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PRELIMINARY DATA ON DETECTION OF ANTIBODIES AGAINST *Anaplasma* spp. IN CATTLE, ROMANIA

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Abstract

Anaplasmosis, a tick-borne disease affecting cattle worldwide caused by Anaplasma spp. (Rickettsiales: Anaplasmataceae), is associated with considerable economic losses to dairy and beef industries. Data about exposure to Anaplasma spp. infection of cattle in Romania is limited. Therefore, a cross-sectional serological study was conducted to preliminary investigate the serological status on Anaplasma spp. infection in Romanian catlle. For this, 182 catlle originated from three counties in Northeastern and Southeastern Romania were included in the study. Cattle serum samples were tested with a competitive enzyme linked immunosorbent assay (cELISA) detecting an epitope of major surface protein 5 (MSP5) of Anaplasma spp. The results were expressed as percentage (%) of inhibition (I) calculated according to the indications of the manufactures; samples with I ≥30% were considered positive. Subsequently, the overall seropositivity was 42.3% (95% CI: 35.03 - 49.84); significant difference of seroprevalence values (p< 0.05) were registered according to the cattle originating county, ranging from 22.2% (95% CI: 13.26-33.57) to 72.0% (95% CI: 57.50-83.77). These findings emphasize on the need for further studies to identify the species and associated risk factors for Anaplasma inffection of cattle in Romania, as base for developing control strategies.

Key words*: Anaplasma spp., serological investigation, cELISA, cattle, Romania.*

INTRODUCTION

Anaplasmosis is a tick-borne disease affecting cattle worldwide caused by *Anaplasma* spp. (Rickettsiales: Anaplasmataceae). It is associated with considerable economic losses to dairy and beef industries worldwide (Kocan et al., 2010). The species of the genus *Anaplasma* are Gram-negative, immobile, obligatory intracellular bacteria that cause diseases in humans and animals, displaying specific cell tropism in the vertebrate hosts (erythrocytes, granulocytes, monocytes/ macrophages, or platelets), depending on the species (Dumler et al., 2001).

The main species affecting domestic ruminants of socio-economic impact are *Anaplasma marginale, Anaplasma centrale, Anaplasma bovis, Anaplasma phagocytophilum, Anaplasma ovis,* and *Anaplasma capra* (Kocan et al., 2010).

Of them, *A. phagocytophilum,* a multi-host pathogen also with zoonotic potential, causes the tick-borne fever in domestic ruminants, while *A. marginale* and *A. centrale* are responsible for bovine anaplasmosis (Dumler et al., 2001; de la Fuente et al., 2005). Ticks are referred as main biological vectors of *Anaplasma*, but also mechanical transmission by biting flies or fomites are described (Kocanet al., 2010; Aubry and Geale, 2011; DaSilva et al., 2014).

Anaplasma spp. causes persistence infection in the host population, which assures more spreading and occurring of new outbreaks.

Bovine anaplasmosis is clinically characterized by anaemia, fever, weight loss, jaundice, abortion, and death (Kocan et al., 2010).

The disease is endemic in tropical and sub-tropical regions of the world, causing significant economic losses to dairy and beef industries worldwide. It is being reported more frequent also in Europe, in Switzerland, Italy (Sicily), Hungary, Spain (Hofmann-Lehmann et al., 2004; de la Fuente et al., 2005; Hornok et al., 2007). Recently, fatal cases of bovine anaplasmosis have been reported in southwestern Spain (Moraga Fernadez et al., 2022).

Different diagnostic tests have been developed and are available for diagnostic; of them, light microscopy of Giemsa-stained blood smears is useful during of the disease's acute phase, while serological tests and molecular-biology based techniques are able to detect subclinical

infected animals and/or low-level infection in carrier animals (Aubry and Geale, 2011).

Several enzyme-linked immunosorbent assay (ELISA)-based tests have been developed and are currently used, of them competitive inhibition enzyme-linked immunosorbent assay (cELISA) and card agglutination test (CAT) are recommend for field, epidemiological studies (O.I.E., 2015).

In Romania data about *Anaplasma* spp. infection in cattle is very limited and no recent studies about bovine anaplasmosis in Romania are available. Therefore, a cross-sectional study was conducted to investigate the serological status on *Anaplasma* spp. infection in Romanian cattle.

MATERIALS AND METHODS

The study was conducted in three counties located in North-eastern and South-eastern Romania (Figure 1). In all three counties there were reported tick infesting cattle.

A number of 182 dairy cattle originated from two different areas in Northeastern (Suceava - SV county; n=60) and Southeastern (Buzau-Bz; n=50; Teleorman- Tr; n=72) Romania, were included in the study.

From cattle, blood samples were collected without anticoagulant, from which serum samples were obtained for serological testing. All samples were stored at -20ºC until testing.

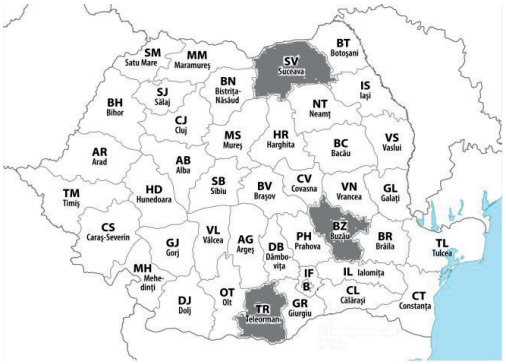

Figure 1. Map of Romania: the study area (cattle originating counties are shown)

The serum samples were tested for detecting IgG antibodies against the MSP5 protein of *Anaplasma* spp. by using a competitive ELISA (cELISA, VMRD v2, Inc.,Pullman, WA, USA) test. The kit is approved for detection of antibodies against *A. marginale, A. ovis,* and *A. centrale*, being reliable for identifying persistently infected cattle (Knowles et al., 1996; Dreher et al., 2005).

The assay was performed in accordance with the manufacturer's instructions. Briefly, 50 µl of the controls and serum samples were transferred to the corresponding wells of the *Anaplasma* Antigen-Coated Plate and incubated for 1 h. After two washes with wash solution, 50 µl of conjugate-peroxidase was added and incubated for 20 min. After washing the plate four times, 50 µl of substrate solution was added to each well and incubated in darkness for 20 min. Finally, 50 µl of stop solution was added and the optical density of the plate was read by a microplate reader Epoch at 630 nm. Results were calculated according to the formula provided by the kit's manual and expressed as percentage of inhibition (%I). Samples with %I! ≥ 30 were considered positive.

RESULTS AND DISCUSSIONS

To investigate the serological status on infection with or exposure to *Anaplasma* spp. infection, cattle reared in two geographical areas in Romania (northeastern and southeastern) were tested by using a cELISA technique. Of the 182 tested cattle, 77 were seropositive and 105 were seronegative.

The overall observed apparent seroprevalence of antibodies against *Anaplasma* spp. was of 42.3% (95% CI: 35.03 - 49.84).

Significant diferences of seroprevalence ($p<0.05$) were registered according to the originating county, ranging from 22.2% (95% CI: 13.26 - 33.57) to 72.0% (95% CI: 57. 50 - 83.77) (Table 1).

Table 1. Seroprevalence of *Anaplasma* spp. in cattle in two different regions of Romania as revealed by cELISA results, stratified by originating area/county

Region/ county	No. tested	No. positive	Prevalence (%)	95% Confidence Interval	P-value
Northeastern					<0.05
Sv	60	25	41.7	29.06-55.12	
Southeastern					
Bz	50	36	72.0	57.50 - 83.77	
Tr	72	16	22.2	13.26 -33.57	
Total	182	77	42.3	35.03- 49.84	

In the present study, using the cELISA (VMRD v2) test an overall seroprevalence of 42.3% of antibodies against *Anaplasma* spp. was recorded, with presence of positive cattle in all the studied counties, suggesting the presence of *Anaplasma* spp. infection in Romanian cattle.

Several recent studies has reported *Anaplasma* spp. infection in Romania by ELISA-based test but primarily in horses (Bogdan et al., 2021) and dogs (Ionita et al., 2012; 2023; Leica et al., 2019), either as single or concurrent infection with other tick-borne pathogens. Moreover, molecular studies have reported the presence of *A. phagocytophilum* in ticks infesting various species, cattle, dog (Ionita et al., 2013; 2016).

Additionally, should be mentioned that in Romania it is a divers tick fauna, comprising up to 25 species belonging to the genera: *Ixodes, Dermacentor*, *Haemaphysalis, Hyalomma, Boophilus & Rhipicephalus,* of them *Ixodes ricinus and Dermacentor marginatus* is the most widespread and the most abundant, respectively (Ionita et al., 2010; Mihalca et al., 2012; Pavel et al., 2020).

Up to date, DNA of *A. marginale* have been detected in various tick species, such as *I. ricinus, D. marginatus, D. reticulatus,* R. *bursa, R. sanguineus, H. marginatum* (Antunes et al., 2016; de la Fuente et al., 2005; 2004; Hornok et al., 2012), all these species are reported in Romania (Ionita et al., 2010; 2013).

Moreover, in the last decades, an increasing abundance of tick population is being reported, and subsequently tick-borne diseases are being more frequently detected, including in cattle (Ionita and Mitrea, 2017; Ionita et al., 2012; 2018; Pavel al., 2020).

Therefore, considering the diverse and sympatric occurrence of tick fauna in Romania, cattle are at are high risks for tick-borne diseases, including bovine anaplasmosis.

The cELISA method used in the present study is recommended and largely applied for serological screening of bovine anaplasmosis (OIE, 2015), with high sensitivity (96.0-98.0%) and specificity (95.2-100%) (VMRDv2; (Knowles et al., 1996; Aubry and Geale, 2011).

Analysing the results obtained with other similar studies on cattle in Europe reveals variations according to the country/region and investigation test used.

Bovine anaplasmosis has been recorded in many countries in Central and South America, Africa and Asia, in the United States, and in European countries (Kocan et al., 2010).

Oh the cattle infecting *Anaplasma* species, *A. marginale* is regarded as the most pathogenic, causing acute disease, which can be also fatal, depending on various factors, including susceptibility of the host, virulence of the strain, and concurrent infection (Hofmann-Lehmann et al., 2004; Jurković et al., 2020).

Fatal disease has been recently reported in Spain and northern Europe, suggesting the risks when infection occurs within a herd (Moraga Fernandez et al., 2022).

Moreover, animals recovered from acute disease are of high epidemiological significance since they remain lifelong persistently infected carriers and serve as natural reservoir (Kocan et al., 2010).

A.centrale is a closely related to *A. marginale* and considered less pathogenic, causing usually benign diseases characterized by mild anemia; it is used as live vaccine against *A.marginale* - South America, Africa, Israel, so (de la Fuente et al., 2005b; Bellezze et al., 2023).

In Europe, *A. marginale* is endemic in some Mediterranean countries, such as in Spain (de la Fuente et al., 2004; Calleja-Bueno et al., 2022), Italy (de la Fuente et al., 2005a) but it is being reported also in other European countries, such as in Italy (de la Fuente et al, 2005), Portugal (Pereira et al., 2016), Hungary (Hornok et al., 2007; 2008; 2012), including in the northern areas, the Alpine region (Dreher et al., 2005a; Hofmann-Lehmann et al., 2004).

A. marginale is endemic also in the island of Sicily (Italy), where high prevalence (78.0%) of seropositivity of *Anaplasma* spp. was detected by cELISA in 50 tested cattle (de la Fuente et al., 2005). A similar serological cELISA-based study peformed on 26 cattle in northern Hungary reported a seropositivity of 80.8% among the tested animals; in the positive animals, *A. marginale* was confirmed by PCR (Hornok et al., 2007).

In Belgium, a sero-prevalence of 15.6% (53/339) was reported in cattle from all regions from Belgium, with higher seropositivity for cattle in Liege (44.4%) than in Flanders (8%) (Adjadj et al., 2023).

The seroprevalence value differences between the originating counties in our study are similar to those described by Spare et al. (2019), in Kansas, reporting an overall seroprevalence of the 52.5% at the herd level; by region the seroprevalence varied from 19.1% (in Western Kansas) up to 87.3% (in Eastern Kansas) and selected management practices have been found associated with herd infection status (Spare et al., 2019). However, multiple factors can be involved in spreading of the disease.

Higher prevalence values are reported in cattle from Egypt, of 54.8% by serology and 68.3% by qPCR (Al-Hosary et al., 2020). Similar high infection rates were reported in cattle from Mozambique, varing from 76.5% in southern region up to 86.3% in Manputo (Tembue et al., 2011).

Nevertheless, the serological surveys provide valuable data of epidemiological relevance to evaluate the distribution and the risk associated factors, in a particular area.

CONCLUSIONS

This study revealed the presence of *Anaplasma* spp. antibodies in cattle in Romania. As the economic impact of anaplasmosis in cattle is major and of increasing importance, further research are need to assess the distribution and risk factors for this pathogen in Romanian cattle. This study was the first to screen for the presence of antibodies to *Anaplasma* spp. in cattle in Romania. Additionally, the findings emphasize on the need for further studies to identify the species and associated risk factors for *Anaplasma* infection of cattle in Romania, as base for developing appropriate control strategies.

REFERENCES

Adjadj, N.R., Cargnel, M., Ribbens, S., Quinet, C., Malandrin, L., Mignon, B., & Mori, M. (2023). Prevalence of *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensulato, Rickettsia* spp. and *Babesia* spp. in cattle serum and questing ticks from Belgium. *Ticks Tick Borne Dis.,*14(4), 102146, doi: 10.1016/j.ttbdis.2023.102146.

Al-Hosary, A., Răileanu, C., Tauchmann, O., Fischer, S., Nijhof, A.M., & Silaghi, C. (2020). Epidemiology and genotyping of *Anaplasma marginale* and co-infection with piroplasms and other Anaplasmataceae in cattle and buffaloes from Egypt. *Parasit Vectors,* 13(1), 495, doi: 10.1186/s13071-020-04372-z.

Antunes, S., Ferrolho, J., Domingues, N., Santos, A.S., Santos-Silva, M.M., Domingos, A. (2016). *Anaplasma marginale* and *Theileria annulata* in questing ticks from Portugal. Exp. Appl. Acarol. 70, 79-88.

Aubry, P. & Geale, D.W. (2011). A review of bovine anaplasmosis. *Transbound Emerg Dis.,* 58(1),1-30, doi: 10.1111/j.1865-1682.2010.01173.x.

Bellezze J, Thompson CS, Bosio AS, Torioni SM, Primo ME. (2023). Development and field evaluation of an ELISA to differentiate *Anaplasma marginale*-infected from *A. centrale*-vaccinated cattle. *J Vet Diagn Invest., 35*(2):204-208.

Bogdan, A.M., Ionita, M., Mitrea I.L. (2021). Serological evidence of natural exposure to tick-borne pathogens in horses, Romania. *Microorganisms,* 9(2):373. doi: 10.3390/microorganisms9020373.

Calleja-Bueno L, Sainz Á, García-Sancho M, González-Martín JV, Díaz-Regañón D, Rodríguez-Franco F, Agulla B, Tormo B, Villaescusa A. (2022). First detection of *Anaplasma phagocytophilum* and *Babesia divergens* and high infection rates of *Anaplasma marginale* and *Babesia bigemina* in cattle in extensive grazing systems of Central Spain. *Transbound Emerg Dis. 69(4):*e1090-e1100. doi: 10.1111/tbed.14394.

da Silva, J.B., & da Fonseca, A.H. (2014). Risk factors for anaplasmosis in dairy cows during the peripartum. *Trop Anim Health Prod.,* 46(2), 461-465, doi: 10.1007/s11250-013-0514-0.

de la Fuente, J., Vicente, J., Höfle, U., Ruiz-Fons, F., Fernández De Mera, I.G., Van Den Bussche, R.A., Kocan, K.M., Gortazar, C. (2004). *Anaplasma* infection in free-ranging Iberian red deer in the region of Castilla-La Mancha, Spain. *Vet Microbiol*., 100(3-4), 163-73.

de la Fuente, J., Torina, A., Caracappa, S., Tumino, G., Furlá, R., Almazán, C. & Kocan, K.M. (2005a). Serologic and molecular characterization of *Anaplasma* species infection in farm animals and ticks from Sicily, *Vet Parasitol*., 133(4), 357-62.

de la Fuente, J., A. Lew, H. Lutz, M. L. Meli, R. HofmannLehmann, V. Shkap, T. Molad, A. J. Mangold, C. Alamaza´n, V. Naranjo, C. Gorta´zar, A. Torina, S. Caracappa, A. L. Garcı´a-Pe´rez, M. Barral, B. Oporto, L. Ceci, G. Carelli, E. F. Blouin, and K. M. Kocan (2005b). Genetic diversity of Anaplasma species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. *Anim. Health Res. Rev. 6,* 75–89.

Dreher, U., De La Fuente, J., Hofmann-Lehmann, R., Meli, M.L., Pusterla, N., Kocan, K., Woldehiwet, Z., Braun, U., Regula, G. & Staerk, K. (2005). Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. *Clin. Vaccine Immunol.*, 12(10), 1177-1183.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R. (2001). Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of

Ehrlichia phagocytophilum. *Int. J. Syst. Evol. Microbiol. 51,* 2145–2165.

Hofmann-Lehmann, R., Meli, M.L., Dreher, U.M., Gönczi, E., Deplazes, P., Braun, U., Engels, M., Schüpbach, J., Jörger, K., Thoma, R., Griot, C., Stärk, K.D., Willi, B., Schmidt, J., Kocan, K.M., Lutz, H. (2004). Concurrent infections with vector-borne pathogens associated with fatal hemolytic anemia in a cattle herd in Switzerland. *J. Clin. Microbiol. 42,* 3775-80.

Hornok, S., Elek, V., de la Fuente, J., Naranjo, V., Farkas, R., Majoros, G. & Földvári, G. (2007). First serological and molecular evidence on the endemicity of *Anaplasma ovis* and *A. marginale* in Hungary.*Vet Microbiol.*, 122(3-4), 316-22.

Hornok, S., Földvári, G., Elek, V., Naranjo, V., Farkas, R., de la Fuente, J. (2008). Molecular identification of Anaplasma marginale and rickettsial endosymbionts in blood-sucking flies (Diptera: Tabanidae, Muscidae) and hard ticks (Acari: Ixodidae). *Vet. Parasitol. 154,* 354-9

Ionita, M., Mitrea, I. L. (2017). Actualities on vectorborne disease: risks for the human and animal health in Romania (a review). *Revista Romana de Medicina Veterinara, 27(2),* 8-14.

Ionita, M., Mitrea I.L., Buzatu M.C., Dascalu, L., (2010), Seasonal dynamics of tick (Acari: Ixodidae) populations in different areas of Romania and the associated risks of tick-borne diseases. Proceedings of the XIIth International Congress of Parasitology (ICOPA), Melbourne, Australia, 15- 20th August, 2010, *Medimond International Proceedings*, pp.91-95.

Ionita, M., Enachescu, V., Mitrea, I.L. (2012). Preliminary data on serological survey of exposure to arthropod-borne pathogens in stray dogs from Bucharest, Romania. *Scientific Works-University of Agronomical Sciences and Veterinary Medicine, Bucharest Series C, Veterinary Medicine*, 58 (4), 217-222.

Ionita, M., Mitrea, I.L., Pfister, K., Hamel, D., Silaghi, C. (2013). Molecular evidence for bacterial and protozoan pathogens in hard ticks from Romania. *Veterinary Parasitology*, 196(1-2), 71-76.

Ionita, M., Silaghi, C., Mitrea, I.L., Edouard, S., Parola, P. & Pfister, K. (2016). Molecular detection of *Rickettsia conorii* and other zoonotic spotted fever group rickettsiae in ticks, Romania. *Ticks Tick Borne Dis.*, 7(1):150-153. doi: 10.1016/j.ttbdis.2015.10.006.

Ionita, M., Nicorescu, MI., Pfister, K., Mitrea, I. L. (2018). Parasitological and molecular diagnostic of a clinical *babesia caballi* outbreack in Southern Romania. *Parasitology Research, 117,* 2333-2339.

Ionita, M., Leica, L., Wassermann, M., Mitrea, E. Nicorescu, M., Mitrea, I.L. (2023). Detection and molecular characterization of canine babesiosis causative agent *Babesia canis* in Naturally Infected Dogs in the Dobrogea Area (Southeastern Romania), *Life, 13* (6), 1354.

Jurković D, Mihaljević Ž, Duvnjak S, Silaghi C, Beck R. (2020). First reports of indigenous lethal infection with Anaplasma marginale, *Anaplasma bovis* and *Theileria orientalis* in Croatian cattle. *Ticks Tick Borne Dis.11*(5):101469.

Knowles, D., Torioni de Echaide, S., Palmer, G., McGuire, T., Stiller, D., McElwain, T. (1996). Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. *J. Clin. Microbiol. 34*, 2225–2230.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S.A. (2010). The natural history of *Anaplasma marginale. Vet. Parasitol.* 167, 95-107.

Leica L, Mitrea IL, Ionita M. (2019). Clinical occurrence of canine babesiosis in the coastal area of the Black Sea (Dobrogea) in Southeastern Romania and associated epidemiological implications. *J Parasitol.,* 105(4):491-496.

Mitrea, I.L. (2002). Parasitological control - biological, medical and economic concept. *Scientia Parasitologica*, 1, 79-89.

Moraga Fernández A, Ortiz JA, Jabbar A, Ghafar A, Cabezas-Cruz A, de la Fuente G, de la Fuente J, Fernández de Mera IG. (2022). Fatal cases of bovine anaplasmosis in a herd infected with different Anaplasma marginale genotypes in southern Spain. *Ticks Tick Borne Dis*.., 13(1):101864.

OIE (2015). *Manual of Diagnostic Tests and Vaccines for Terestrial Animals. Bovine Anaplasmosis*, Chapter 2.4.1.

Pavel, A., Ionita, M., Mitrea, I.L. (2020). Clinical and anatomo-pathological study on bovine babesiosis in the Danube Delta (Romania). *Revista Romana de Medicina Veterinara* 30(2), 33-38.

Spare, M.R., Hanzlicek, G.A., Wootten, K.L., Anderson, G.A., Thomson, D.U., Sanderson, M.W., Ganta, R.R., Reif, K.E., & Raghavan, R.K. (2019). Bovine anaplasmosis herd prevalence and management practices as risk-factors associated with herd disease status. *Vet. Parasitol.,* 6(3), 100021.

Tembue, A.A., da Silva, J.B., da Silva, F.J., Pires, M.S., Baldani, C.D., Soares, C.O., Massard, C.L. & da Fonseca, A.H. (2011). Seroprevalence of IgG antibodies against *Anaplasma marginale* in cattle from south Mozambique. *Rev Bras Parasitol Vet*., 20(4), 318-324.