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Screening for *Coxiella burnetii* and *Chlamydia* species in Polish *Cervidae**

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***Coxiella burnetii* and *Chlamydia* spp. are known to have a wide range of hosts, e.g., mammals, birds, reptiles, including free-living animals, which serve as vectors for its transmission to human and animal population. The aim of this study was to verify the occurrence of *Coxiella burnetii* and *Chlamydiaceae* in samples collected from red deers, roe deers and fallow deers living in various regions of Poland. Serum samples (n=385) were analyzed using an ELISA test to detect *C. burnetii* antibodies, while tissue samples (i.e. lungs, liver, spleen) from 372 animals were tested by specific real-time PCRs for the presence of *Chlamydiaceae*-specific 23S rRNA and *C. burnetii* DNA. All serum samples tested negative for *C. burnetii* antibodies. Additionally, molecular analysis performed on the DNA samples did not detect either of searching pathogens. Lack of tested zoonotic agents in the national population might indicate that cervids do not play a significant role in the transmission of these pathogens to humans and farm animals in Poland.**

KEYWORDS: *Coxiella burnetii* / *Chlamydia* spp. / *Cervidae* / ELISA / real-time PCR

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Coxiella burnetii (C. burnetii), the causative agent of Q fever, and *Chlamydia* species – members of *Chlamydiaceae* family, which cause chlamydiosis, are intracellular pathogens. Despite belonging to distinct classes - *C. burnetii* is a member of the Gammaproteobacteria, while *Chlamydia* species belong to the class Chlamydia - all are important zoonotic pathogens with broad host ranges and the potential to significantly impact agriculture and public health [Marti and Jelocnik 2022]. These pathogens have been detected in different animal species including mammals, birds, and reptiles [Celina and Cerný *et al*. 2022, Mitura *et al*. 2017, Szymańska-Czerwińska *et al*., 2017a, 2017b]. Free-living animals may serve as reservoirs and sources of *C. burnetii* and *Chlamydia* infections for both domestic animals and humans [Aazis *et al*. 2015, Di Francesco *et al*. 2012, Marti *et al*. 2022, Pires *et al*. 2023].

Q fever and chlamydiosis are widespread globally, including in Europe and Poland [Celina and Cerny 2022, Marti and Jelocnik 2022, Szymańska-Czerwińska *et al*. 2017a, 2017b, 2019, 2022, 2024-*in press*]. However, the role of wildlife in their transmission remains poorly understood. Data on the prevalence of *C. burnetii* and *Chlamydia* spp. in wild animals in Poland are limited to serological studies on bison and molecular studies on red deer, roe deer, wild boar, and ticks, with samples collected from limited areas [Bielawska-Drózd *et al*. 2018, Krzysiak *et al*. 2021, Szymańska-Czerwińska *et al*. 2013]. Given the significant prevalence of *C. burnetii* in domestic ruminants, such as dairy cattle, and the confirmed shedding of chlamydiae in poultry, cattle as well as wild birds and reptiles, a screening survey of cervids is a crucial step in evaluating the circulation of these pathogens [Mitura *et al*. 2017, Szymańska-Czerwińska *et al*. 2017a, 2017b, 2019].

The aim of this study was to screen for *C. burnetii* antibodies in serum samples of *Cervidae* using ELISA test. Additionally, the study evaluated the presence of *C. burnetii* and *Chlamydiaceae* in internal organs of these animals through molecular testing of tissues collected by hunters.

Material and methods

Ethical review and approval were waived for this study, due to the origin of the blood and tissues of carcasses. As a matter of fact, carcasses were not obtained from experimental trials but from a regional passive surveillance system during hunting season. This study followed the activity of hunting, in strict collaboration with the hunters and assistant veterinarians.

A total of 442 animals belonging to *Cervidae* family i.e., red deer (*Cervus elaphus*) (n=307), roe deer (*Capreolus capreolus*) (n=99) and fallow deer (*Dama dama*) (n=36) were hunted in 2023 in the areas of 37 forest inspectorates in Poland. Locations of sampling are presented on Figure 1. Samples were collected from 127 stags, 95 red deer does, 34 roebucks, 60 roe deer does, 25 fallow deer bucks and 10 fallow deer does. Gender of 85 red deers, 5 roe deers and one fallow deer was unknown due to missing data from hunters. The age of tested red deers ranged from 5 months to 12 years. In the case of roe deers and fallow deers, animals with age ranging from 5

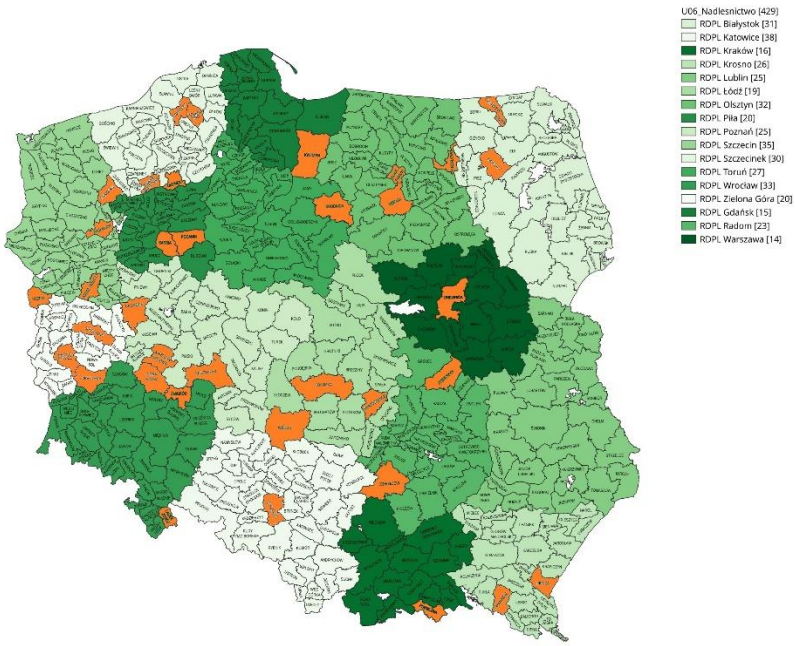

Fig. 1. Areas of sampling. The forest districts where samples were collected are marked in orange. Different shades of green represent forrest districts belonging to the same Regional State Forest Directorate as indicated in the map legend.

months to 8 years and 1 to 8 years, respectively, were hunted.

Blood samples (n=385) were stored at room temperature to allow clotting and then sent to the laboratory. Serum was obtained by centrifugation of blood samples at 1,000 × *g* for 10 min. If the serological test was performed within 48 h, the temperature of the sample was maintained between 4°C and 8°C, otherwise the sera were stored at −20 ± 5°C until tested. Q-Fever (*Coxiella burnetii*) Antibody Test Kit (IDEXX, Liebefeld, Switzerland) was utilised to screen sera for the presence of *C. burnetii* antibodies. Following the manufacturer's instructions, the optical density (OD) percentage was calculated as (OD sample − ODneg)/(ODpos − ODneg) × 100 after averaging the duplicate values. Sera were considered to be negative when %OD <30, dubious when %OD ≥30 and %OD ≤40 or positive when %OD >40.

Blood, lungs, liver and spleen tissues were collected from the animals as specified in Table 1. Collected samples from a single animal were pooled (n=372), and DNA extraction was performed using the QIAamp DNA Mini Kit and the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA extracts were stored at -20°C until further analysis.

Real-time PCR was conducted using the 7500 Fast Real-Time PCR System v2.3 (Applied Biosystems, USA) to detect *Chlamydiaceae* and *C. burnetii* in tissue samples

Table 1. Summary of samples by species, sex, and location. B – blood, O – organs. N/A – data about gender not available

Forest inspectorate	Red deer									Roe deer									Fallow deer								
	Male			Female			N/A			Male			Female			N/A			Male			Female			N/A		
	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O	B	B+O	O
Bierzwnik		5																					1				
Bircza		5			4			1																			
Borne Sulinowo		4			1																						
Brodnica		1									1			1													
Czerwony Dwór		1		3	2	4						1	7		7	1											
Dobieszyn														1						8							
Drawsko		2												1	1												
Drewnica											9																
Drygały		4			3			6																			
Góra Śląska		14			1		14																				
Grodzisk																				8			3				
Jędrzejów	2		1	1	3	2								7									1				
Karczma Borowa		3	10			1	11																				
Kolumna					1						2			4									3				
Krotoszyn		2			2			2			1			1			2			1			2			1	
Krzystkowice		1			4									10													
Kwidzyn		1									7																
Lądek Zdrój		3			7																						
Międzyrzecz		5			8																						
Nidzica			9			2	10	13																			
Nowe Ramuki		1			1		2	10																			
Okonek														3													
Piwniczna			2		3	2	4	1				2		1		2											
Podanin					1															8							
Rudziniec					1						2			10	2												
Rymanów		1			4																						
Rzepin		10																									
Sarbia		12									1																
Smardzewice							1																				
Strzałowo	3		3		1			2		4		3															
Sulechów		7			2			1																			
Szprotawa					5	4	5		1																		
Trzebielino		5			5																						
Warcino		3			6			1																			
Wieluń		7			6																						
Żmigród					5						1			4													
Total	**5**	**97**	**25**	**4**	**76**	**15**	**47**	**37**	**1**	**4**	**24**	**6**	**7**	**43**	**10**	**3**	**2**	**0**	**0**	**25**	**0**	**0**	**10**	**0**	**0**	**1**	**0**
	127			**95**			**85**			**34**			**60**			**5**			**25**			**10**			**1**		

from free-living ruminants. *Chlamydiaceae*-specific 23S rRNA gene fragment was performed, as previously described by Ehricht *et al*. [2006]. A panel of required positive and negative controls was used in each run, including TaqMan Exogenous Internal Positive Control (Thermo Fisher Scientific, USA) as a commercial internal amplification control to monitor PCR inhibitors. All samples with a cycle-threshold (Ct) value above 36 were considered negative. The cut-off value was selected based on the limit of detection determined in the validation process. To detect *C. burnetii*, a qualitative real-time PCR test targeting the IS*1111* repetitive element was carried out using an Adiavet COX Real-Time PCR kit (Bio-X Diagnostics, Rochefort, Belgium). A panel of required positive and negative controls was included in each run. An analytical cut-off value of 36.0 was selected corresponding to the defined limit of detection of the test. These real-time PCR procedures were validated under laboratory conditions.

Results and discussion

All tested serum samples were negative for the presence of *C. burnetii* antibodies. Real-time PCR tests, detecting *C. burnetii* and *Chlamydiaceae* DNA, were negative for all tested animals.

C. burnetii and *Chlamydia* spp. are significant zoonotic pathogens with the potential to infect a wide range of animal species, including wildlife [Berri *et al*. 2009]. Investigating their prevalence in free-living animals is crucial, as these populations can act as reservoirs, contributing to the spread of infections to domestic animals and humans. Due to their mobility and interaction with various environments, wildlife may play a key role in the epidemiology of these pathogens.

C. burnetii and *Chlamydia* spp. have been found in various wild animals worldwide, although most studies focus on domestic livestock [Celina and Cerný 2022, Cubero-Pablo *et al*. 2000, Martin and Jelocnic 2022, Meredith *et al*. 2015]. Wild animals are increasingly recognized as important reservoirs of pathogens [Kruse *et al*. 2004]. Presence of antibodies to *C. burnetii* has been reported in various wild animal species in Spain, including chamois (*Rupicapra rupicapra*), fallow deer (*Dama dama*), European wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) [Espí *et al*. 2021], European mouflon (*Ovis aries musimon*), and red deer (*Cervus elaphus*) [Espí *et al*. 2021, Fernández-Aguilar *et al*. 2015]. To date, *C. burnetii* has been sporadically detected in wild animals, including wild ruminants, in Poland [Bielawska Drózd *et al*. 2018, Krzysiak *et al*. 2021]. The majority of studies conducted in Poland have been focused on domestic ruminants, and shown a significant presence of the pathogen [Szymańska-Czerwińska *et al*. 2019, 2022]. For instance, a research on dairy cattle confirmed high prevalence of *C. burnetii* in these animals, as well as in dairy products [Szymańska-Czerwińska *et al*. 2019]. One of the very few studies conducted on wildlife indicated that all but one of 523 serum samples collected from European bison from different Polish populations were negative, suggesting limited pathogen circulation in that species [Krzysiak *et al*. 2021]. Aforementioned study provide some insight into the presence of *C. burnetii* in wildlife, but more extensive studies are necessary to determine the full extent of its prevalence in different free-living animal species in Poland. This study contributes to our knowledge about the presence of both *C. burnetii* and *Chlamydia* spp. in cervids. Contrary to data from other countries and previous research from Poland, no antibodies for *C. burnetii* were detected in wild ruminants in this study. The lack of seropositive animals in Poland and the low seroprevalence found in red deer and European mouflon (1.6% and 8.4%) in the Spanish study [Fernández-Aguilar *et al*. 2015], in roe deer (1.2%) in Flanders [Tavernier *et al*. 2015] and in red deer (1.9%) and wild boar (1.1%) in Portugal [Pires *et al*. 2023], may suggest that wild ruminants are exposed to *C. burnetii*, but are not a major reservoir of the pathogen and do not play an important role in maintaining the bacteria's transmission in these environments. The absence of seropositive animals in this study could be due to several factors. One possibility is low pathogen circulation in the population, meaning that the animals had limited exposure to *C. burnetii*. As

sampling was conducted in 37 out of 429 forest inspectorates (Fig. 1), presence of seropositive wildlife populations in non-tested regions cannot be excluded. The absence of detectable antibodies in animals exposed to *C. burnetii* could be linked to the pathogen's ability to establish latent infections [Arricau-Bouvery *et al*. 2005]. *C. burnetii* is known for its capacity to persist in host tissues in a latent or subclinical state, which means the immune system may not be fully activated to produce detectable levels of antibodies, particularly in chronic or low-level infections. Latent infections can occur when *C. burnetii* avoids immune detection or triggers an insufficient immune response, leading to a lack of seroconversion. The bacterium can reside intracellularly in macrophages and other immune cells, where it may evade the immune system by reducing its visibility and downregulating immune responses [Arricau-Bouvery *et al*. 2005]. In such cases, chronic carriers might not exhibit overt clinical signs of infection, and their immune system might not generate enough antibodies to be detected through standard serological tests like ELISA. Moreover, immunosuppression or immunotolerance could prevent the full stimulation of the host's immune system. For example, in animals with repeated exposure to low levels of *C. burnetii*, the immune response may not be robust enough to produce significant antibody levels. This lack of immune stimulation could explain why some animals, even if infected, do not show seropositivity during testing [Porter *et al*. 2011]. Despite the confirmed common presence of *C. burnetii* in domestic ruminants [Szymańska-Czerwińska *et al*. 2019, 2022], no evidence of its presence in organs of tested *Cervidae* was found in this study through real-time PCR testing. One reason is that these animals live in different ecological niches that rarely overlap with domestic livestock. Moreover, many studies based on genotyping and genome sequencing have shown a potential host tropism among *C. burnetii* isolates [Tomaiuolo *et al.* 2021; Joulié *et al*. 2017]. This dependency was observed by González-Barrio *et al*. [2016], who analyzed genotypes circulating in Spanish wildlife. The study showed that certain *C. burnetii* genomic groups are more prone to be found in livestock, while others are more frequent in wildlife and ticks. MLVA analysis showed that *C. burnetii* genotypes present in Polish cattle are clustered together with cattle-derived genotypes from other countries and are genetically distinct from those identified in small ruminants and wildlife in Europe [Szymańska-Czerwińska *et al*. 2019].

It cannot be excluded that particular pathogen-host adaptations may occur in *C. burnetii* strains circulating in Polish domestic ruminants, hindering infection of wildlife. This assumption is supported by results obtained by Bielawska-Drózd *et al*. [2018], who detected bacterial DNA in only 3% of tested wildlife, specifically three boars, three stags and one roe deer.

Molecular studies conducted on wildlife in Europe showed low prevalence of *C. burnetii* and *Chlamydia* spp. Research conducted in central Italy revealed the presence *C. burnetii* in the spleens of 4/72 (4.16%) tested roe deer, while all specimens were negative for *C. abortus* [Ebani *et al*. 2022]. In Hungary, members of the Chlamydiales order were detected with the 16S ribosomal RNA gene-based qPCR assay in 4 of 91

placentas collected from roe deer, red deer and fallow deer, while *C. burnetii* DNA was found in two of them (2.2%) [Kreizinger *et al*. 2015]. The absence of *C. burnetii* and *Chlamydiaceae* in the internal organs of wild ruminants in this study could also be due to the pathogen's preference for specific tissues involved in transmission. *C. burnetii* shows stronger tropism for the respiratory and reproductive systems rather than systemic infection in major organs like the liver or kidneys. The bacterium is often concentrated in tissues associated with reproduction, such as the placenta, facilitating transmission during birthing or abortion [Tolpinrud *et al*. 2024]. It can also spread through respiratory secretions, which may explain why it is less detectable in other internal organs. While *Chlamydiaceae* family members were absent in this study, they have been detected more frequently in samples from the gut, reproductive tract and faeces of roe deer, respectively in France and Australia [Aaziz *et al*. 2015, Jelocnic *et al*. 2019]. Comparative sequence analysis led to the detection of an atypical (non-classified) *Chlamydiaceae* strain closely related to *C. trachomatis*, *C. suis*, and *C. muridarum* [Aaziz *et al*. 2015, Jelocnic *et al*. 2019].

Chlamydial agents, including atypical strains, have been reported also in Poland in various wild animals, including birds and amphibians [Mitura *et al*. 2017, Szymańska-Czerwińska *et al*. 2017]. It is worth mention that during latent infections, both tested intracellular bacterial agents can remain at very low levels, evading detection by conventional methods like PCR. This could further explain the absence of the pathogens in tissues not directly involved in their transmission pathways. Chlamydial infections have been found in various wild animal species globally, including Poland. Wild birds, mammals, and reptiles are the primary hosts of these bacteria [Stokes *et al*. 2021, Di Francesco *et al*. 2012]. Little is known about chlamydiosis in wild ruminants, although serological investigations in Europe suggest a role of wild ruminants as reservoirs of chlamydia. The presence of antibodies against *C. abortus* was confirmed in 6.7% of 190 roe deers from Flanders [Tavernier *et al*. 2015]. Antibodies against *C. psittaci* and *C. suis* were also detected in red deer in Italy [Di Francesco *et al*. 2012]. The authors of the research suggested that the antibody response could be related to contact with birds and wild boars. Serological surveys were not conducted in this study, although real-time PCR analyses, performed on internal organ samples, did not confirmed the presence of *Chlamydiaceae* in tested wildlife.

In conclusion, lack of evidence on presence of *C. burnetii* and *Chlamydia* spp. in *Cervidae* in this study suggests that they may not play a significant role as reservoirs for these pathogens in tested areas. The absence of detectable antibodies to *C. burnetii* and the lack of pathogens presence in internal organs highlights the possibility of low pathogen circulation, latent infections, or the preference of these pathogens for specific tissues. Further research, including studies other species and covering larger areas of the country, is needed to fully understand the epidemiology of these zoonotic agents in wildlife and their potential transmission to livestock and humans.

REFERENCES

1. AAZIZ R., VORIMORE F., VERHEYDEN H., PICOT D., BERTIN C., RUETTGER A., SACHSE K., LAROUCAU K., 2015 - Detection of atypical *Chlamydiaceae* in roe deer (*Capreolus capreolus*). ***Veterinary Microbiology*** 31, 181(3-4), 318-22.
2. ARRICAU-BOUVERY N., RODOLAKIS A., 2005 - *Is Q fever an emerging or re-emerging zoonosis?*. ***Veterinary Research*** 36(3), 327-349.
3. BERRI M., REKIKI A., BOUMEDINE K.,S., RODOLAKIS A., 2009 - Simultaneous differential detection of *Chlamydophila abortus, Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. ***BMC Microbiology*** 9, 130.
4. BIELAWSKA-DRÓZD A., CIEŚLIK1 P., ŻAKOWSKA D., GŁOWACKA P., WLIZŁO-*SKOWRONEK* B., ZIĘBA P., ZDUN A., 2018 - Detection of *Coxiella burnetii* and *Francisella tularensis* in tissues of wild-living animals and in ticks of North-West Poland. ***Polish Journal of Microbiology*** 67, 4, 529-534.
5. CELINA S.S., CERNÝ J., 2022 - *Coxiella burnetii* in ticks, livestock, pets and wildlife: A mini-review. ***Frontiers in Veterinary Science*** 11, 9,1068129.
6. CUBERO-PABLO M. J., PLAZA M., PÉREZ L., GONZÁLEZ M., LEÓN-VIZCAÍNO L., 2000 - Seroepidemiology of chlamydial infections of wild ruminants in Spain. ***Journal of Wildlife Diseases*** 36(1), 35-47.
7. Di FRANCESCO A., DONATI M., NICOLOSO S., ORLANDI L., BALDELLI R., SALVATORE D., SARLI G., CEVENINI R., MORANDI F., 2012 - Chlamydiosis: Seroepidemiologic survey in a red deer (*Cervus elaphus*) population in Italy. ***Journal of Wildlife Diseases*** 48(2), 488-491.
8. EBANI V.V., TREBINO C., GUARDONE L., BERTELLONI F., CAGNOLI G., ALTOMONTE I., VIGNOLA P., BONGI P., MANCIANTI F., 2022 - Retrospective molecular survey on bacterial and protozoan abortive agents in roe deer (*Capreolus capreolus*) from Central Italy. ***Animals*** 12, 3202.
9. EHRICHT R., SLICKERS P., GOELLNER S., HOTZEL H., SACHSE K., 2006 - Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. ***Molecular and Cellular Probes*** 20(1), 60-3.
10. ESPÍ A., DEL CERRO A., OLEAGA O., RODRÍGUEZ-PÉREZ M., LÓPEZ C., HURTADO A., RODRÍGUEZ-MARTÍNEZ L., BARANDIKA J., GARCÍA-PÉREZ A., 2021 - One health approach: An overview of Q fever in livestock, wildlife and humans in Asturias (Northwestern Spain). ***Animals*** 11, 1395.
11. FERNÁNDEZ-AGUILAR X., CABEZÓN O., COLOM-CADENA A., LAVÍN S., LÓPEZ-OLVERA J.R., 2015 - Serological survey of *Coxiella burnetii* at the wildlife-livestock interface in the Eastern Pyrenees, Spain. ***Acta Veterinaria Scandinavica*** 58, 26.
12. GONZÁLEZ-BARRIO D., JADO I., FERNÁNDEZ-DE-MERA, I.G., DEL ROCIO FERNÁNDEZ-SANTOS M., RODRÍGUEZ-VARGAS M., GARCÍA-AMIL C., BELTRÁN-BECK B., ANDA P., RUIZ-FONS F., 2016 - Genotypes of *Coxiella burnetii* in wildlife: disentangling the molecular epidemiology of a multi-host pathogen. ***Environmental Microbiology Reports*** *8*(5), 708-714.
13. JELOCNIK M., TAYLOR-BROWN A., O'DEA C., ANSTEY S., BOMMANA S., E MASTERS N., KATOULI M., JENKINS C., POLKINGHORNE A., 2019 - Detection of a range of genetically diverse chlamydiae in Australian domesticated and wild ungulates. ***Transboundary and Emerging Diseases*** 66:1132-1137.
14. JOULIÉ A., SIDI-BOUMEDINE K., BAILLY X., GASQUI P., BARRY S., JAFFRELO L., PONCET C., ABRIAL D., YANG E., 2017 - Animal diagnostic laboratories consortium, Leblond, A., Rousset, E., Jourdain, E., Molecular epidemiology of *Coxiella burnetii* in French livestock reveals the existence of three main genotype clusters and suggests species-specific associations as well as regional stability. Infection, genetics and evolution: ***Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*** 48, 142-149.

15. KREIZINGER Z., SZEREDI L., BACSADI Á., NEMES C., SUGÁR L., VARGA T., SULYOK K.M., SZIGETI A., ÁCS K., TÓBIÁS E., BOREL N., GYURANECZ M., 2015 - Occurrence of *Coxiella burnetii* and *Chlamydiales* species in abortions of domestic ruminants and in wild ruminants in Hungary, Central Europe. ***Journal of Veterinary Diagnostic Investigation*** 27(2), 206-210.
16. KRUSE H., KIRKEMO A.M., HANDELAND K., 2004 - Wildlife as source of zoonotic infections. ***Emerging Infectious Diseases*** 10(12), 2067-2072.
17. KRZYSIAK M.K., PUCHALSKA M., OLECH W., ANUSZ K., 2021 - A Freedom of *Coxiella burnetii* infection survey in European Bison (*Bison bonasus*) in Poland. ***Animals*** 11, 651.
18. MARTI H., JELOCNIK M., 2022 - Animal Chlamydiae: A concern for human and veterinary medicine. ***Pathogens*** 11(3), 364.
19. MEREDITH A.L., CLEAVELAND S.C., DENWOOD M.J., BROWN J.K., SHAW D.J., 2015 - *Coxiella burnetii* (Q-Fever) seroprevalence in prey and predators in the United Kingdom: Evaluation of Infection in Wild Rodents, Foxes and Domestic Cats Using a Modified ELISA. ***Transboundary and Emerging Diseases*** 62(6), 639-49.
20. MITURA A., NIEMCZUK K., ZARĘBA K., ZAJĄC M., LAROUCAU K., SZYMAŃSKA-CZERWIŃSKA M., 2017 - Free-living and captive turtles and tartoises as carriers of new *Chlamydia* spp. ***PLoS One*** 12(9), e0185407.
21. PIRES H., CARDOSO L., LOPES A.P., FONTES M.D.C., MATOS M., PINTADO C., FIGUEIRA L., MESQUITA J.R., MATOS A.C., COELHO A.C., 2023 - Seropositivity for *Coxiella burnetii* in Wild Boar (*Sus scrofa*) and Red Deer (*Cervus elaphus*) in Portugal. ***Pathogens*** 12, 421.
22. PORTER S. R., CZAPLICKI G., MAINIL J., GUATTÉO R., SAEGERMAN C., 2011 - *Q fever: current state of knowledge and perspectives of research of a neglected zoonosis.* ***International Journal of Microbiology*** 2011. Article ID 248418.
23. STOKES H.S., BERG M.L., BENNETT A.T.D., 2021 - A review of chlamydial infections in wild birds. ***Pathogens*** 10(8), 948.
24. SZYMAŃSKA-CZERWIŃSKA M., GALIŃSKA M.E., NIEMCZUK K., ZASĘPA M., 2013 - Prevalence of *Coxiella burnetii* infection in foresters and ticks in south-eastern Poland and comparison of diagnostic methods. ***Annals of Agricultural and Environmental Medicine*** 20(4), 699-704.
25. SZYMAŃSKA-CZERWIŃSKA M., MITURA A., NIEMCZUK K., ZARĘBA K., JODEŁKO A., PLUTA A., 2017a - Dissemination and genetic diversity of chlamydial agents in Polish wildfowl: Isolation and molecular characterisation of avian *Chlamydia abortus* strains. ***PLoS One*** 12(3), e0174599.
26. SZYMAŃSKA-CZERWIŃSKA M., MITURA A., ZARĘBA K., SCHNEE C., KONCICKI A., NIEMCZUK K., 2017b - Poultry in Poland as *Chlamydiaceae* carrier. ***Journal of Veterinary Research***, 61(4), 411-419.
27. SZYMAŃSKA-CZERWIŃSKA M., JODEŁKO A., ZARĘBA-MARCHEWKA K., NIEMCZUK K., 2019 - Shedding and genetic diversity of *Coxiella burnetii* in Polish dairy cattle. ***PLoS ONE*** 14(1): e0210244.
28. SZYMAŃSKA-CZERWIŃSKA M., JODEŁKO A., OSIŃSKI Z., NIEMCZUK K., 2022 - Screening for *Coxiella burnetii* in dairy cattle herds in Poland. ***Journal of Veterinary Research*** 66(4), 549-557.
29. SZYMAŃSKA-CZERWIŃSKA M., JODEŁKO A., ZARĘBA-MARCHEWKA K., OSIŃSKI Z., NIEMCZUK K., 2024 – Prevalence of chlamydiae in dairy cattle herds and factors contributing to the spread of infections. ***Journal of Veterinary Research*** 68, 531-538.
30. TAVERNIER P., SYS S. U., DE CLERCQ K., DE LEEUW I., CAIJ A. B., DE BAERE M., DE REGGE N., FRETIN D., ROUPIE V., GOVAERTS M., HEYMAN P., VANROMPAY D., YIN L., KALMAR I., SUIN V., BROCHIER B., DOBLY A., DE CRAEYE S., ROELANDT S., GOOSSENS E., ROELS S., 2015 - Serologic screening for 13 infectious agents in roe deer (*Capreolus capreolus*) in Flanders. ***Infection Ecology & Epidemiology*** *5*, 29862.

31. TOLPINRUD A., TADEPALLI M., STENOS J., LIGNEREUX L., CHABER A. L., DEVLIN J.M., CARAGUEL C., STEVENSON M.A., 2024 - Tissue distribution of *Coxiella burnetii* and antibody responses in macropods co-grazing with livestock in Queensland, Australia. ***PLoS ONE*** 19(5):e0303877.
32. TOMAIUOLO S., BOARBI S., FANCELLO T., MICHEL P., DESQUEPER D., GRÉGOIRE F., CALLENS J., FRETIN D., DEVRIENDT B., COX E., MORI M., 2021 - Phylogeography of Human and Animal *Coxiella burnetii* Strains: Genetic Fingerprinting of Q Fever in Belgium. ***Frontiers in Cellular and Infection Microbiology*** 10, 625576.