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Two centrifugal flotation techniques for counting gastrointestinal parasite eggs and oocysts in alpaca faeces

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Abstract

Introduction: The alpaca (*Vicugna pacos*) is a camelid native to South America, but the species has wide distribution outside its natural habitat and is found in various countries on other continents, Poland being one in Europe. Parasitic infections affect the productivity and health of alpacas. The aim of the study was to estimate the parasite loads in alpacas kept in Poland with the use of two direct centrifugal flotation methods. **Material and Methods:** A total of 248 faecal samples from alpacas from 12 provinces in Poland were examined for parasite eggs and oocysts with a modified Willis method (WM), and 59 samples were examined simultaneously with WM and a modified Stoll method (SM). **Results:** The WM detected eggs of Trichostrongylidae, and the SM oocysts of *Eimeria* spp. as the respective most prevalent parasite material. The eggs of *Nematodirus* sp., *Nematodirus battus*, *Aonchotheca* sp., and *Trichuris* sp. were detected in lower numbers in both methods. Oocysts of *E. macusaniensis* and eggs of *Moniezia* sp. were the rarest in WM, the former also being so in SM; eggs of *Moniezia* were absent in SM. The prevalence of *Eimeria* spp. was significantly higher as detected by SM than as detected by WM; however, the prevalence of eggs of *Nematodirus* spp. and trichostrongyles was significantly higher as detected by WM than as detected by SM. **Conclusion:** WM is more accurate at detecting heavy gastro-intestinal nematode eggs, including those of trichostrongyles and *Nematodirus*, whereas SM is more accurate at detecting smaller coccidia from the genus *Eimeria*.

Keywords: faecal examination, flotation techniques, parasites, alpaca, monitoring.

Introduction

Alpacas are South American camelids, together with llamas, guanacos and vicuñas. Despite their stomachs consisting of only three compartments (with the abomasum as the third one), they are treated as ruminants (11). This predisposes alpacas to sharing a wide variety of parasites and infectious agents with copastured livestock ruminants (14). Traditionally, alpacas are kept in an extensive grazing system on pastures on the slopes of the Andes, with Peru being the major alpaca-producing country in the world. Typical uses for alpacas there are for meat, hides, fibre, and transport, and their droppings are used as fuel and fertiliser (4). The species has become widely distributed outside its natural habitat and is now reared in various countries including Australia (14), Japan (10), the USA (16), England and Wales (9), Sweden (2) and Poland (11). Alpacas are kept there under intensive to semi-intensive conditions, very often with other domestic ruminants, and thereby are exposed to high pressures of infection with gastrointestinal nematodes (GINs) (14). In Poland, alpacas have been recognised as farm animals since 2020, and their population is estimated at approx. 5,500 individuals (11). Outside South America, alpacas are kept for breeding, fine wool production, recreation, as companion animals and as tourist attractions (2, 9). It has been proven that parasitic infections are among the factors limiting the productivity of alpacas, as they lead to reductions in the quality and quantity of meat and

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wool, lower fertility, abortion, and even the death of heavily infected individuals (4). Moreover, camelids are very susceptible to coccidiosis, particularly caused by *Eimeria macusaniensis,* which can induce symptoms of weight loss, lethargy, and diarrhoea (5). Heavy parasitisation of *E. macusaniensis* in the ileal crypts predisposes the host to infection with other dangerous enteric pathogens, including *Clostridium perfringens* which can develop toxaemia (5).

The aims of the study were to estimate the parasite loads in alpacas kept in Poland so that data were obtained to guide treatment decisions and to compare faecal egg and oocysts counts obtained with two centrifugal flotation methods.

Material and Methods

Samples collected and the area of the study. The study was conducted between July 2021 and January 2022 on 248 faecal samples from alpacas kept on 43 farms located in 12 provinces in Poland (Fig. 1). Individual faecal samples were collected directly from the recta, placed in 30 mL labelled plastic tubes and transported to the laboratory in a cooler. Samples with a weight of at least 3 g (n = 248) were investigated with the direct flotation method (a modified Willis method - WM), and samples with a weight of at least 5 g (n = 59) were analysed both by direct flotation (WM) and a quantitative flotation method (a modified Stoll method - SM). In both techniques, a sucrose flotation solution with specific gravity of 1.27 was used (13).



Fig. 1. Percentage share of alpaca farms participating in the study by Polish province. The number of farms per province in Poland is given in brackets

Modified Willis method. A modified WM (18) was performed with centrifugation as described previously (7). Three grams of faeces were passed through a sieve in 10 mL of sucrose solution, and the suspension was poured into 10 mL centrifuge tubes and centrifuged for 2 min at 2,000 rpm (19). Subsequently, more sucrose solution was added to each tube to make a bulging meniscus, on the top of which a glass coverslip was placed for 20 min. Finally, the coverslip was placed on a glass slide and the presence of parasitic oocysts and eggs was investigated under an OPTA-TECH LAB40 (Opta-Tech, Warsaw, Poland) light microscope at 100 × and 400 × magnification. Microphotographs of parasitic eggs and oocysts were taken directly from the microscope using the OPTA View-15 2019 (Opta-Tech) imaging software package.

Modified Stoll's quantitative method. The modified Stoll's test was performed following Whitehead, who recommended the technique for use in camelids (16). Two grams of faeces were carefully mixed with 38 mL of tap water in a flask, and then another 60 mL of tap water was added to the mixture and swirled to distribute the faeces evenly throughout the water in the flask. Subsequently, 10 mL of swirled uniform suspension was transferred into a 10 mL centrifuge tube and centrifuged at 1,000 rpm for 5 min. Then, the supernatant was decanted, the pellet was resuspended in 10 mL of sucrose solution, and the samples were centrifuged at 1,000 rpm for 5 min. Finally, sucrose solution was added to make a bulging meniscus which was covered with a coverslip for 10 min. After this time, the coverslip was placed on a glass slide and examined for the presence of parasitic oocysts and eggs under the OPTA-TECH LAB40 light microscope at 100 × and 400 × magnification. The sensitivity of the method was reduced to 5 per gram of faeces, which meant that the number of observed oocysts and eggs was multiplied by 5.

Determination of oocysts/eggs. Nematode eggs looking alike were identified under the microscope to the family level and recorded as being Trichostrongylidae (trichostrongyles). The eggs of *Nematodirus*, *Aonchotheca*, *Moniezia* and *Trichuris* were determined to the genus level; whereas the more characteristic eggs of *Nematodirus battus* were determined to the species level (10, 15). Among *Eimeria* spp., only *E. macusaniensis* was identified to the species level and this was possible because of the distinctive morphology of the oocysts (8). The remaining coccidia were identified only to the genus level, as Eimeriidae species identification should be based on observation of oocyst sporulation time and their measurements (6). In fact, the time limitations imposed on veterinary practitioners and the sameness of the treatment regimen regardless of the infecting species of *Eimeria* urged us not to pursue a detailed study on the morphology of oocysts.

Statistical analysis. The prevalence of particular parasitological structures established with the use of both coprological techniques (WM and SM) (n = 59) was compared with the prevalence in the McNemar test. A P value lower than 0.05 was considered significant. The oocyst and egg counts were not compared because WM is qualitative and thus not comparable to the quantitative SM.

Results

Eight classes of parasitological objects were determined with WM, and seven with SM (Table 1). Although in different orders of occurrence frequency, the same three classes of parasites were the most prevalent in the faeces of alpacas in both methods' results. *Ostertagia*-type Trichostrongylidae eggs (Fig. 2A) predominated in WM and *Eimeria* spp. oocysts (Fig. 2B) in SM (Table 1). In both methods, the next four parasitic eggs in descending order of frequency were *Nematodirus* sp. (Fig. 2C), *Nematodirus battus* (Fig. 2D), *Aonchotheca* sp. (Fig. 2E), and *Trichuris* sp. (Fig. 2F) in both methods. Oocysts of *E. macusaniensis* (Fig. 2G) and eggs of tapeworms of *Moniezia* sp. (Fig. 2H) were the rarest as detected by WM, and oocysts of *E. macusaniensis* also were when SM detected the material, whereas eggs of *Moniezia* were not detected by the latter method (Table 1).

The two highest counts of material identified per sample in WM were for *E. macusaniensis* (12,187 oocysts per 3 g of faeces), and trichostrongyles (10,532 eggs per 3 g of faeces) (Table 1). There were 205 GIN positive samples (prevalence = 82.7%) out of the 248 total samples examined with WM. The range of egg counts was 1–10,534. The general prevalence of GINs reached respectively 72.9% (43 positive samples) and 52.5% (31 positive samples) out of 59 samples examined with WM and SM where both methods tested the same sample. The ranges of egg counts were 3–593 and 5–355 by WM and SM, respectively.

Statistical analysis of the obtained data revealed that the prevalence of *Eimeria* spp. was significantly higher with the use of SM than with WM ($P < 0.004$); however, the prevalence of eggs of *Nematodirus* sp. and Trichostrongylidae was significantly higher with the use of WM than with SM ($P = 0.000$, and $P = 0.006$, respectively). The prevalence of the remaining parasitic eggs and oocysts did not differ statistically between the methods ($P > 0.05$) (Table 1).



Fig. 2. Parasitic eggs and oocysts found in faeces of alpacas with the use of flotation techniques (400 × magnification, bar = 12 µm)
2A – an egg of the Trichostrongylidae family; 2B – an oocyst of the *Eimeria* genus; 2C – an egg of the *Nematodirus* genus; 2D – an egg of *Nematodirus battus*; 2E – an egg of the *Aonchotheca* genus; 2F – an egg of the *Trichuris* genus; 2G – an oocyst of *Eimeria macusaniensis*; 2H – eggs of the *Moniezia* genus

Table 1. Coprological findings in alpacas (n = 248) after applying a modified Willis method (WM), comparison of the results obtained using the Willis and a modified Stoll methods (SM) (n = 59) and statistical comparison with the McNemar test (P)

		Aonchotheca sp.	*Eimeria* spp.	*E. macusaniensis*	*Moniezia* sp.	*Nematodirus* sp.	*N. battus*	Trichostrongylidae	*Trichuris* sp.
Prevalence (%)	WM n = 248	14.1	41.9	4	2	33.9	15.7	46	11.7
	WM n = 59	16.9	40.7	1.7	1.7	40.7	15.3	45.8	13.6
	SM n = 59	8.5	62.7	3.4	-	20.3	8.5	28.8	8.5
	P	0.063	0.004*	1.000	1.000	0.000*	0.125	0.006*	0.219
Eggs/oocysts per 3 g (WM)	Average n = 248	3.6	221.4	1,236.5	561.6	15.9	6.7	163.4	6.3
	Average n = 59	3.8	110	2	3	23.2	6	38.3	6.4
	Median n = 248	2	11	2	321	8.5	2	8	2
	Median n = 59	2	6.5	2	3	8.5	3	3.5	3
	Range n = 248	1–19	1–6,270	1–12,187	3–1,496	1–160	1–81	1–10,532	1–31
	Range n = 59	1–12	1–1,322	2–2	3–3	1–160	1–22	1–584	1–31
Eggs/oocysts per g-(SM)	Average n = 59	8	129.2	5	-	23.3	8	31.2	5
	Median n = 59	10	20	5	-	10	10	5	5
	Range n = 59	5–10	5–1,250	5–5	-	5–115	5–10	5–345	5–5

* – statistically significant difference

Discussion

Our coprological findings in alpacas inhabiting Poland are consistent with reports from other countries. According to Hyuga and Matsumoto (10), the most prevalently detected parasite material in alpacas in Japan were eggs of trichostrongyles (50.9%) and oocysts of *Eimeria* spp. (71.7%), whereas eggs of *Moniezia* spp. were the rarest. The result perfectly reflects our own data, besides the greater frequency of observation of oocysts of *E. macusaniensis* in Japan (7.5%) than in Poland (1.7%–4%). According to Diaz *et al.* (4), 64.3% of the examined alpacas inhabiting their native land of Peru shed oocysts of *Eimeria* spp. This corresponds to the results of this study obtained using SM. Rashid *et al*. (14) reported that the prevalence of GIN eggs reached 61% in alpacas kept in Australia (14); the result falls in the range of prevalence levels for GIN obtained by us with the use of both WM and SM. The pathogenicity of gastrointestinal parasites in alpacas has been proved by veterinary pathologists in Sweden, who revealed that 78% of cases of enteritis in dissected individuals resulted from parasitic infestations, mainly of the *Eimeria*, *Nematodirus*, *Trichuris*, *Ostertagia*, *Trichostrongylus*, and *Haemonchus* genera (2). According to Williamson (17), faecal egg counts greater than 3,000 per gram can be associated with morbidity in camelids; however, the method of counting was not disclosed. In this study, counts exceeding that value were notated for *Eimeria* spp. (maximal oocyst count = 6,270), *E. macusaniensis* in a highly pathogenic result (maximal oocyst count = 12,187), and trichostrongyles (maximal egg count = 10,532); all results were obtained with the use of WM in samples with a weight of 3 g.

Both WM and SM are direct centrifugal flotation techniques; however, WM is a single-step approach, while SM, is a dilution method, requiring an initial wash step in water (1). The McMaster technique (MM) is also a two-step method (1). It is worth mentioning that dilution methods tend to be less accurate, because they require extrapolation (1). Additionally, WM and SM represent the test tube and cover slip approach to faecal egg counting in contrast to methods representing the counting chamber approach, such as MM (12). On the other hand, WM is a qualitative method, whereas SM and MM are quantitative coprological techniques (7, 17). Gałązka *et al.* (7) reported that WM yielded a higher prevalence of eggs and oocysts in European bison-derived samples than MM. The statistical analysis of the results of this study revealed that WM offers an advantage in the detection of eggs of Trichostrongylidae and *Nematodirus*, whereas SM does in the detection of smaller coccidia. Cebra and Stang (3) made a similar observation for MM, namely that it yielded more positive results exclusively for smaller coccidia.

A pragmatic interpretation of our study is the conclusion that the modified Willis technique is more accurate at detecting heavy GIN eggs, including those of Trichostrongylidae and *Nematodirus*, whereas the modified Stoll technique is more accurate at detecting smaller coccidia from the *Eimeria* genus. In being a single-step flotation technique that is less time-consuming than SM and other dilution methods, WM is to be recommended as the method more adaptable to field work and veterinary practice.

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