



Serological evidence of *Anaplasma* spp. antibodies in domestic ruminants in Slovakia

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Abstract

Introduction and Objective. *Anaplasma* bacteria are intracellular, gram-negative microorganisms transmitted by ticks that can pose a threat to the health of both animals and humans. In our geographical conditions, the transmission of *Anaplasmas* occurs mainly through ticks of the species *Ixodes ricinus*, which represent the most abundant species in Slovakia. The aim of the study is to investigate the seroprevalence of *Anaplasma* spp. antibodies in cattle, sheep and goats across Slovakia.

Materials and Method. The study involved serological testing of 156 cattle, 124 sheep and 104 goats from various regions of Slovakia. A total of 384 serum samples were analysed through the use of the competitive ELISA method.

Results. The seropositivity was 10.90% in cattle, 70.16% in sheep and 43.27% in goats.

Conclusions. The study additionally identified regional variations, indicating that environmental conditions, vector ecology, and animal management practices, play a role in the transmission of *Anaplasma* spp. These findings emphasise the need for targeted strategies for the prevention and control of anaplasmosis, tailored to specific species and regions, to reduce its economic and health impacts on livestock. The results contribute to the growing body of knowledge on *Anaplasma* epidemiology, highlighting the importance of ongoing surveillance to manage its effects. Given the zoonotic potential of anaplasmosis, it is important to analyse the results obtained also from the One Health perspective.

Key words

serology, Slovakia, sheep, goat, cattle, tick-borne disease

INTRODUCTION AND OBJECTIVE

Anaplasma spp. are obligate intracellular bacteria belonging to the order Rickettsiales and the family Anaplasmataceae. These pathogens are primarily transmitted by hard ticks of the genera *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Amblyomma*, and infect a variety of hosts, including domestic and wild ruminants, companion animals and humans [1, 2]. The detection of species within the family Anaplasmataceae in reptiles and their associated ticks illustrates that circulation of these bacteria extends beyond domestic hosts and involves a broader ecological context [3]. The bacteria reside in the tick's salivary glands and midgut, and transmission to a susceptible host occurs during feeding on blood [4]. The successful transmission of *Anaplasma phagocytophilum* typically requires a tick attachment duration of 24–48 hours, while the incubation period in the host ranges from 5–14 days [5].

Once inside the host, *Anaplasma* spp. primarily infect haematopoietic cells and actively modulate the immune response by upregulating, downregulating or inhibiting key immune factors, allowing them to evade immune clearance and establish persistent infections. This immunomodulatory

effect increases the host's susceptibility to secondary infections, which is a significant cause of mortality in sheep [6]. The clinical presentation of anaplasmosis varies from subclinical infections to severe disease characterised by fever, anaemia, weight loss, reproductive disorders and decreased milk production [7, 8].

The genus *Anaplasma* includes well-recognised species: *A. phagocytophilum*, *A. marginale*, *A. centrale*, *A. ovis*, *A. platys* and *A. bovis*. In addition, emerging species, such as *A. capra* and *A. odocoilei*, have been identified, alongside numerous unclassified genetic variants [9]. Among these, *A. phagocytophilum* is important due to its zoonotic potential and its role in causing granulocytic anaplasmosis in humans, tick-borne fever (TBF) in ruminants, and granulocytic anaplasmosis in horses, dogs, and cats.

In Europe, seroprevalence in livestock has been reported to range from 0%–55%, with higher rates observed in regions with high tick densities [10]. Most outbreaks of TBF occur when previously unexposed ruminants are introduced to tick-infested pastures. Anaplasmosis is a growing concern in livestock production, with significant economic and health implications worldwide. Reports of *Anaplasma* spp. infections have been documented in various geographic regions, including Europe, North America, Africa and Asia [11, 12]. In Central Europe, tick-borne diseases, including anaplasmosis, pose an increasing threat to livestock, yet epidemiological data from Slovakia remain limited. Given

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the economic impact of the disease and its role in livestock morbidity, continuous surveillance is essential for assessing prevalence and identifying risk factors.

The aim of the study is to determine the seroprevalence of *Anaplasma* spp. in cattle, sheep and goats across different regions in Slovakia using a competitive enzyme-linked immunosorbent assay (cELISA). The cELISA has good sensitivity in detecting carrier animals. In general, unless animals have been treated or are at a very early stage of infection (<14 days), serology using the cELISA may be the preferred methods of identifying infected animals in most laboratories [13]. By evaluating host species susceptibility and regional variation in prevalence, the current study provides essential epidemiological data that can inform targeted disease control measures, and improve strategies for livestock health management.

MATERIALS AND METHOD

The study involved serological testing of livestock from various regions of Slovakia to assess the prevalence of *Anaplasma* spp. in cattle, sheep and goats.

Sampling locations and animals. A total of 384 ruminants from the northern, central and eastern regions of Slovakia were investigated (Fig. 1). The samples were obtained from 23 various, randomly-selected locations in Slovakia. The farms of ruminants across the country were obtained through the willingness of owners to provide the animals for sampling. 156 cattle samples were collected from 9 breedings, 124 sheep samples were collected from 7 breedings, and 104 goat samples were obtained from 7 farms (Tab. 1). The study included randomly-selected animals of both genders and varying ages, ensuring a representative sample of the population. Ruminants were with unknown anamnesis and without clinical signs of infectious diseases. Animal breeders reported the occasional presence of ticks on animals. The animals were on day pastures and mostly raised for milk production. The average annual temperature in Slovakia is 8.5 °C, with the average temperature increasing to 22–24 °C during the summer. In association with an annual average relative humidity, the whole territory of Slovakia represents a very suitable biotope for tick occurrence.

Sampling technique. The study was performed in compliance with the institutional guidelines for animal welfare issued by the Ethics Committee of the University of Veterinary

Medicine and Pharmacy in Košice, Slovakia. All animal samples in this study were examined with the assistance of their owners and animal welfare was ensured during animal handling. Blood samples were collected by a veterinarian all year round from 2023–2025. Sampling was performed via jugular venipuncture using sterile equipment to ensure sample quality and minimise contamination. Blood was drawn using a needle and a syringe, with the animals properly restrained to minimise stress and ensure safety. Approximately 5 mL of blood was collected into sterile tubes without anti-coagulants. The tubes were centrifuged at 10,000 rpm for 10 minutes to separate serum. The serum was carefully transferred to clean microtubes to avoid contamination with cellular debris and stored at -20 °C for subsequent serological analysis.

Serological testing. The presence of antibodies against *Anaplasma* spp. in the collected serum samples was assessed using a cELISA. For this purpose, the commercially-available Anaplasma Antibody Test Kit, cELISA v2 (VMRD, Veterinary Medical Research & Development, USA) was utilised. This assay is specifically designed to detect antibodies against *Anaplasma* spp. in ruminant serum and is widely recognised for its sensitivity and specificity. The diagnostic sensitivity is 100%, and the diagnostic specificity is 99.7% using a cut-off of 30% inhibition, as determined by a receiver operating characteristic plot. The assay specifically detects the presence of serum antibodies that target a surface protein MSP5 of *Anaplasma* spp. This cELISA has already been successfully applied to ovine and caprine sera from areas where *A. ovis* and *A. phagocytophilum* are present [14, 15, 16].

The cELISA was performed according to the manufacturer's instructions. Serum samples were diluted appropriately and added to wells pre-coated with purified *Anaplasma* antigens. Following the addition of the conjugate and subsequent incubation, unbound material was removed by washing, and substrate solution was added to each well. The reaction was stopped after the recommended incubation period, and the optical density of each well was measured at 630 nm using the Bio Tek Synergy HTX Multimode Microplate Reader (BioTek Instruments, USA).

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (version 5.01) (GraphPad Software, Inc., San Diego, California, USA) to compare positive and negative samples across individual animal groups. Categorical variables were compared using the chi-square (χ^2) test; p-values less than 0.05 were considered statistically significant.

RESULTS

A total of 384 blood samples from cattle, sheep, and goats were analysed for the presence of antibodies against *Anaplasma* spp. using the cELISA method. The findings indicated species-specific variations in seropositivity rates (Fig. 2). From the 156 cattle samples, 17 (10.90%; 95% CI: 6.92–16.76) were seropositive for *Anaplasma* spp. (Tab. 2), while the remaining 139 (89.10%) were seronegative. Out of the 124 sheep samples, 87 (70.16%; 95% CI: 61.60–77.51) were determined to be seropositive (Tab. 3), and the remaining 37 (29.84%) tested seronegative. In goats, 104 blood samples were analysed, of which 45 (43.27%; 95% CI: 34.16–52.86) were

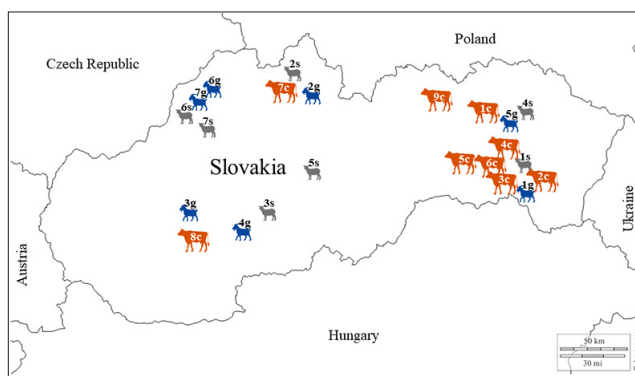


Figure 1. 23 sampling locations. Farms: cattle – 9, sheep – 7, and goats – 7

Table 1. Characterisation of the ruminant breeding

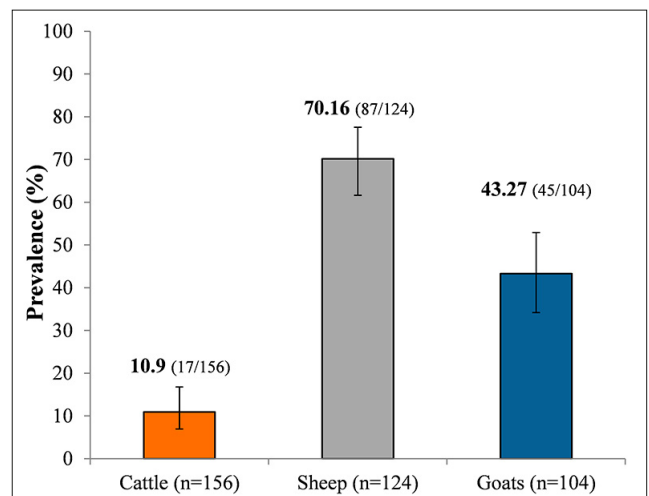
RUMINANTS							
Breeding	District	Altitude above sea level	Season of sampling	Species	Total	Female	Male
1c	Prešov	286 m	Spring/Autumn	cattle	41	41	0
2c	Trebišov	204 m	Autumn	cattle	30	30	0
3c	Košice-okolie	210 m	Winter	cattle	15	15	0
4c	Košice-okolie	280 m	Winter	cattle	16	16	0
5c	Košice-okolie	370 m	Spring	cattle	1	0	1
6c	Košice-okolie	223 m	Spring	cattle	28	28	0
7c	Dolný Kubín	601 m	Autumn	cattle	16	6	10
8c	Zlaté Moravce	196 m	Summer	cattle	1	1	0
9c	Sabinov	468 m	Spring	cattle	8	8	0
1s	Košice-okolie	202 m	Winter	sheep	15	15	0
2s	Dolný Kubín	531 m	Spring	sheep	30	30	0
3s	Zvolen	418 m	Summer	sheep	34	34	0
4s	Prešov	299 m	Spring	sheep	11	11	0
5s	Brezno	475 m	Spring	sheep	20	20	0
6s	Považská Bystrica	498 m	Spring	sheep	10	10	0
7s	Považská Bystrica	306 m	Spring	sheep	4	4	0
1g	Košice-okolie	462 m	Winter	goat	15	15	0
2g	Dolný Kubín	531 m	Spring	goat	30	30	0
3g	Zlaté Moravce	196 m	Summer	goat	23	23	0
4g	Levice	283 m	Summer	goat	5	5	0
5g	Prešov	270 m	Spring	goat	16	16	0
6g	Žilina	316 m	Spring	goat	10	10	0
7g	Považská Bystrica	343 m	Spring	goat	5	5	0
23	Total				384	373	11

Table 2. Results of screening of *Anaplasma* spp. antibodies by cELISA in cattle from selected regions in Slovakia

Breeding	Samples	Positive	%	Prevalence (95% CI)
1c	41	10	24.39	
2c	30	0	0.00	
3c	15	0	0.00	
4c	16	4	25.00	
5c	1	1	100.00	
6c	28	0	0.00	
7c	16	1	6.25	
8c	1	0	0.00	
9c	8	1	12.50	
Total	156	17	10.90	6.92–16.76

Table 3. Results of screening of *Anaplasma* spp. antibodies by cELISA in sheep from selected regions in Slovakia

Breeding	Samples	Positive	%	Prevalence (95% CI)
1s	15	4	26.67	
2s	30	20	66.67	
3s	34	33	97.06	
4s	11	1	9.00	
5s	20	18	90.00	
6s	10	7	70.00	
7s	4	4	100.00	
Total	124	87	70.16	61.60–77.51

**Figure 2.** Serosurvey for *Anaplasma* spp. in domestic ruminants in Slovakia

seropositive for *Anaplasma* spp. (Tab. 4), while 59 (56.73%) tested seronegative.

The overall results demonstrated the highest seroprevalence of *Anaplasma* spp. antibodies in sheep, followed by goats, and the lowest in cattle. These findings suggest significant differences in exposure to *Anaplasma* spp. or immune responses among the three species analysed. The data underscore the importance of species-specific surveillance and management strategies to address *Anaplasma* spp. infections effectively.

Table 4. Results of screening of *Anaplasma* spp. antibodies by cELISA in goats from selected regions in Slovakia

Breeding	Samples	Positive	%	Prevalence (95% CI)
1g	15	7	46.67	
2g	30	12	40.00	
3g	23	0	0	
4g	5	0	0	
5g	16	13	81.25	
6g	10	8	80.00	
7g	5	5	100.00	
Total	104	45	43.27	34.16–52.86

The prevalence of *Anaplasma* spp. was almost detected in each breed with regional variability in disease incidence. This difference in prevalence could be influenced by environmental, management, or vector-related factors, which will require further investigation in future studies.

DISCUSSION

In recent years, increases in tick activity and incidences of tick-borne diseases have been observed in various European countries. These increases are linked to many ecological and anthropogenic factors, including landscape management, climate change, animal migration, changes in land usage, and the increased popularity of outdoor activities.

Slovakia lies within a temperate continental climate zone. Warm summers (above 20 °C), high moisture levels and abundant rainfall provide an ideal habitat for *Ixodes ricinus* ticks, which require temperatures between 7 °C–25 °C, and a relative humidity above 45–50% for effective questing [17]. To date, 22 tick species have been found to occur in Slovakia, where ticks were found to harbour and transmit zoonotic and/or potentially zoonotic agents, such as bacteria belonging to the order Rickettsiales. *Ixodes ricinus* is the principal vector of the largest variety of microorganisms including *Anaplasma* spp., that pose a lower or potential risk to humans [1, 18]. The *Anaplasma* strains from wild animals, including wild ruminants, represent the variants that can cause disease in domestic animals [19]. Reports from geographically-distant regions confirm that *Anaplasma* spp. are widely distributed in both livestock and ticks, providing a useful comparison for interpreting national and global seroprevalence data [20].

To our knowledge, the current study is the first to evaluate the seroprevalence of *Anaplasma* spp. in domestic ruminants in Slovakia. The advantage of the study is the comprehensive coverage of Slovakia's territory. In this study, *Anaplasma* spp. antibodies were detected by cELISA, the advantages of the which with a standardised antigen are improved specificity, high sensitivity, and the detection of persistently-infected animals. A target antigen is highly conserved among *A. marginale* strains; therefore, this kit detects infection with all strains of *A. marginale* [13]. Rubel et al. [16] successfully applied the same cELISA for detecting seroprevalence in small ruminants.

The current study provides valuable insights into the seroprevalence of *Anaplasma* spp. antibodies in livestock across Slovakia, revealing significant interspecies and regional variations. Among the tested species, sheep exhibited the highest seroprevalence at 70.16%, followed by goats at

43.27% and cattle – 10.90%. The findings of the study are consistent with previous studies following seroprevalence in domestic ruminants. For example, the study from the Kingdom of Saudi Arabia [15] with a seroprevalence of 49.2% in sheep and 44.7% in goats. In Korea [21], 7.0% cattle tested seropositive for *Anaplasma* spp. by cELISA. In Sicily [14], 69.59% seropositivity was detected in sheep, 45.45% in goats and 57.16% in cattle. According to the study of Rubel et al. [16], almost all small ruminant flocks tested seropositive for *Anaplasma* spp. antibodies.

The results obtained in the current align with previous research indicating that small ruminants are more susceptible to *Anaplasma* infections due to their grazing behaviours, which expose them more frequently to tick vectors [7]. Conversely, the lower seroprevalence in cattle is likely attributable to their more confined management systems, limiting their contact with tick habitats [8]. The notable differences in seroprevalence among species suggest varying levels of exposure and host susceptibility. Sheep, which graze extensively in tick-infested areas, showed the highest seroprevalence. This aligns with findings from North Egypt, where similar grazing behaviours were associated with high infection rates [7]. Goats, although also exposed to similar environments, displayed lower seroprevalence, which may be attributed to differences in immune responses or grazing patterns [22]. Cattle exhibited the lowest prevalence, which is consistent with studies from Pakistan, where confined livestock showed reduced exposure to *Anaplasma* spp. [4].

In the current study, notable regional variability was observed. For sheep, breeding 3s and 7s recorded the highest seroprevalence at 97.06%–100%, while breeding 1s had a significantly lower rate of 26.67%. This disparity may reflect differences in local tick densities, vegetation, and herd management practices. Among goats, breeding 1g exhibited a seropositivity rate of 46.67%, surpassing the 0% observed in breeding 3g. In cattle, breeding 1c showed a seroprevalence of 24.39%, while breedings 2c and 3c reported no positive cases. These findings corroborate prior studies suggesting that ecological factors, such as vegetation type, climate, and tick population dynamics, are critical in shaping *Anaplasma* transmission [4, 5].

The role of *I. ricinus* as the primary vector for *A. phagocytophilum* in Europe is well documented. Sheep, which graze extensively in tick-infested areas, are particularly vulnerable, while goats and cattle may experience reduced exposure due to differing grazing and management practices [2]. The Zvolen district (3s), which is characterised by dense tick populations, exhibited higher infection rates, affirming the link between tick activity and *Anaplasma* prevalence [8]. Comparative studies reinforce these findings.

Research conducted in Venezuela reported seroprevalence rates of 80.46% in sheep and 59.25% in goats, which are higher than the rates observed for Slovakian goats; however, they are consistent with Slovakian sheep [22]. Similarly, studies in China found *Anaplasma* spp. prevalence in sheep ranging from 67%–89%, demonstrating regional variations linked to tick densities and climatic factors [11]. Research in Norway reported *A. phagocytophilum* seroprevalence in sheep reaching 80%, which is comparable to the findings in breeding 3s, but higher than in other Slovak regions [23].

Serological tests primarily detect previous exposure rather than active infections, potentially inflating prevalence estimates. Molecular methods, such as PCR,

could complement serology to confirm active infections and provide a clearer picture of the epidemiology of *Anaplasma* spp., including such emerging pathogens as *A. capra* [24].

The economic and health consequences of *Anaplasma* infections are considerable. Subclinical infections can reduce productivity and increase susceptibility to secondary diseases. Implementing region-specific control measures, such as enhanced tick control, vaccination programmes, and better herd management practices, are vital for minimising these effects [2, 7].

The zoonotic potential of certain *Anaplasma* spp. is of increasing concern, as *A. phagocytophilum* is known to cause human granulocytic anaplasmosis, a significant tick-borne disease [25]. While no direct transmission from livestock to humans has been reported, understanding the role of livestock as potential reservoirs for zoonotic strains is critical for public health surveillance [2].

Climate change may further impact the epidemiology of tick-borne disease and *Anaplasma* spp., as shifting temperatures and precipitation patterns can expand tick habitats, increasing infection risks in livestock [26]. Monitoring these environmental changes will be crucial for predicting outbreaks and implementing pro-active control strategies.

Limitations of the study. 1) Since herd enrolment depended on voluntary farmer participation, a random selection process could lead to the identification of farms with different breeding methods. 2) Uneven spatial coverage shaped by herd size, farm accessibility, and willingness to participate, may have under-represented certain regions. 3) Animal demographics and health records were not recorded, making it impossible to link seropositivity with age, tick bites, or clinical signs. Despite these limitations, the results obtained supply a survey of *Anaplasma* spp. exposure in Slovakian domestic ruminants.

CONCLUSIONS

The study offers valuable insights into the seroprevalence of *Anaplasma* spp. antibodies in domestic ruminants across Slovakia, demonstrating significant interspecies and regional differences. The study also underscores the pivotal role of small ruminants in the epidemiology of *Anaplasma* spp. in Slovakia. The findings emphasise the need for species-specific and regionally tailored control measures to address the challenges posed by anaplasmosis, ensuring the health and productivity of livestock while minimising economic losses.

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