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Gut parasites of alpacas (*Vicugna pacos*) raised in Poland

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Summary

This study aimed to determine the prevalence of gastrointestinal parasites in alpacas on selected farms in Poland. In July and August 2019 and August 2021, 223 samples from six commercial farms were examined using coproscopic techniques. The total percentage of alpacas infected with intestinal parasites was 57.7%. Eggs of *Nematodirus* sp. were found in 28.9%, *Trichostrongylus* sp. in 15.5%, *Strongyloides* sp. in 13.4%, *Camelostrongylus* sp. in 11.3%, other strongyle-type in 12.4%, *Trichuris* sp. in 3.1%, *Capillaria* spp. in 2.1%, *Oesophagostomum* sp. in 1.0% and eggs of *Moniezia* sp. in 1.0% of individuals. Oocysts of *Eimeria macusaniensis* were found in 8.2%, *Eimeria* sp. in 4.1%, and *Cryptosporidium* sp. in 3.1% of animals. Redundancy analysis showed that parasites and their number in faeces were related to the individual's country of origin, sex and age. Females had significantly more eggs of parasites than males. More significant parasite infection was recorded in younger individuals. Moreover, the most infected were individuals from Germany. Some of the described parasites in tested alpacas have zoonotic potential. Due to the possibility of introducing parasites native to alpacas and acquiring species parasitising wild and farmed animals in Europe, permanent veterinary monitoring of animals imported from other regions is necessary.

Introduction

Importing exotic animal species to Europe for breeding purposes has a long tradition. In recent decades, more attention has been paid to possible threats related to "imported" parasites. This new approach results from two points of view: (1) the threat to the economic result of breeding and (2) possible epidemic or zoonotic problems. In Europe, including Poland, the importance of domesticated South American camelids has increased in recent decades because of their high-quality fibre, meat and hides. For instance, alpaca meat is becoming popular worldwide due to its lower cholesterol than red meat (Saeed *et al.*, 2018).

The growing popularity of these animals resulting from using their products and their role in alpacotherapy changed people's attitudes toward diagnosing alpaca parasites. Ectoparasitic invasions were mainly described in the context of their influence on fibre quality (Bornstein, 2010). In turn, intestinal parasites play an important role in veterinary medicine and as the etiological agents of zoonoses (Fugassa & Cafrune, 2023). It should be emphasized that alpaca-related exotic parasites imported to new areas with their hosts result in new transfers that could threaten native fauna (Carmichael, 2014). On the other hand, introducing alpacas to Europe and exposing these animals to native parasitic fauna may result in new host-parasite associations (Love, 2017). This issue is critical due to the uncontrolled movement of animals within the EU.

The first alpacas were brought to Poland at the beginning of the 21st century, and the first commercial farms were established in 2004 (Krajewska-Wędzina *et al.*, 2020). It was estimated that there were 50 herds of alpacas in Poland, with nearly 2000 individuals (Markowska-Daniel *et al.*, 2018). Due to the commercial importance of alpacas and the close contact of animals with breeding keepers and people (especially using alpacotherapy), there was an urgent need to diagnose gastrointestinal parasites in these animals. Many years ago, Leguía (1991) drew attention to the negative impact of intestinal parasites on the quality of fur and other products used. In turn, Windsor et al. (1992) pointed to the positive effect of deworming on the quality of fur of farmed animals. Unfortunately, only a few studies on diagnosing ectoparasites and potential health problems due to procaryotic pathogens in alpacas in Poland have been published (Markowska-Daniel *et al.*, 2018). On the other hand, the complete lack of data on the spread of gastrointestinal parasites in farms in Poland should be remedied as soon as possible, given the health of herds and people in contact with them.

This study aimed to estimate the prevalence of gastrointestinal parasites in alpacas from selected commercial farms in Poland and assess the health risks to farm animals and the zoonotic potential.

Material and methods

Sample collection

The diagnostic material comprised 223 stool samples from 97 individual animals from six breeding herds in Poland. The distance between the herd locations was from 100 to 500 km. The samples were tested fresh in summer 2019 (97 in July and 96 in August) and 2021 (30 in August). Among the animals tested in the summer of 2019 were individuals from Germany, Spain, Chile, and the United States, as well as animals born in Poland. The average age of individuals imported from abroad was five years ± 3 months, and they average stay in Poland at the time of sampling had been 0.5 year ± 1 month. Additionally, in the summer of 2021, we tested 30 animals born in Poland. Among the individuals tested, 95 were clinically healthy, while two were diagnosed with diarrhoea, and one died shortly after the test. The tested animals were 58 males and 39 females (Table 1). Some females were inseminated at the time of sample collection, but pregnancies were still not confirmed, so their status was not recorded. Faecal samples were collected from the animals immediately after defecation.

Sample examination

All samples were examined using the modified method of McMaster (Raynaud *et al.*, 1970; Sweeny *et al.*, 2011). One gram of faecal sample was used for each test. The intensity of infection was calculated as the number of eggs per 1 gram of faeces. The morphological diagnosis of parasite eggs was performed based on Zajac & Conboy (2012).

Statistical analysis

The exploratory data analysis used the CANOCO for Windows 4.5 software (Ter Braak & Šmilauer, 2002). The preliminary analysis results (Detrended Correspondence Analysis) showed that the variability of biological data was best described by a linear model (gradient length was 2.260). Therefore, redundancy analysis (RDA) was used for further analysis. A forward selection procedure was used during the RDA analysis to assess the relationships between explanatory and biological variables. Their statistical significance and the significance of the canonical axes were also evaluated using the Monte Carlo permutation test for 499 repetitions. For analyses, the data were logarithmically transformed [ln(x+1)] and centred, and the results were presented on an ordination diagram.

Table 1. Alpacas examined during study

Country of origin	Number of males		Number of females	
	Infected	Non-infected	Infected	Non-infected
Poland	17	22	12	9
Germany	6	1	3	0
Spain	0	1	0	0
Chile	5	5	12	3
USA	1	0	0	0

Results

In five of six herds studied, alpacas had gastrointestinal parasites. Of the 97 animals examined, 56 (57.7%) had at least one parasite species. Among these positive cases, 28 were co-infected with two or more parasite species. The presence of at least 11 species of parasites has been found. The prevalence of each gastrointestinal parasite is summarized in Table 2. The typical appearance of the egg or oocyst of the parasites is shown in Figure 1.

Nematodirus sp. was the most prevalent of the helminths, followed by *Trichostrongylus* sp., *Strongyloides* sp., and *Camelostrongylus* sp. We also detected two types of *Capillaria* eggs that differed in size and eggs of tapeworm *Moniezia* sp. (Table 3).

Of the protozoan parasites, oocysts of two *Eimeria* species (*E. macusaniensis* and smaller *Eimeria* sp.) and *Cryptosporidium* sp. were found (Table 3).

In the RDA analysis, which included only the significant explanatory variables, the first two axes explained 22.9% of the total variability. The relationship between biological data and explanatory variables accounted for 89.8% of the variation. Monte Carlo permutation tests showed that the RDA ordination axes were significant (axis 1: $F = 38.820$, $p = 0.0020$; all axis: $F = 6.175$, $p = 0.0020$).

RDA analysis showed that the presence of parasites and their number in faeces was related to, among others, the individual's country of origin, sex and age (Fig. 2). Parasite species located in the lower right part of the ordination diagram (e.g., *Camelostrongylus, Eimeria, Capillaria*) more often infected female alpacas. On the other hand, the parasites located at the upper part of the ordination space were noted inside young hosts. They were also more common and numerous in the German alpaca population.

Discussion

The results of this study revealed for the first time that alpacas raised in Poland have gastrointestinal parasites similar to those described in other countries outside of South America (Franz *et al.*, 2015; Hyuga & Matsumoto, 2016).

Nematodirus sp. eggs were the most common in the alpacas studied. *Nematodirus* spp. are parasites of the small intestine of ruminants and have a direct life cycle. Their larvae within ova are highly resistant to low temperatures, so their effective transmission depends on overwintering (Van Dijk & Morgan, 2008). In Europe, *Nematodirus lamae* was reported from the United Kingdom as a very probable cause of the sudden death of alpacas (Mitchell & Hopkins, 2016). Other species of this genus in ruminants, like *N. battus*, were reported from elsewhere in Europe, while *N. helvetianus, N. filicollis* and *N. spathiger* have been noted in mixed infections in this area (Lindqvist *et al.*, 2001); however, they are considered more common across Australasia (McMahon *et al.*, 2017). Our study revealed the presence of *Nematodirus* sp. eggs similar in shape and size to *N. abnormalis*, described by Onar (1975). However, this nematode species is endemic in the Mediterranean climatic zone (Louw, 1989), and its presence in alpacas in Poland—an area with a temperate climate—can result from the transfer from warmer regions. The fact that they are present in animals in five different herds indicates the success of this transfer.

The size of *Trichostrongylus* sp. eggs found in alpacas indicates their similarity to *T. axei.* This nematode was noted in ruminants inhabiting Zoos and wild habitats in Poland (Bartosik & Górski, 2010). According to Souza et al. (2013), *T. axei* belongs to zoonotic

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Table 2. Prevalence of parasites in tested alpacas

Parasite	Numer of infected animals					Prevalence [%]	Average No eggs*/1 g faeces ± SD
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5		
Eimeria sp.	1	2	1	0	0	4.1	7± 0
E.macusaniensis	3	0	3	0	2	8.2	25± 18
Cryptosporidium sp.	3	0	0	0	0	3.1	8± 3
Nematodirus sp.	14	1	7	0	6	28.9	23± 11
Strongyloides sp.	7	0	3	0	3	13.4	25± 13
Camelostrongylus sp.	4	3	1	0	3	11.3	34± 17
Trichostrongylus sp.	11	1	3	0	2	15.5	23± 14
Capillaria sp.	0	0	2	0	0	2.1	30± 4
Trichuris sp.	1	2	0	0	0	3.1	26± 16
Oesophagostomum sp.	0	1	0	0	0	1.0	7± 0
Moniezia sp.	1	0	0	0	0	1.0	7± 0
Undiagnosed	10	2	2	0	2	12.4	–

*Or oocysts of Protista.

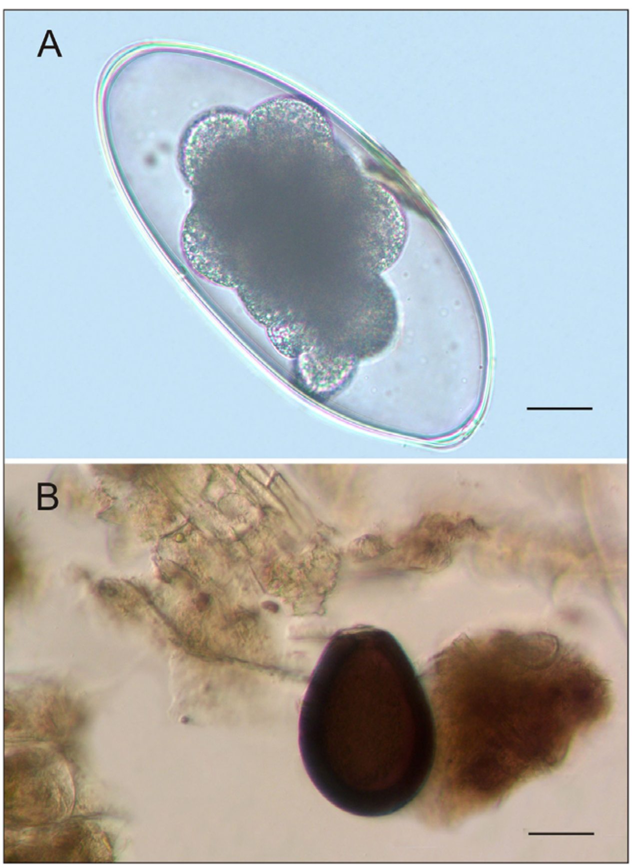

Figure 1. Detected forms of parasites: (A) egg of *Nematodirus* sp. (barr = 30 µm), (B) oocyst of *Eimeria macusaniensis* (barr = 30 µm).

Table 3. Size of protist oocysts and nematode eggs

Oocysts of Protista		
Parasite species	Length range [µm]	Width range [µm]
Eimeria macusaniensis	80.0–85.0	53.0–61.7
Eimeria sp.	23.6–29.1	13.7–22.0
Eggs of Nematoda		
Parasite species	Length range [µm]	Width range [µm]
Nematodirus sp.	200.0–215.0	89.0–111.0
Capillaria sp. (large)	77.7–79.1	45.3–46.6
Capillaria sp. (small)	35.9–37.8	23.1–24.0
Camelostrongylus sp.	74.0–99.0	44.0–46.6
Trichostrongylus sp.	78.0–94.0	44.0–49.4
Strongyloides sp.	55.3–56.0	38.4–39.0
Trichuris sp.	46.8–51.1	25.9–27.2
Moniezia sp.	48.4.–55.1	58.1–60.2

nematodes most frequently acquired through contact with herbivorous animals.

Eggs of *Camelostrongylus* sp. were noted in alpacas from four of six studied herds. The first finding of this parasite in alpaca was described in the United Kingdom (Welchman *et al.*, 2008). The active trade in alpacas in Europe probably contributed to this nematode's spread. Some *Camelostrongylus* species were described in Europe only in captive exotic animals (Ortiz *et al.*, 2006).

In four of the studied farms, we noted eggs of *Strongyloides* sp. In Poland, only *S. papillosus* was noted in ruminants. The parthenogenetic females were described as pathogenic for lambs (Romaniuk *et al*,. 1995). However, the size of eggs in tested alpacas was longer than described by other authors (Peter *et al.*, 2015). We can only suspect that the eggs found belong to this species.

Animals in two tested herds were infected with *Trichuris* sp. These parasites are highly prevalent in the world but rarely cause clinical signs. Species infecting ruminants in Poland include *T. discolor*, *T. skrjabini* and *T. globulosa* (Patyk, 1956; Karbowiak

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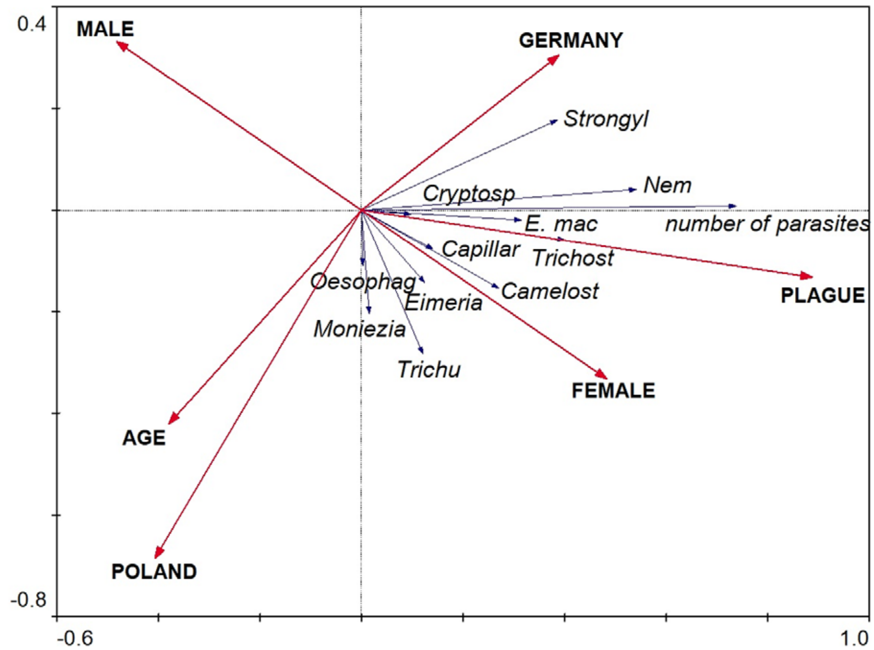

Figure 2. Redundancy analysis (RDA) ordination diagram showing the results of the study on parasite infection in alpaca *Vicugna pacos.* Parasites: Strongyl = *Strongyloides* sp., Nem = *Nematodirus* sp., Cryptosp = *Cryptosporidium* sp., E. mac = *Eimeria macusaniensis*, Capillar = *Capillaria* sp., Trichost = *Trichostrongulus* sp., Camelost = *Camelostrongylus* sp., Eimeria = *Eimeria* sp., Oesophag = *Oesophagus* sp., Moniezia = *Moniezia* sp., Trichu = *Trichuis* sp. PLAGUE = presence of parasites.

et al., 2014). Additionally, Dróżdż (1966) described *T. bovis* in wild Cervidae. In South American Camelidae, *T. tenuis* was noted (Cafrune *et al.*, 1999); however, this parasite was not described in animals outside their natural geographic range.

Only two animals from one herd were infected with *Capillaria* sp. In the faeces of alpacas, we found characteristic barrel-shaped eggs similar to those presented by Lambacher *et al.* (2016). However, Capillaria species infecting birds or carnivores in Poland were generally noted (Tomczuk *et al.*, 2017), and Demiaszkiewicz et al. (2016) found eggs of *C. bovis* in red deer. Given the direct life cycle of this nematode, transmission could have occurred while grazing alpacas in the wild.

Only in one specimen were eggs of tapeworm *Moniezia* sp. found. They were pyramidal in shape, and their dimensions were similar to those of *Moniezia expansa* (Verocai *et al.*, 2020). This representative of Cestodes has a worldwide distribution (Zhang *et al.*, 2010). It has been reported in alpacas in Peru (Ortiz, 2013) and European ruminants, including Poland (Piekarska *et al.*, 2012).

The representatives of two genera of protists were observed in alpacas tested: *Eimeria* and *Cryptosporidium*. *Eimeria macusaniensis* belongs to common alpaca parasites in South America (Cafrune *et al.*, 2009) and probably was introduced to Japan (Hyuga & Matsumoto, 2016). Our report of its occurrence in Poland is the first described case, and considering the high specificity of the parasite to the host, it means that this coccidium was introduced with the imported Camelidae. The smaller oocysts of *Eimeria* sp. were similar to *Eimeria lamae* described by Gomez-Puerta et al. (2021) in *Vicuna pacos* from the Peruvian Andes. However, without molecular data, this suggestion cannot be confirmed. In Poland, similar cysts were observed in rabbits (Sadzikowski *et al.*, 2008) and goats (Mickiewicz *et al.*, 2017). However, their proposed identity is contradicted by the host specificity of species in the genus *Eimeria*. *Cryptosporidium* genus is a species-rich protist group that can only be identified based on molecular diagnostics. Representatives of this genus are common animal parasites and zoonotic agents (e.g., *Cryptosporidium parvum*). This species was diagnosed in *Vicugna pacos* by Zhang et al. (2020). In this context, it is necessary to control the presence of oocysts in animals, especially in alpacotherapy, to minimise the risk of transmitting infection from alpacas to humans.

The presence of parasitic protists, tapeworm, and nematodes in alpacas bred in European countries fully justifies the necessary monitoring of infections in these animals. As our results and the research of various authors indicate, the introduction of alpacas resulted in both the transmission of parasites from domestic native ruminants to newcomers and the import of South American parasites to new areas (Dubey, 2018). The lack of reports from many countries of the Old World concerning alpaca parasites may result from the lack of appropriate regulations and the limited knowledge about the etiological factors of these animals (Neubert *et al.*, 2021). As Rickard (1994) indicated, camelids respond differently to certain parasites than cattle or sheep. Depending on the parasitic burden, infections in alpacas can be subclinical, mild, or lead to death if untreated (Fowler, 2010). Clinical signs due to endoparasites are usually unspecific. Poor growth, anorexia and anaemia can occur. Diarrhoea is more often observed in younger animals, but in many cases, it is absent at the early stages of infection. The malabsorption of minerals and micronutrients and loss of proteins can result from damage to the intestinal mucosa, leading to reduced growth and performance (Franz *et al.*, 2015).

The essential aspect of diagnosing alpaca parasites in commercial herds is the zoonotic nature of such species as *Trichostrongylus axei* or *Cryptosporidium parvum.* Particular attention should be paid to the possibility of transmission of alpaca parasites with a holoxenic lifecycle to children in petting zoos or during alpacotherapy (Halsby *et al.*, 2017). According to Walker (2018), a faecal examination in alpaca herds should be done on each animal

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before any anthelmintic is administered, which is impractical, especially in large herds. The author suggests that, as a minimum, 10% of the animals or at least 10 animals, should be tested two to three times a year.

Our results suggest mandatory regular parasitological testing in commercial alpaca herds, particularly those used in alpacotherapy.

Statistical analysis showed that parasites and their number in faeces were related to the individual's country of origin, sex and age. Additionally, the largest herd had the most significant count of parasitic taxons, which greater infection possibilities between animals and lower success rates of deworming strategies can cause. We cannot exclude existing parasites showing anthelmintic resistance in a much larger herd. Large herds should be dewormed more regularly and often, which can lead to the rise of populations partially resistant to anthelmintic drugs (Hodgkinson *et al.*, 2019). Moreover, individuals of various origins in the largest herd could have influenced the composition of the parasitic fauna. The number of eggs (or oocysts) was higher in female than male alpacas. A host's immunity can significantly impact egg production due to internal parasites (Rosenberg *et al.*, 2013). The results of a more significant count of eggs between sexes but not in the parasite taxon can show that the immunity of the females was weaker because of the possible pregnancy (Pazos *et al.*, 2012). Younger animals were also characterized by greater intensity of infection, which may be the result of lower immunocompetence of the defence system of these animals compared to older animals (Colditz *et al.*, 1996). What is surprising is the strong parasitism of individuals imported from Germany. Unfortunately, import documents only indicate the country directly transported to Poland. It is possible that these individuals were brought to Europe from other countries. When importing exotic animal species to Europe, parasite diagnostics should be carried out carefully. The present research is the first attempt in Poland to assess the infestation of alpaca herds with intestinal parasites. The relatively large number of parasite taxa found (at least 11) is surprising. Already, preliminary studies indicate an association between the infection and the sex of the hosts, possibly due to the reduced immunity of pregnant females. Due to the diversity of alpacas' parasitic fauna and potentially zoonotic parasites, we postulate that the veterinary care of herds should be increased. Even in the case of asymptomatic infections, there is a risk of parasite transmission between farmed alpacas, wild and farmed ruminant fauna, and even humans and alpacas.

Conclusions

Analysis of the consequences associated with alien species usually concerns organisms accidentally introduced to new areas. Meanwhile, omitting alien species of economic importance is a grave mistake, which results from the belief that alien-farmed species are kept isolated from natural ecosystems.

However, the present research results indicate that the threat associated with such introductions may have a more complex dimension beyond the newcomers' gene pool. One aspect worth emphasising is the problem of intestinal parasites, which do not necessarily affect the economic results of breeding foreign species. However, their transfer may have far-reaching effects by creating new host-parasite associations. These consequences may have both veterinary and medical dimensions.

Declarations. Conflict of interest: The author(s) declare none.

Ethical standard. The conducted research does not require the consent of the National Ethical Committee for Animal Experiments.

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