucella* species or biovars was not performed in the present study. Although molecular approaches, including multilocus PCR, may be used for differentiation of *Brucella* species and biovars, these methods were outside the scope of the monitoring programme analysed in this study (Osina et al., 2022).

Epidemiological Indicators

The epidemiological situation was described using three indicators: seroprevalence, focus index, and unsafe area index.

Seroprevalence (%) was calculated as the number of seropositive animals divided by the total number of examined animals within each region and multiplied by 100.

Focus index was calculated as the number of seropositive animals divided by the number of affected communities within each region. Regional focus indices were calculated separately for each of the five investigated regions. The overall mean focus index was expressed as the arithmetic mean of the five regional focus-index values.

Unsafe area index (%) was calculated as the number of affected communities divided by the total number of surveyed communities within each region and multiplied by 100. Regional unsafe area indices were calculated separately for each region, and the overall mean unsafe area index was expressed as the arithmetic mean of the five regional values.

All epidemiological indicators were calculated separately for small ruminants and swine.

Economic Loss Assessment

Economic losses associated with brucellosis were estimated using veterinary economic assessment approaches previously applied to the evaluation of brucellosis-related losses in livestock production (Grigoryan & Zadayan, 1999; Zadayan, 2001). The assessment included estimated losses resulting from the compulsory slaughter of seropositive animals and losses associated with reproductive failure.

Economic loss due to compulsory slaughter was calculated using the following formula:

$$U = (M \times W \times C) - Sf \quad (1)$$

where U is the estimated economic loss expressed in Armenian drams (AMD), M is the number of compulsorily slaughtered seropositive animals, W is the assumed average live weight per animal (65 kg), C is the market value per kilogram of live weight of a healthy animal (2,500 AMD kg^{-1}), and Sf is the total actual revenue obtained from the sale of products derived from compulsorily slaughtered animals.

For estimation of slaughter revenue, a value of 500 AMD per kilogram of live weight was applied. Accordingly, *Sf* represents total estimated slaughter revenue and was calculated as $M \times W \times 500$ AMD kg^{-1}.

Losses associated with infertility and fetal loss were estimated on the basis of planned and actual reproductive performance, the estimated value of offspring at birth, and feed costs associated with intrauterine development. Total estimated economic damage was calculated as the sum of losses attributed to compulsory slaughter and reproductive failure.

Disease control and rehabilitation measures

During the study period, animals classified as seropositive for brucellosis were subjected to compulsory culling in accordance with the applicable national veterinary control framework. Rehabilitation measures in affected livestock holdings and communities included repeated serological testing of susceptible animals and veterinary-sanitary measures in contaminated areas. Disinfection procedures included the use of active chlorine-based disinfectants and 2–3% sodium hydroxide solutions. Aborted fetuses, placental tissues, and contaminated manure were destroyed by incineration in accordance with applicable veterinary and biosafety requirements.

Statistical analysis

The study was primarily descriptive. Serological and epidemiological data were summarized as absolute numbers and percentages and were stratified by animal group and region. Seroprevalence, focus index, and unsafe area index were calculated using the definitions described above. Results of RBT screening and subsequent SAT and ELISA assessment were compared descriptively on the basis of the number and proportion of reactive samples within the sequential diagnostic algorithm. Because SAT and ELISA were applied to RBT-positive or doubtful samples rather than independently to the entire screened population, no formal statistical comparison of diagnostic sensitivity, specificity, or equivalence among the three serological methods was performed.

RESULTS

Serological findings and sequential diagnostic assessment

The results of the sequential serological examination of small-ruminant serum samples are presented in Table 1. In 2025, a total of 25,000 serum samples were initially screened using the Rose Bengal Test (RBT), of which 2,050 (8.2%) showed positive reactions. RBT-positive and doubtful samples were subsequently subjected to further examination using the Serum Agglutination Test (SAT) and enzyme-linked immunosorbent assay (ELISA). Among the samples subjected to confirmatory assessment, 2,046 were confirmed by SAT and 2,045 by ELISA.

Small numerical differences were observed between the initial RBT screening results and the subsequent SAT and ELISA findings. RBT was applied as the primary screening assay, whereas SAT and ELISA were used for complementary confirmatory assessment within the sequential diagnostic approach.

Table 1. Results of sequential serological screening and confirmatory testing of small-ruminant serum samples in high-altitude regions of Armenia in 2025

Region	Number screened by RBT	RBT-positive, n (%)	SAT-confirmed, n	ELISA-confirmed, n
Aragatsotn	5,000	320 (6.4)	318	318
Kotayk	5,000	550 (11.0)	549	550
Gegharkunik	5,000	520 (10.4)	520	520
Shirak	5,000	510 (10.2)	509	508
Syunik	5,000	150 (3.0)	150	149
Total	25,000	2,050 (8.2)	2,046	2,045

RBT – Rose Bengal Test; SAT – Serum Agglutination Test; ELISA – Enzyme-Linked Immunosorbent Assay.

Regional variation in RBT seroreactivity was observed. The highest proportion of RBT-positive small ruminants was recorded in Kotayk (11.0%), followed by Gegharkunik (10.4%) and Shirak (10.2%). Aragatsotn showed a seroreactivity rate of 6.4%, whereas the lowest proportion was recorded in Syunik (3.0%). The regional distribution of SAT- and ELISA-confirmed samples generally followed the pattern observed during initial RBT screening.

Clinical observations and field findings

Clinical observations recorded during epidemiological monitoring included reproductive and systemic abnormalities in the investigated livestock populations. Reproductive disorders observed in small ruminants and swine included abortions, premature parturition, retained placenta, birth of non-viable offspring, and increased perinatal mortality. Occasional cases of paresis and paralysis were recorded in lambs, and sporadic piglet mortality was observed in the monitored swine populations.

Post-mortem examination of compulsorily slaughtered seropositive animals identified exudative lesions in joint capsules, edema of tendon sheaths, vertebral lesions, osteomyelitis, and arthritis. Reproductive tract lesions included orchitis and epididymitis in males and inflammatory thickening of the uterine mucosa in females. Purulent lesions were also observed in lymph nodes and internal organs, including the spleen, liver, kidneys, and mammary glands.

These clinical and pathological observations were recorded as supplementary field findings and were not used as independent diagnostic criteria for classification of brucellosis seropositivity.

Epidemiological characteristics of brucellosis in small ruminants

During the five-year monitoring period from 2021 to 2025, 6,727 seropositive animals were identified among approximately 50,000 examined small ruminants, corresponding to an overall seroprevalence of 13.5%.

Regional variation in seroprevalence was observed. The highest proportion of seropositive animals was recorded in Gegharkunik (14.5%), followed by Aragatsotn (14.2%) and Kotayk (13.8%). Seroprevalence was 13.3% in Shirak and 11.5% in Syunik.

A total of 470 communities were included in the epidemiological assessment, of which 105 were classified as affected communities. The mean focus index across the five investigated regions was 65.1 seropositive animals per affected community. The

mean unsafe area index across the five investigated regions was 23.9%, with substantial regional variation.

Regional differences were also observed in the geographical distribution and concentration of seropositive animals. The highest unsafe area index was recorded in Kotayk (38.3%), followed by Gegharkunik (28.7%), Aragatsotn (19.1%), Shirak (19.0%), and Syunik (14.7%).

The regional distribution of seroprevalence, focus index, and unsafe area index is presented in Fig. 1.

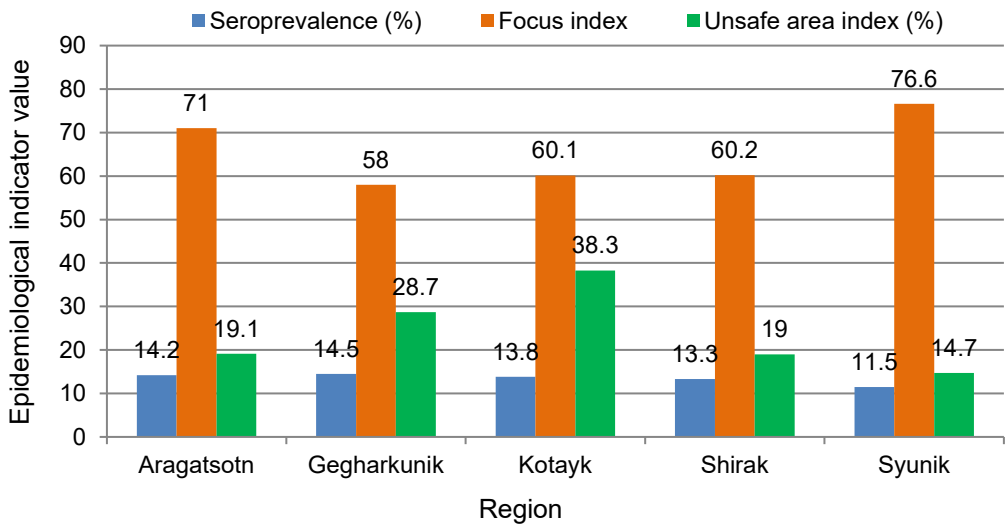


Figure 1. Regional distribution of seroprevalence, focus index, and unsafe area index for small-ruminant brucellosis in the five investigated regions of Armenia, 2021–2025.

Note: Seroprevalence and unsafe area index are expressed as percentages; the focus index represents the number of seropositive animals per affected community.

Epidemiological characteristics of brucellosis in swine

During the five-year monitoring period from 2021 to 2025, 20 seropositive pigs were identified among approximately 5,000 examined animals, corresponding to an overall seroprevalence of 0.4%. Six of the 470 surveyed communities were classified as affected by swine brucellosis.

The highest regional seroprevalence was recorded in Kotayk (1.4%; 14/1,000), followed by Aragatsotn (0.5%; 5/1,000) and Syunik (0.1%; 1/1,000). No seropositive pigs were detected in Gegharkunik or Shirak during the monitoring period.

The focus index was highest in Kotayk (4.7 seropositive pigs per affected community), followed by Aragatsotn (2.5) and Syunik (1.0); no focus index was recorded for Gegharkunik or Shirak because no affected communities were identified in these regions. The mean focus index across the five investigated regions was 1.6. The unsafe area index was 5.0% in Kotayk, 1.9% in Aragatsotn, and 1.0% in Syunik, while no affected communities were recorded in Gegharkunik or Shirak. The mean unsafe area index across the five investigated regions was 1.6%.

Regional variation in swine seropositivity was therefore characterized by a limited number of affected communities and a comparatively low number of seropositive animals.

The regional distribution of seroprevalence, focus index, and unsafe area index for swine is presented in Fig. 2.

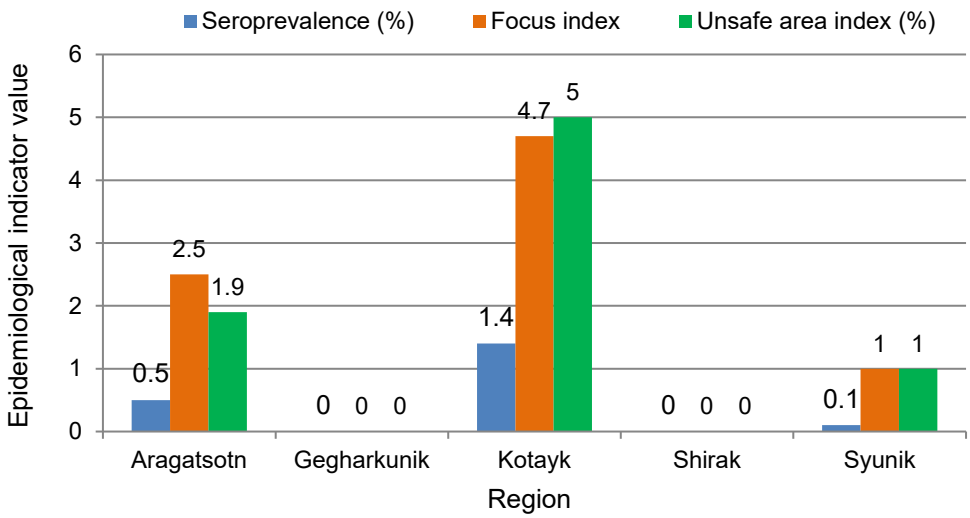


Figure 2. Regional distribution of seroprevalence, focus index, and unsafe area index for swine brucellosis in the five investigated regions of Armenia, 2021–2025.

Note: Seroprevalence and unsafe area index are expressed as percentages; the focus index represents the number of seropositive pigs per affected community.

Economic losses associated with brucellosis

The estimated economic losses associated with brucellosis during the study period are summarized according to losses from compulsory slaughter and reproductive failure. Based on the economic assessment methodology applied in the study, estimated losses attributable to compulsory slaughter amounted to 877,110,000 AMD. Additional estimated losses associated with infertility and fetal loss amounted to 7,980,000 AMD.

The total estimated economic damage was therefore 885,090,000 AMD. Estimated slaughter revenue obtained from compulsorily slaughtered animals amounted to 219,277,500 AMD.

Of the total estimated economic damage, approximately 99.1% was attributable to compulsory slaughter and 0.9% to infertility and fetal loss.

DISCUSSION

The present study provides an integrated descriptive assessment of brucellosis in small ruminants and swine in five high-altitude regions of Armenia. The results demonstrate a marked difference in the epidemiological burden between the two livestock groups. Overall seroprevalence in small ruminants reached 13.5%, with 6,727 seropositive animals identified among 50,000 examined animals, whereas only 20

seropositive pigs were detected among 5,000 examined swine, corresponding to an overall seroprevalence of 0.4%. The regional data supplied for the present analysis show that small-ruminant seropositivity was distributed across all five investigated regions, whereas swine cases were restricted to Aragatsotn, Kotayk, and Syunik.

The relatively high seroprevalence observed in small ruminants is consistent with the recognized epidemiological importance of sheep and goats in the maintenance and transmission of *Brucella melitensis* in endemic livestock systems. Previous studies have emphasized that brucellosis remains particularly difficult to control in areas characterized by extensive grazing, seasonal livestock movements, and close contact among animals belonging to different owners (WHO et al., 2006; Aragón-Aranda et al., 2020; Sidorchuk et al., 2024). These characteristics are also typical of the investigated Armenian highland communities, where livestock are predominantly maintained at household and community level and commonly use shared grazing systems. The study population was predominantly sheep, with goats representing approximately 0.1% of the investigated small-ruminant population.

Marked regional heterogeneity was observed in the distribution of small-ruminant brucellosis. Gegharkunik and Aragatsotn showed the highest seroprevalence values, whereas Syunik had the lowest seroprevalence but the highest focus index. This distinction is epidemiologically relevant because seroprevalence and focus index describe different dimensions of disease occurrence. A relatively lower regional seroprevalence combined with a high number of seropositive animals per affected community may indicate a more concentrated distribution of infection within individual epidemiological foci. Conversely, the high unsafe area index observed in Kotayk indicates a broader geographical distribution of affected communities. The heterogeneous regional pattern observed in the present study is consistent with earlier community-level spatial analyses in Armenia. Porphyre et al. (2010) demonstrated that brucellosis was widely but unevenly distributed among Armenian communities and that geographical patterns of higher disease risk differed between livestock populations. Although the earlier study analysed cattle and small ruminants using 2006–2007 national control data, whereas the present investigation focuses on small ruminants and swine during 2021–2025, both studies indicate substantial geographical heterogeneity and support the use of geographically targeted, risk-based surveillance strategies.

The substantially lower seroprevalence observed in swine is consistent with previous findings from Armenia. In an earlier investigation of swine brucellosis in Aragatsotn region, five seropositive pigs were identified among approximately 2,000 examined animals, corresponding to a prevalence of 0.25% (Sargsyan et al., 2022a). In the present study, the overall swine seroprevalence was 0.4%, with the highest regional value recorded in Kotayk (1.4%). Although the study populations and geographical coverage differ, both investigations indicate relatively limited but persistent circulation of brucellosis in Armenian swine populations. The earlier Armenian study reported 5 positive pigs among 2,000 examined animals and described reproductive and pathological manifestations associated with infection.

The sporadic and geographically restricted occurrence of swine brucellosis observed in the present study should nevertheless be interpreted cautiously. Routine annual brucellosis screening is applied to breeding females and breeding rams in small-ruminant populations, whereas comparable systematic annual serological screening is

not routinely implemented in swine. Consequently, the observed difference between small ruminants and swine may reflect both genuine differences in infection dynamics and differences in surveillance intensity. This limitation is important when interpreting interspecies comparisons and highlights the need for more systematic epidemiological investigation of swine populations in areas where brucellosis is endemic in ruminants.

The sequential serological diagnostic approach used in the present study demonstrated close agreement between RBT screening and subsequent SAT and ELISA confirmation. RBT remains widely used as a rapid screening method because of its operational simplicity and suitability for large-scale surveillance, while SAT and ELISA provide complementary information for the assessment of reactive or doubtful samples (Ismael et al., 2016; Bertelloni et al., 2018; Paul et al., 2020). The small numerical differences observed between RBT-positive samples and SAT- or ELISA-confirmed samples in the present study support the practical applicability of this sequential diagnostic algorithm. However, because the three tests were not applied independently to the entire study population in parallel, the present results should not be interpreted as a formal comparison of diagnostic sensitivity or specificity. Rather, they demonstrate concordance within the diagnostic sequence used under field surveillance conditions.

The clinical and post-mortem observations recorded during the study were compatible with the reproductive and systemic manifestations commonly associated with animal brucellosis. Abortions, premature parturition, retained placenta, non-viable offspring, orchitis, epididymitis, arthritis, and lesions involving lymphoid and internal organs are consistent with the recognized pathogenesis of chronic *Brucella* infection (Kroese et al., 2018). Similar reproductive and pathological findings were also described in the earlier Armenian investigation of swine brucellosis (Sargsyan et al., 2022a). These observations reinforce the importance of combining epidemiological information, clinical assessment, and laboratory confirmation, particularly because brucellosis may persist in livestock populations without highly specific clinical signs.

The economic analysis further demonstrates the importance of brucellosis as a livestock production constraint. Total estimated economic damage reached 885.09 million Armenian drams (AMD), of which 877.11 million AMD was associated with forced slaughter and 7.98 million AMD with infertility and fetal loss. The predominance of forced-slaughter losses indicates that the economic consequences of identifying and removing seropositive animals can be substantial for livestock production systems. Previous veterinary economic assessments have similarly emphasized direct losses associated with compulsory slaughter, reproductive failure, and loss of productive potential (Grigoryan & Zadayan, 1999; Zadayan, 2001). The present findings therefore support the economic rationale for earlier detection and prevention of transmission before infection becomes established within community livestock populations.

From a disease-control perspective, the continued detection of brucellosis in the investigated high-altitude regions indicates the need for surveillance strategies that account for geographical heterogeneity and community-level disease patterns. Earlier spatial analysis of Armenian national control data identified priority areas for targeted risk-based surveillance and disease-control interventions (Porphyre et al., 2010). The present findings extend this epidemiological perspective by demonstrating marked differences in seroprevalence, focus index, and unsafe area index among five high-altitude regions during 2021–2025. Together, these findings support prioritization of

communities and regions according to epidemiological indicators rather than the uniform application of surveillance intensity across all livestock populations.

The zoonotic nature of brucellosis further requires coordinated action beyond the veterinary sector. Strengthening collaboration between veterinary services, public health institutions, diagnostic laboratories, and livestock owners is essential for early detection, risk communication, and coordinated response. Within a One Health framework, integrated animal and human surveillance could improve the identification of transmission hotspots and support more targeted preventive interventions. Particular attention should be given to affected communities with high focus indices or high unsafe area indices, because these epidemiological indicators identify different patterns of infection concentration and geographical spread.

The present study has several limitations. The investigated communities were selected using a risk-based monitoring approach rather than random population-based sampling; therefore, the reported seroprevalence estimates should be interpreted primarily as descriptive indicators for the monitored high-risk populations and should not be directly extrapolated to the entire national livestock population. In addition, molecular characterization of *Brucella* species and biovars was not performed, limiting conclusions regarding the specific strains circulating in the investigated regions. Finally, differences in routine surveillance intensity between small ruminants and swine may influence direct comparisons between the two animal groups. Future studies should incorporate probability-based sampling where feasible, molecular typing of *Brucella* isolates, spatial analysis of affected communities, and integrated animal–human epidemiological data.

CONCLUSION

1. Brucellosis remains an epidemiologically and economically important zoonotic disease in the investigated high-altitude regions of the Republic of Armenia. The substantially higher seroprevalence observed in small ruminants than in swine indicates that small-ruminant populations represent the principal livestock group affected by brucellosis in the monitored areas.

2. During the 2021–2025 observation period, 6,727 seropositive animals were identified among 50,000 examined small ruminants, corresponding to an overall seroprevalence of 13.5%. In contrast, 20 seropositive animals were detected among 5,000 examined swine, corresponding to an overall seroprevalence of 0.4%.

3. The epidemiological distribution of brucellosis showed marked regional heterogeneity. Gegharkunik and Aragatsotn had the highest seroprevalence values among small ruminants, whereas Syunik showed the highest focus index and Kotayk the highest unsafe area index. Swine brucellosis was detected only in Kotayk, Aragatsotn, and Syunik, indicating a more geographically restricted pattern of seropositivity.

4. The sequential serological diagnostic approach, based on RBT screening followed by SAT and ELISA assessment of RBT-positive or doubtful samples, demonstrated close agreement between screening and confirmatory findings. The results support the practical applicability of this diagnostic sequence for large-scale brucellosis surveillance under the investigated field conditions.

5. The estimated total economic damage associated with brucellosis amounted to 885.09 million Armenian drams (AMD), including 877.11 million AMD attributable to forced slaughter and 7.98 million AMD associated with infertility and fetal loss. These findings demonstrate the considerable direct economic burden of brucellosis on livestock production in the investigated regions.

6. The persistence and spatial distribution of brucellosis in the monitored high-altitude communities indicate the need to strengthen risk-based epidemiological surveillance, community- and farm-level biosecurity, animal movement traceability, and cooperation between livestock owners and veterinary diagnostic services.

7. Given the zoonotic nature of brucellosis, effective disease control requires coordinated action between veterinary and public health sectors. Integration of animal and human surveillance within the One Health framework may improve early detection, identification of high-risk communities, and implementation of targeted preventive and control measures.

Future investigations incorporating probability-based sampling, molecular characterization of circulating Brucella spp., spatial epidemiological analysis, and integrated animal–human surveillance are needed to improve understanding of transmission dynamics and support evidence-based national control policies**.**

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DECLARATION OF AI USE. The authors used artificial intelligence tools (e.g., ChatGPT) for language editing and improvement of readability. The scientific content, data interpretation, and final conclusions of the manuscript were developed and verified solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

REFERENCES

Ameen, A.M., Abdulaziz, N.S. & Ghaffar, N.M. 2023. Molecular detection and associated risk factors of *Brucella melitensis* in aborted sheep and goats in Duhok Province, Iraq. *Pathogens* **12**(4), 544. https://doi.org/10.3390/pathogens12040544

Aragón-Aranda, B., de Miguel, M.J., Lázaro-Antón, L., Salvador-Bescós, M., Zúñiga-Ripa, A., Moriyón, I., Iriarte, M., Muñoz, P.M. & Conde-Álvarez, R. 2020. Development of attenuated live vaccine candidates against swine brucellosis in a non-zoonotic *Brucella suis* biovar 2 background. *Veterinary Research* **51**(1), 92. https://doi.org/10.1186/s13567-020-00815-8

Bertelloni, F., Forzan, M., Turchi, B., Sagona, S., Mazzei, M., Felicioli, A., Fratini, F. & Cerri, D. 2018. A serological survey on swine brucellosis using standard procedures, dot blot, and Western blot in finisher pigs in central-north Italy. *Veterinary Sciences* **5**(4), 86. https://doi.org/10.3390/vetsci5040086

Egoyan, S.G., Mkrtchyan, A.R., Sargsyan, M.A. & Andressyan, T.A. 2018. Analysis of the epizootic situation of brucellosis in large and small ruminants in the Republic of Armenia. In *Proceedings of the International Scientific Conference on Current Problems of Agriculture Development in Armenia*.Yerevan, p. 51 (in Russian)

Grigoryan, S.L. & Zadayan, M.H. 1999. Economic efficiency of measures against brucellosis. *Agropress* **2**, 234–235 (in Armenian).

Ismael, A.B., Swelum, A.A., Mostafa, S.A. & Alhumiany, A.R. 2016. Latex agglutination using periplasmic protein antigen of *Brucella melitensis* as a rapid and specific serodiagnostic test for ovine brucellosis. *International Journal of Immunopathology and Pharmacology* **29**(3), 480–485. https://doi.org/10.1177/0394632016648709

Kefaloudi, C., Mellou, K., Dougas, G., Vorou, R., Mitrou, K. & Kontopidou, F. 2022. Human brucellosis in Greece (2005–2020): A persistent public health problem. *Vector-Borne and Zoonotic Diseases* **22**(3), 163–169. https://doi.org/10.1089/vbz.2021.0050

Kroese, M.V., Beckers, L., Bisselink, Y.J.W.M., Brasseur, S., van Tulden, P.W., Koene, M.G.J., Roest, H.I.J., Ruuls, R.C., Backer, J.A., IJzer, J., van der Giessen, J.W.B. & Willemsen, P.T.J. 2018. *Brucella pinnipedialis* in grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) in the Netherlands. *Journal of Wildlife Diseases* **54**(3), 439–449. https://doi.org/10.7589/2017-05-097

Li, L.M., Wang, Q., Shi, J.F., Li, T., Zhao, B., Ma, Q.X., Liu, H.Y., Su, N., Cai, R.P., Zeng, F.L., Gong, Q.L., Shi, K., Li, J.M., Liu, F. & Du, R. 2023. Seroprevalence and potential risk factors of brucellosis in sheep from America, Africa and Asia: A systematic review and meta-analysis. *Research in Veterinary Science* **165**, 105048. https://doi.org/10.1016/j.rvsc.2023.105048

López, G.E., Peña, S., Escobar, G.I., Hasan, D.B. & Lucero, N.E. 2018. Serological study of brucellosis in Argentine Creole sheep. *Revista Argentina de Microbiología* **50**(3), 285–289. https://doi.org/10.1016/j.ram.2017.10.006

Osina, N.A., Sitmbetov, D.A., Domanina, I.V., Bulgakova, E.G., Kasyan, Zh.A., Kasyan, I.A., Lyashova, O.Yu. & Shcherbakova, S.A. 2022. Development of a method for determining biovars of *Brucella suis* using multilocus PCR based on real-time analysis. *Problems of Particularly Dangerous Infections* **2**, 107–114. https://doi.org/10.21055/0370-1069-2022-2-107-114 (in Russian)

Paul, S., Peddayelachagiri, B.V., Gogoi, M., Nagaraj, S., Ramlal, S., Konduru, B. & Batra, H.V. 2020. Genome-wide unique insertion sequences among five *Brucella* species and demonstration of differential identification of *Brucella* by multiplex PCR assay. *Scientific Reports* **10**, 6368. https://doi.org/10.1038/s41598-020-62472-3

Popova, A.Yu., Demina, Yu.V., Pakskina, N.D., Sheenkov, N.V., Sharova, I.N., Osina, N.A., Kasyan, Zh.A., Shcherbakova, S.A. & Kutyrev, V.V. 2017. Epidemiological *surveillance and laboratory diagnostics of brucellosis: Methodological guidelines*. *Federal Center of Hygiene and Epidemiology of Rospotrebnadzor*, Moscow, 60 pp. ISBN 978-5-7508-1539-5 (in Russian).

Porphyre, T., Jackson, R., Sauter-Louis, C., Ward, D., Baghyan, G. & Stepanyan, E. 2010. Mapping brucellosis risk in communities in the Republic of Armenia. *Geospatial Health* **5**(1), 103–118. https://doi.org/10.4081/gh.2010.191

Sargsyan, M.A., Balasanyan, H.S. & Tovmasyan, G.R. 2022a. Study of swine brucellosis infection rate in the Avan community, Aragatsotn region. *AgriScience and Technology* **4**(80), 397–401. https://doi.org/10.52276/25792822-2022.4-397

Sargsyan, M.A., Balasanyan, H.S., Tovmasyan, G.R. & Grigoryan, H. 2024. Diagnosis and epizootological analysis of small cattle brucellosis in regions of Armenia. *AgriScience and Technology* **3**(87), 262–268. https://doi.org/10.52276/25792822-2024.3-262

Sargsyan, M.A., Mkrtchyan, A.R., Balasanyan, A.S. & Tovmasyan, G.R. 2022b. Epidemiology and infectious diseases. Laboratory guidelines on the topic 'Diagnosis, prevention and control measures of brucellosis'. *Armenian National Academy Edition*, Yerevan, 20 pp. (in Russian).

Sidorchuk, A.A., Masimov, N.A., Krupalnik, V.L., Bessarabov, B.F., Tremasov, M.Y., Grishchenko, L.I., Maslennikova, V.I. 2024. Infectious diseases of animals: Textbook. 2nd ed., revised and expanded. Moscow: INFRA-M, 954 pp. (in Russian).

Smirnova, E.A., Vasin, A.V., Sandybaev, N.T., Klotchenko, S.A., Plotnikova, M.A., Chervyakova, O.V., Sansyzbay, A.R. & Kiselev, O.I. 2013. Current methods of human and animal brucellosis diagnostics. *Advances in Infectious Diseases* **3**, 177–184. https://doi.org/10.4236/aid.2013.33026

Wattam, A.R., Foster, J.T., Mane, S.P., Beckstrom-Sternberg, S.M., Beckstrom-Sternberg, J.M., Dickerman, A.W., Keim, P., Pearson, T., Shukla, M., Ward, D.V., Williams, K.P., Sobral, B.W., Tsolis, R.M., Whatmore, A.M. & O'Callaghan, D. 2014. Comparative phylogenomics and evolution of the Brucellae reveal a path to virulence. *Journal of Bacteriology* **196**(5), 920–930. https://doi.org/10.1128/JB.01091-13

World Health Organization, Food and Agriculture Organization of the United Nations, & World Organisation for Animal Health. 2006. Brucellosis in humans and animals. *World Health Organization*. ISBN 978-92-4-154713-0. Official WHO publication record

Zadayan, M.H. 2001. Economic efficiency of epidemiological measures against brucellosis, tuberculosis, and pasteurellosis. *Agropres*s **4**, 345–348 (in Armenian).