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Review article

Occurrence of chosen parasitic protozoa of roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) in Poland: a current review

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ABSTRACT. Both roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) are wild ruminants that are present in large numbers in Poland. Although both are very often infected with parasitic protozoa, the species composition of these parasites and their prevalence are relatively poorly known. The aim of the present work is to gather existing data on the occurrence and species diversity of parasitic protozoa of the genus *Eimeria*, *Babesia*, *Theileria*, *Giardia*, *Cryptosporidium* and family Sarcocystidae in red deer and roe deer in Poland and compare the findings with those from other European countries.

Keywords: *Capreolus capreolus*, roe deer, *Cervus elaphus*, red deer, parasitic protozoa, Poland

Introduction

Both red deer and roe deer are wild ruminants belonging to the family Cervidae. Both species are widespread throughout Poland, with estimated populations of 276,000 red deer and 885,000 roe deer in the country [1]. An important factor affecting the abundance and condition of the wild ruminants populations is the presence of parasites and their associated diseases. Of the parasites found in cervids, an particularly important group are the Protozoa; however, despite their significance, the species composition of these parasites and their prevalence are relatively poorly known in Poland.

The aim of the present work, therefore, is to collect existing data on the occurrence of Protozoa in red deer and roe deer in Poland and to compare the findings with those from other European countries.

Eimeria spp.

The coccidia of the genus *Eimeria* are frequently found in cervids. These pathogenic protozoa exist in the cells of the gastrointestinal epithelium, causing an inflammation of the intestines called coccidiosis, which is most often seen in young animals. These parasites are characterized by high host species specificity [2].

In Poland, *Eimeria* oocysts were firstly recorded in cervids in the Borecka Forest in the 1990s, where coccidia were detected in 33.3% of roe deer faecal samples: the species composition was *E. capreoli* (prevalence 17.5%), *E. panda* (7%), *E. rotunda* (7%) and *E. ponderosa* (1.7%). They were also found in 30% of red deer faecal samples: *E. sordida* (20%) and *E. elaphi* (10%) [3]. Later, in the year 2000, in Western Pomerania, oocysts of the genus *Eimeria* were recorded in faecal samples of 47% of roe deer and in 39.8% of red deer. In the roe deer, *E. capreoli* also predominated (38.9%), with *E. panda* (12.5%), *E. rotunda* (11.2%) and *E. ponderosa* (5.1%) also being found at lower levels. The most common coccidian species in the red deer was *E. sordida* (36.3% of samples), while *E. elaphi* was presented in 13.1% and *E. cervi* in 7.7%. Co-infections were found in 11% of samples, mainly between *E. sordida* and *E. elaphi* [4]. In a study conducted five years later in the same area by the same authors, oocysts of the genus *Eimeria* were recorded in faecal samples of red deer (74.6%) and

roe deer (52.1%) samples. The observed species composition was similar to that observed previously [5]. Research on the occurrence of oocysts of the genus *Eimeria* was also conducted in the area of Kampinoski National Park, however the individual species of *Eimeria* was not recorded. The prevalence of oocysts in the faeces of the examined roe deer ranged from 20% to 70% depending on the month. The mean number of oocysts in the samples ranged from 25 to 160 oocysts per gram (OPG) of faeces in McMaster method [6]. In another study conducted in the same area, *Eimeria* infection was reported in 10.9% of roe deer and 6.8% of red deer [7]. As a result of comprehensive research on red deer and roe deer from the Białowieża Primeval Forest, the Kampinos Forest, the Piska Primeval Forest, the Lower Silesian Forests and the Biebrza Marshes was conducted by the Institute of Parasitology of the Polish Academy of Sciences in 2009–2012, the species composition of the *Eimeria* was established. The roe deer were found to host eight species (*E. capreoli*, *E. catubrina*, *E. panda*, *E. patavina*, *E. ponderosa*, *E. rotunda*, *E. superba*, *E.* sp. (Boch and Lucke, 1961), with a total prevalence of *Eimeria* in 30.3% and the number of OPG of faeces ranging from 50 to 9,000 in McMaster method . The red deer were found to host five species of coccidia, for which they are specific hosts (*E. austriaca*, *E. elaphi*, *E. robusta*, *E. sordida*, *E. asymmetrica*), and one species (*E. virginianus*) typical of white-tailed deer, with a total prevalence of 22.6% (*Eimeria* spp.) and the number of OPG of faeces ranging from 50 to 7,950 in McMaster method. The morphology and differential diagnoses of the different species have also been elaborated [8,9]. In a post-mortem study conducted in the Malopolska region near Krakow, coccidia of the genus *Eimeria* were found in two of six red deer. The number of OPG of faeces was 20 in modified McMaster method, and oocysts of two species (*E. austriaca* and *E. elaphi*) were identified [10]. Elsewhere, in a study in the Warmia and Mazury Province in the Strzałowo Forest District, the prevalence of *Eimeria* spp. was found to range from 10.8% to 30.8% in roe deer and from 18% to 30.4% in red deer. However, individual species of coccidia were not identified [11]. In a subsequent study conducted in the Lubartów Forest District, oocysts of the genus *Eimeria* were found in 45.8% of the studied roe deer, with three species identified: *E. capreoli*, *E. panda* and *E. rotunda.* The number of OPG of faeces ranged from 350 to 700 in individual samples in McMaster method. Only three samples showed a high OPGs at a level of 2,800–4,500 oocysts. In these cases, inflammation of the small intestinal mucosa was also observed [12].

In other European countries, studies on wild ruminants revealed a similar species composition of coccidia as in Poland. In the former Czechoslovakia, five species of *Eimeria* were found in roe deer: *E. capreoli* in 27% of the examined animals, *E. ponderosa* in 14.9%, *E. superba* in 6.7%, *E. panda* in 8.6% and *E. rotunda* in 0.9% [13]; a few years later, this group was found to also include *E. asymetrica*, *E. austriaca*, *E. cervi*, *E. elaphi*, *E. robusta*, *E. schoenbuchi* and *E. sordida* [14]. Of these, *E. cervi* and *E. schoenbuchi* were not found in studies of red deer coccidia in Poland. In contrast, in Spain, seven species have been identified: *E. patavina* in 37% of red deer, *E. capreoli* in 30%, *E. catubrina* in 21%, *E. superba* in 9*%, E. panda* in 9%, *E. rotunda* in 5%, and *E. ponderosa* in 3% [15]. In Italy, only four species of coccidia were found: *E. capreoli*, *E. panda*, *E. ponderosa* and *E. rotunda* [16]. Finally, five species of coccidia were found in deer in Austria [17].

***Sarcocystis* spp.**

Protozoa belonging to the genus *Sarcocystis* cause sarcocystosis in domestic and wild animals, with the definitive hosts being carnivores and the intermediate hosts herbivores. Of these, sarcocystosis is more pathogenic for the intermediate hosts, in which the Protozoa become localized in the muscle tissue. As herbivores, the Cervidae become infected with oocysts by eating vegetation contaminated with faeces from infected carnivores. Symptoms may include anaemia, abortion or weight loss, and sometimes falls. Sarcocystosis is also of great public health importance in red deer due to the increased commercial interest in venison [2]. However, the species composition of *Sarcocystis* spp. in Poland in cervids remains poorly understood, and no studies have examined the influence of infection on the quality of the meat or the general condition of the animals.

The first examination of Cervidae for *Sarcocystis* spp. in Poland was conducted in 1997–1999 among 53 roe deer and 53 red deer by trichinoscopic compressor in the Warmińsko-Mazurskie and Podkarpackie voivodeships; *Sarcocystis* cysts were found in the diaphragm muscles in 88.7%, and 94.3% of these animals. Histopathological examination found a higher

intensity of infection in roe deer than in red deer. In addition, the roe deer demonstrated an inflammatory infiltrate with numerous mast cells; while similar changes were noted in red deer, the inflammation was diffuse in three of them. Lymphocytic myositis with eosinophils contributes to the degeneration of the diaphragm and has a significant effect on respiratory capacity in red deer [18]. In a later study, molecular tests of four roe deer in the Wielkopolskie voivodeship showed the presence of three species: *S. gracilis*, *S. oviformis* and *S. silva* [19]

In other European countries, the prevalence and species composition of *Sarcocystis* spp. in cervids have been better studied. In Spain, the prevalence of *Sarcocysti*s spp. in roe deer was 85.6% with an intensity of infection ranging from 15 to 16.8 cysts per gram of muscle tissue [20]. In contrast, in Germany, *Sarcocystis* spp. was found in 87.3% of 102 tested roe deer and in 86% of 100 red deer tested. In roe deer, the mean intensity of infection per gram of muscle tissue was 219,000 cysts in the diaphragm and 153,000 in the stomach muscles while in red deer, the respective intensities were 298,000 and 237,000 cysts [21]. Morphology and small subunit (18S) ribosomal RNA (18S rRNA) sequence studies in western Norway identified the presence of five species: *S. hjorti*, *S. hardangeri*, *S. ovalis*, *S. elongata* and *S. truncata*. Of these *S. elongata* and *S. truncata* were not recorded in other intermediate hosts besides red deer [22]. In Switzerland, eosinophilic myositis and fasciitis were diagnosed in 69% of tested red deer and the presence of sarcocysts in 89%. The species *S. pilosa*, *S. linearis* and *S. taeniata* were accompanied by eosinophilic myositis and fasciitis, and gray-greenish tissue colour changes. Other red deer without tissue discoloration were infected with *S. hjorti*, *S.venatoria*, *S. iberica* and *S. silva* [23]. In addition, two species have been found in roe deer in Norway: *S. capreolicanis* and *S. silva* [24].

Neospora caninum, Toxoplasma gondii

The species *Toxoplasma gondii* and *Neospora caninum* are similar intracellular parasitic protozoa with cosmopolitan distributions. The two species have similar morphologies and pathogenesis mechanisms, and both demonstrate life cycles that include carnivores as definitive hosts and domestic and wild animals as intermediate hosts; herbivores become infected by ingesting water or food contaminated with oocysts, and carnivores by ingesting tissue infected with tachyzoites or tissue cysts. However, while *T. gondii* causes zoonosis, *N. caninum* is not pathogenic to humans. In addition, *T. gondii* is an important factor of abortion in goats and sheep, while *N. caninum* causes abortion in cattle [2].

The first report of the presence of *N. caninum* in red deer in Poland was noted in a study of 47 free-living red deer and 106 farmed deer from the Research Station of the Institute of Parasitology PAS in Kosewo. Testing based on a modified immunostimulating complex (iscom) enzyme-linked immunoassay (ELISA) technique found 12 samples (11.3%) from farmed deer and six (12.7%) from hunted animals to be positive [25]. In other countries, antibodies against *N. caninum* were found in the serum of one out of 199 tested roe deer in Sweden [26], two of 73 tested roe deer sera (2.7%) in Belgium [27], and 11 out of 79 tested roe deer (14%) and 24 out of 377 tested red deer (6%) in the Czech Republic [28]. In addition, antibodies against *N. caninum* were found in 11.8% of 237 red deer and in 6.1% of 33 roe deer in Spain [29]. An interesting case was the detection of *N. caninum* in the brain of a dead newborn red deer in a zoo in France [30]. In central Italy, anti-*N. caninum* antibodies were detected in sera from 17 of 60 red deer (28%) and anti-*T. gondii* in 13 (22%). Co-infections of both species were noted in five cases (8%) [31].

The first identification of antibodies against *T. gondii* in Poland was recorded in a study of meat juice samples; in this case, 30.4% of 92 examined roe deer and in 24.1% of 552 red deer were found to be positive by ELISA test [32]. This prevalence in roe deer is substantially higher than in Italy (2.4%) [33] or Spain (14%) [34]; however, a higher prevalence was reported in France (46%) [35]. A similar prevalence of *T. gondii* as in Poland has been shown in Sweden (34%), where it was also found that adults are more likely to give a positive result than young animals, and that more roe deer become infected in the forest than in the womb [26]. *Toxoplasma gondii* was also detected in red deer in Norway, where the prevalence was 7.7% [36], and in Spain, with a prevalence of 15.6% [37]. In red deer, considerably higher serological prevalence of *T. gondii* was observed in the Czech Republic (45%); these values were approximately twice as high as those recorded in Poland [28]. In the Italian Alps, the prevalence was 52.4% at the age of one year and 51.3% in adults red deer. Interestingly, calves were not infected, which supports the theory

that horizontal transmission is the main route of infection [37]. However, in Spain, *T. gondii* was detected in 15.6% of red deer and in 21.8% of roe deer sera, and no differences in prevalence were observed between red deer, calves and older individuals [38].

***Babesia* spp., *Theileria* spp.**

Babesia spp. and *Theileria* spp. are protozoa that parasitize blood cells, and are transmitted by ticks. The main difference between *Theileria* spp. and *Babesia* spp. it consists in the type of cells in which they parasitize: *Theileria* spp. infect leukocytes and erythrocytes of intermediate hosts while *Babesia* spp. parasitize only the erythrocytes. Both species of protozoa damage blood cells, leading to anaemia [2].

The first detection in Poland was recorded in the north west of the country, where blood from 67 roe deer and 15 red deer were tested by Polymerase Chain Reaction (PCR). The prevalence of *B. divergens* was found to be 24.4% (20/82), with infection of roe deer reaching 75% (15/20) and red deer 25% (5/20). In addition, DNA from *Theileria* spp. was detected in 11% (9/82) of all animals, of which roe deer accounted for 77% (7/9) and red deer 23% (2/9). Of the animals shot during the spring period, 24.6% of roe deer were infected with *B. divergens* and 10.5% with *Theileria* spp. No information is available regarding red deer in spring. In the autumn period, the prevalence of *B. divergens* increased to 83% in roe deer and to 17% in red deer, while *Theileria* spp. was detected in one red deer and two roe deer. These findings indicate that one species of the genus *Babesia*, *B. divergens*, is present in the Cervidae in Poland [39]. The presence of *B. divergens* in red deer and roe deer was confirmed by analysis of the 18S rRNA gene sequence, which was found to share 100% similarity with another sequence previously obtained from *Ixodes ricinus* isolates from northwestern Poland [40]. In subsequent studies, significant differences were observed in the infection rates of roe deer and red deer in western Poland: *Theileria* spp. DNA was found in 24.6% of roe deer and 84% of red deer, while *Babesia* spp. DNA was detected in 30.4% of roe deer and 2% of red deer [41]. Finally, *B. venatorum*, *B. divergens*, *B. capreoli* and *B. odocoilei* were found in blood samples from 67 roe deer originating from several regions of Poland. Of these, *B. venatorum* was found in 16.6% of roe deer, and this is the first report of this species in Poland; in addition, 16.6% of roe deer were infected with *B. divergens* and 66.7% with *B. capreoli* [42].

In other European countries, protozoa of the genus *Babesia* have been found repeatedly in roe deer and red deer. *Babesia divergens* species have been detected in roe deer in Slovenia [43] and Italy [57,58]. In Spain, a study conducted on 174 roe deer identified five species parasitizing blood cells: *B. capreoli*, *B. venatorum*, *B. bigemina*, *Theileria* sp. OT3 and *Theileria* sp. 3185/02. The highest prevalence (60.3%) was shown by *Theileria* sp. OT3 [45]. In another study in Spain, *Theileria* sp. OT3 was found in 53% of examined red deer and *Theileria* sp. 3185/02 in 85% [46]. However, 100% prevalence of *Theileria* spp. was observed among in red deer kept in an enclosure in eastern Austria [47]

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***Giardia* spp., *Cryptosporidium* spp.**

Giardia spp. and *Cryptosporidium* spp. are widespread gastrointestinal parasitic protozoa found in animals and humans. The protozoa reduce the brushstroke epithelial surface, shorten the microvilli and induce enterocyte apoptosis, and infection with these parasites contributes to impaired intestinal absorption and hypersecretion, and eventually diarrhoea [2].

Studies on faecal samples from 52 wild red deer and 22 roe deer at the Polish Academy of Sciences Research Station in Popielno using the indirect fluorescent antibody (IFA) test, found the prevalence of *Giardia* spp. to be 4.5% in roe deer and 1.7% in red deer. In addition, the prevalence of *Cryptosporidum* spp. reached 9% in roe deer and 14% in red deer. Co-infection with *Cryptosporidium* spp. and *Giardia* spp. was found in less than 1% of red deer samples. It was also confirmed that wild red deer had a higher prevalence than farmed deer. The results indicate that the studied the Cervidae species play an important role in maintaining the natural reservoirs of *Cryptosporidium* spp. and *Giardia* spp. in the environment [48]. Elsewhere, *G. duodenalis* cysts were detected in one red deer faecal sample from a group of 61 red deer and two faecal samples from a group of 50 roe deer in west-central and north-eastern Poland [49]. Genotyping and phylogenetic analysis found that the *G. duodenalis* isolate from red deer belonged to genotype AIII, which has never been identified in humans, while the isolates from roe deer belonged to zoonotic genotype AI [50]. An extensive study of 162 faecal samples from wild animals was carried out in the

Łęczyńsko-Włodawskie Lake District in eastern Poland. The presence of *G. duodenalis* was assessed by direct fluorescence assay (DFA) and PCR and sequencing of the beta-giardin gene fragment. DFA revealed the presence of *G. duodenalis* cysts in 12 of 162 faecal samples (7%), including two from roe deer (4%). PCR identified 34 of 162 (21%) samples as positive, including five red deer (18%) and 11 roe deer (23%). Sequence analysis revealed the presence of genotype B in red deer and roe deer. This is the first detection of genotype B in red deer in Poland. Genotype B has zoonotic potential, therefore wild animals from eastern Poland may constitute a reservoir of *G. duodenalis* cysts infectious to humans [51].

In Norway, the prevalence of *Cryptosporidium* spp. was found to reach 20% in red deer [52] and 6.2% in roe deer [53], which is similar to the prevalence observed in Poland. In Italy, the zoonotic species *C. parvum* is frequently found in cervids [54]. The prevalence of *Giardia* spp. in roe deer varies considerably among European countries, from 5.3% in Spain [55], through 15.5% in Norway [56] to 24% in Croatia [57].

In conclusion, this brief review summarises the most important parasitic protozoa occurring in red deer and roe deer in Poland and their prevalence. These parasites, localized in various tissues and organs of their hosts, cause pathological changes threatening the health of animals and affecting their condition. Some parasitoses of cervids caused by protozoa are also zoonoses. As there is also a strong possibility that they may be transmitted from wild to domestic animals, it is necessary to further research and monitor the state of infection of cervids with parasitic protozoa. Knowledge of the status of parasitic protozoan infection in red deer and roe deer plays an important part in developing prophylactic measures to prevent the occurrence of parasitic diseases in the natural environment, as well as on the reserves and farms where these animals are kept.

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