Parasitological survey to address major risk factors threatening alpacas in Andean extensive farms (Arequipa, Peru)

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ABSTRACT. Extensive alpaca farming in the Colca Valley (Arequipa, Peru) is the most important resource for farmers who live in this remote area of the country. Grazing is the major available source of food, whereas forage production and supply are limited. Food availability is low during the dry season predisposing animals to parasitic diseases and reproduction failure. In this study, we looked into gaining an overview about flock health management and nutritional and parasitological status of different age groups in medium-large alpaca farms at the beginning of the dry season. A total of 20 herds were included in the survey and 288 fecal samples were collected and analyzed to determine prevalence and oocysts/eggs output level of the most common gastro-intestinal parasites that affect alpacas. Body condition scoring (BCS), was used to determine the nutritional status of sampled animals, belonging to different physiological classes (i.e. crias, tuis, lactating females, non-lactating females, males). Coccidiosis was the parasitic disease with the highest prevalence and output level. It was the most relevant parasitic disease in all classes and especially in young pre-breeding animals. Higher burdens were found in subjects with lower BCS. More specifically, the farms with higher cria mortality rates showed also a tendency to have higher prevalence values of *Eimeria macusaniensis*. An improved management of nutritional aspects and parasite control, in particular regarding the weaning phase, is expected to enhance the farming reproductive and productive performances of the alpacas in this region.

KEY WORDS: alpaca, body condition scoring, *Eimeria*, mortality, Peru

Alpaca breeding is widespread through the Andean high planes, at altitudes exceeding 4,000 m a.s.l., since these areas are suitable neither for agriculture nor for domestic animals breeding, except for llamas and alpacas [22]. In Peru, nearly 73% of alpacas are bred in the Southern Departments of Arequipa, Montegua, Tacna and Puno [28]. The climate is characterized by a wet and a dry season, from November to March and from April to October, respectively. Pasture quality is high at the end of the wet season (March-April) and decreases progressively during the dry period [17].

Alpaca breeding is totally based on extensive pastoral management and animals have no access to sources of food, except for natural pasture [20, 22, 29]. Alpaca productivity depends on the energy reserves gathered during the wet season. However, pasture management and their rotations are frequently inadequate, and the number of heads exceeds the carrying capacity, depleting the resources of the grasslands and threatening their conservation. Because of overgrazing, pastures are often in poor conditions in more than 50% of grasslands located in these areas and high parasitic burdens were reported [17, 25, 31]. Inadequate nutrition and parasites are jointly associated with a decline in health status of the herds, since animals in good condition are less prone to present high parasitic burdens [4].

Poor nutritional status can affect fertility and pregnancy maintenance [4, 16, 19] and negative energy balance at the end of pregnancy has a negative influence on colostrum production and on offspring birth weight, thus contributing to insufficient neonatal immunity acquirement and resistance [3, 7, 11]. As a consequence, the reproductive efficiency and the offspring survival in Peruvian alpaca farming are extremely unfavorable, with embryonic losses up to 45% during the first month of pregnancy [19]

and overall cria mortality rate up to 50% [25].

Parasitic infections are among the factors that limit the productivity of alpaca farms in Peru. South American camelids are sensitive to many gastro-intestinal (GI) parasites that also affect other domestic ruminants, including strongyles, whipworms and tapeworms. In addition, they can be affected by some species-specific GI nematodes that are unique to camelids, such as *Camelostrongylus mentulatus* and *Lamanema chavezi* [7]. With regard to protozoa, alpacas harbour their own species-specific coccidia: *Eimeria alpacae, E. punoensis, E. lamae* [9], *E. macusaniensis* [10] and *E. ivitaensis* [14]. An additional species, *Eimeria peruviana*, was described in 1934 by Yakimoff, but it has not been reported since that first description [6]. Young animals are more susceptible to coccidiosis due to their lack of previous exposure and immunity. In particular, coccidiosis prevalence increases in young animals from about 3 weeks of age onwards [5]. Adults rarely show clinical disease, with the exception of immunocompromised subjects and animals exposed to stressful events including birthing, lactation and weaning. *Eimeria macusaniensis* and *E. lamae* are regarded as the most pathogenic coccidia in camelids. The former species destroys the intestinal epithelium, while the latter damages the crypt glands, leading to severe direct damage to the intestine. They are significant pathogens and the coinfection of the two species seems to be the cause of many coccidiosis outbreaks in crias in late spring [5]. In addition, the primary severe damage predisposes the intestine to invasion by secondary pathogens such as *Escherichia coli* and *Clostridium perfringens*, causing colibacillosis and enterotoxaemia, the major causes of young stock loss in South America [5, 21].

According to some authors, alpacas and llamas are more susceptible than sheep to GI parasites. As a consequence of adaptation to a dry environment, they are efficient in reabsorbing water through their spiral colon, therefore they do not exhibit diarrhea but weight loss as predominant clinical sign [30]. For this reason, body condition scoring (BCS) combined with FAMACHA scoring are considered the gold standard procedures to assess alpaca flocks and perform targeted sampling or treatment, especially when a particular GI parasite is suspected, i.e. *Haemoncus* spp. [24]. The aim of this study was to assess the level of parasitic infestation of a group of alpaca herds in Southern Peru at the beginning of the dry season, and its relationship with physiological classes and BCS.

MATERIALS AND METHODS

Study area

In total 20 alpaca farms were investigated at the beginning of the dry season, from May to July 2011, in the districts of Achoma, Callalli, Sibayo, Tisco and Yanque, province of Caylloma, department of Arequipa, Peru (Fig. 1). All of them were medium- or small-scale extensive farms, composed of a variable number of alpacas (between 100 and 800), located at an altitude ranging from 4,200 to 4,800 m a.s.l. The alpaca feeding was entirely based on grazing, without any form of supplementation.

Questionnaire survey

A questionnaire was delivered to the farmers before starting the sampling. It was composed of open questions about general information (herd size and composition; altitude; cria mortality during the year of the survey) and questions to assess farm procedures for disease control: 1) details about any sanitary intervention (anthelmintic, anticoccidial drug, antibiotic, vaccination) performed on the alpacas in the 3 months before the study; 2) presence of parasite/parasitic elements in alpaca feces (yes or no); 3) sign of liver flukes in the liver in slaughtered alpacas (yes or no).

Fig. 1. Study area and farms location (source: Google Earth).

Coprological survey

Fecal samples were collected randomly from a different number of alpacas in each investigated farm, possibly representative of the whole herd. In principle, a minimum sample size of 10 individuals per farm was defined, but the number of sampled animals was strongly affected by the logistic aspects and animals available from each physiological class. Sampling activity was organized to target animals belonging to five different physiological classes: C (crias, young suckling animals, less than 1 year of age); T (tuis, weaned animals of 1 to 3 years of age, prior to puberty); LF (lactating adult females, more than 2 years of age); F (adult non-lactating females, more than 2 years of age); M (adult breeding males, more than 3 years of age).

In order to assess the nutritional status of sampled animals, BCS was measured by palpating the lumbar vertebrae as previously described [7]. A 5-point BCS system similar to the one described in sheep [27] was used with 1 for thin and 5 for obese subjects, with values subdivided into half scores.

Samples were taken directly from the rectum and 2 g of each fecal sample were mixed with 2 ml of Ecofix® fluid (Meridian Bioscience Inc., Cincinnati, OH, USA), for preservation until subsequent examination at the laboratory. Samples were examined no later than 5 months after collection.

Laboratory analysis

Samples were individually assessed by qualitative flotation technique, using 1 g of feces and a high-density solution (composition: water 1,720 ml, sodium thiosulfate 1,800 g, sodium nitrate 1,280 g, sucrose 1,200 g; specific gravity: 1.450).

Quantitative examination with the McMaster method was individually performed to determine the amount of oocysts per gram (OPG) and eggs per gram (EPG).

The samples were assessed for the presence of oocysts from parasite species belonging to the sub-class Coccidia, and the presence of eggs of parasites belonging to the classes Nematoda, Cestoda and Trematoda. Among Nematoda, it was possible to further differentiate parasitic elements of the genera *Nematodirus*/*Marshallagia*, *Trichuris*, and *Capillaria*. All eggs with a strongyle-like appearance and a smaller size than *Nematodirus*/*Marshallagia* ones were classified as strongyles (order Strongylida). As per Cestoda, eggs were identified as *Moniezia* spp., since this is the only genus reported in alpaca [1, 2] with the morphology ascribable to intestinal cestoda of herbivores. Finally, eggs morphologically attributable to *Fasciola hepatica* and *Dicrocoelium dendriticum* were searched during analysis, since both species were recorded in alpacas [1, 2].

Data analysis

Data from the questionnaire and the copromicroscopic analyses were organized in a Microsoft Excel 2011® spreadsheet (Microsoft Corp., Redmond, WA, USA). The average BCS for each physiological class, considering all sampled animals, was determined. Subsequently, the deviation from the average for each individual animal was calculated.

Prevalence of infection and oocysts/eggs output level (mean number of OPG/EPG) were displayed using simple descriptive statistics for all parasitic groups. The influence of different factors (BCS, gender, physiological class) on the qualitative and quantitative results of copromicroscopic examination was investigated using a Pearson Chi-squared test and a non parametric approach (Mann-Whitney U test or Kruskal-Wallis test, when appropriate), respectively. The correlation between the cria mortality rate and the prevalence values of different parasitic taxa at farm level was investigated through the Spearman correlation coefficient. Data were analysed using IBM SPSS Statistics 24 (IBM Corp., Armonk, NY, USA).

RESULTS

Questionnaire survey

In total, more than five thousand alpacas were present in the 20 investigated farms, and 288 animals belonging to the five different physiological classes were selected for fecal samples collection and BCS measurement. It was not possible to get complete data from all farms.

The altitude, herd size, number of sampled animals and cria mortality rate are shown in Table 1 for each farm. The average heads per farm was 358.6, ranging from 116 to 720. In general, LF and C represented the two most considerable physiological classes accounting jointly for about 58% of the herd. F was the second most numerous class (25%), followed by T (11%) and M (6%). Physiological classes were different in average BCS, with LF presenting the lowest value (2.37), followed by C (2.74), T (2.77), F (2.84) and M (3.13). The overall average BCS of sampled animals measured 2.65.

The results of the questionnaire survey are shown in Table 2. All of the farmers treated their herds with anthelmintics (12 with ivermectin-based drugs, 2 with benzimidazolic drugs and for 6 farmers it was not possible to identify the anthelmintic class) in the three months previous to the survey, but none of them used any anti-coccidial drug. About half of the farmers reported to have noticed endoparasites in the feces (13/20, 65%) and in the liver (10/20, 50%) from slaughtered animals.

Copromicroscopic analysis

In total, 288 individual fecal samples were collected and analyzed. The main qualitative and quantitative epidemiological parameters for each group of parasites are reported in Table 3. All parasite groups were found, except for Trematoda. Helminth groups showed low values both in prevalence and eggs output level. On the contrary, different coccidian species were observed, with an overall prevalence higher than 50%. Coccidia were generically identified as *Eimeria* spp. as unsporulated oocysts of the different species were morphologically difficult to discern, except for *E. macusaniensis*, which was clearly different from other

Table 1. Altitude, total number of animals raised, number of animals for each physiological class, number of sampled animals, cria mortality rate and *Eimeria macusaniensis* prevalence in the investigated farms

Farm ID	Altitude (m a.s.l.)	Total No. animals	Physiological classes[a]					No. animals sampled	Cria mortality	*E. macusaniensis* prevalence
			LF	F	C	T	M			
1	4,433	480	110	130	110	50	80	18	39%	17%
2	4,431	360	120	90	120	30	0	14	13%	14%
3	4,643	536	120	200	120	50	46	38	14%	0%
4	4,377	278	72	28	72	34	72	27	3%	22%
5	4,642	474	200	70	200	0	4	6	3%	0%
6	4,358	179	65	25	65	20	4	10	11%	10%
7	4,382	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18	33%	11%
8	4,356	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12	32%	33%
9	4,714	554	70	230	70	164	20	17	53%	47%
10	4,331	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23	5%	17%
11	4,477	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13	7%	8%
12	4,443	116	35	32	35	10	4	16	22%	38%
13	4,213	630	180	200	180	60	10	8	24%	38%
14	4,556	280	60	120	60	20	20	15	3%	27%
15	4,483	720	220	150	220	100	30	16	12%	13%
16	4,223	236	90	16	90	38	2	5	3%	0%
17	4,488	342	96	66	96	30	54	10	4%	30%
18	4,442	274	100	60	100	10	4	7	2%	14%
19	4,395	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4	26%	0%
20	4,234	149	58	11	58	10	12	11	21%	36%
Total		5,608	1,596	1,428	1,596	626	362	288		19%

a) C, crias; T, tuis; LF, adult lactating females; F, adult non-lactating females; M, adult males. n.d., not determined.

Table 2. Results of the questionnaire survey

Question No.[a]		Answer[b]	No. farms	Farms (%)
1	Treatments to alpacas	D	9	45
		D, A	8	40
		D, V	2	10
		n.d.	1	5
2	Parasites in feces	No	7	35
		Yes	13	65
3	Parasites in the liver	No	10	50
		Yes	10	50

a) 1, which sanitary intervention were used for alpacas in the last 3 months; 2, presence of parasite/parasitic elements in alpaca feces; 3, sign of liver flukes in the liver in slaughtered alpacas. b) D, deworming; A, antibiotics; V, vaccination. n.d., not determined.

Table 3. General results of fecal examination (n=288)

Parasite	Qualitative		Quantitative (OPG/EPG)[a]	
	Positive (N)	Prevalence	Mean	Range
Eimeria spp.	174	60.4%	139.78	0–22,770
E. macusaniensis	54	18.8%	32.22	0–5,452
Trichuris spp.	16	5.6%	0.31	0–75
Capillaria spp.	10	3.5%	0.08	0–50
Moniezia spp.	10	3.5%	9.38	0–4,500
Nematodirus / Marshallagia spp.	6	2.1%	0.03	0–25
Gastro-Intestinal strongyles	4	1.4%	0.06	0–50

a) OPG, oocysts per gram; EPG, eggs per gram.

oocysts, because of its bigger size, very thick wall and piriform shape [6], and therefore was classified separately (Fig. 2).

Risk factor analysis

Animals were divided according their BCS in two groups (positive or negative deviation from the average BCS of all animals belonging to the same physiological class−defined as high or low BCS, respectively). The influence of this factor and that of gender and physiological class (C, T, LF, F, M) in determining the presence and the output level of different parasites was investigated, obtaining the following significant results.

Significant differences were observed in prevalence values for *Eimeria* spp. among physiological classes ($P<0.001$) and between animals with high or low BCS ($P<0.05$): younger animals (C and T) and ones with low BCS values had a higher prevalence. Gender did not seem to influence this group of parasites ($P>0.05$). The output level of *Eimeria* spp. oocysts was different only among physiological classes, with higher OPG ($P<0.05$) in crias and tuis (Table 4).

The prevalence and the output level of *E. macusaniensis* were both significantly higher in crias ($P<0.05$) than in other physiological classes. In addition to this, animals with lower BCS showed marked differences in both prevalence and oocysts output values compared to the ones with high BCS (Table 5).
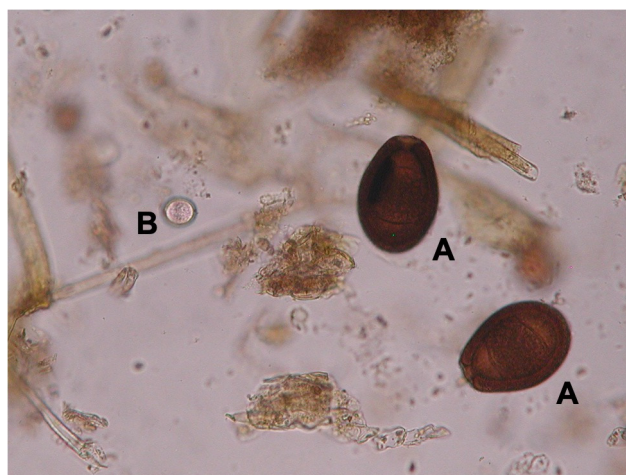
Fig. 2. Two unsporulated oocysts of *Eimeria macusaniensis* (A) on the right and one unsporulated oocyst (B) of another species of *Eimeria*, at the copromicroscopic examination (400×).

Table 4. *Eimeria* spp. in the different physiological classes and in animals with different body condition score (BCS)

Eimeria spp. factor	Variables	Qualitative				Quantitative (OPG)[a]	
		Examined (N)	Positive (N)	Prev.	*P*-value	Mean	Range
Physiological class[b]	C	49	45	91.8%	<0.001	1911.2[1]	0–22,770
	T	49	40	81.6%		341.4[2]	0–4,075
	LF	115	58	50.4%		65.2[3]	0–900
	F	47	15	31.9%		32.4[3]	0–650
	M	28	16	57.1%		143.8[2,3]	0–1,450
BCS	Low	150	99	66.0%	0.043	575.6[1]	0–22,770
	High	138	75	54.3%		268.7[1]	0–6,571

Significant differences in mean output values are highlighted by different superscript numbers (*P*<0.05). a) OPG, oocysts per gram. b) C, crias; T, tuis; LF, adult lactating females; F, adult non-lactating females; M, adult males.

Table 5. *Eimeria macusaniensis* in the different physiological classes and in animals with different body condition score (BCS)

Eimeria macusaniensis factor	Variables	Qualitative				Quantitative (OPG)[a]	
		Examined (N)	Positive (N)	Prev.	*P*-value	Mean	Range
Physiological class[b]	C	49	39	79.6%	<0.001	554.8[1]	0–5,452
	T	49	7	14.3%		15.6[2]	0–350
	LF	115	1	0.9%		0.2[2]	0–25
	F	47	5	10.6%		7.4[2]	0–250
	M	28	2	7.1%		4.6[2]	0–97
BCS	Low	150	41	27.3%	<0.001	163.2[1]	0–5,452
	High	138	13	9.4%		28.8[2]	0–1,250

Significant differences in mean output values are highlighted by different superscript numbers (*P*<0.05). a) OPG, oocysts per gram. b) C, crias; T, tuis; LF, adult lactating females; F, adult non-lactating females; M=adult males.

No differences in prevalence and eggs output level were found for *Trichuris* spp. and it was not possible to perform statistical analyses for other parasites, in consideration of their prevalence values being under 5%.

Concerning crias, farmers reported high variability in the mortality rates, with values ranging from 2% to 53%. The parasitological index that correlated better to this parameter was the *E. macusaniensis* prevalence (Table 1). The Spearman correlation coefficient (R) between the two values was 0.3258.

DISCUSSION

In our study, a high prevalence and oocysts output were observed for coccidia, whereas other species of GI parasites (i.e.

helminths) showed lower values for both epidemiological indexes. The questionnaire survey highlighted that most of the farmers performed deworming treatment on alpacas in the three months before the survey, which corresponded to the end of the rainy season. The anthelmintic treatment of alpacas in this period of the year (generally in April) was also previously reported [22]. The drugs most commonly used were ivermectin-based. This may explain the very low values found for strongyles, which are known to be well adapted to grazing animals. Nevertheless, our study confirmed directly the presence of different groups of endo-parasites (Nematoda and Cestoda), whereas the presence of liver flukes (Trematoda) was indirectly suggested by farmers. These parasites have been reported in alpacas, mostly in other geographical contexts and/or under different management systems [12, 13, 26]. All these parasites may have an important impact on health status, if left untreated [30]. Therefore, the burden of these helminths needs to be monitored in other periods of the year for a proper assessment of their pathological importance at the high altitude of the Andean planes.

The results of our study suggest that the most relevant parasitic problem in these alpaca populations is represented by coccidiosis. Prevalence and oocyst output values of *Eimeria* spp. and *E. macusaniensis* found in our research are in agreement with other studies conducted in similar conditions [5, 6]. Severe coccidiosis infection mostly occurs in young animals [1, 18]. Five different species of *Eimeria* can affect the intestinal tract in alpacas: *E. alpacae*, *E. lamae*, *E. punoensis*, *E. macusaniensis* and *E. ivitaensis*. We did not differentiate *Eimeria* species, except for *E. macusaniensis*, since it was the only species to be readily distinguishable by morphological observation of unsporulated oocysts, also considering that counts were performed at 100× in a McMaster chamber. *Eimeria macusaniensis* is described as the most pathogenic species of coccidia in camelids, as it causes severe damage to intestinal mucosa of infected subjects [1]. *Eimeria macusaniensis* is also associated with *Clostridium perfringens* infections [21], one of the most important causes of death of suckling animals [23].

As expected and previously reported [6], the load of *Eimeria* spp. and *E. macusaniensis* in crias was higher than in other classes. The prevalence of *E. macusaniensis* was the most correlated to the reported cria mortality. Although this correlation did not result very strong (R=0.3258), it still seems that farms with higher prevalence of this species present higher mortality rates in crias. These findings suggest that coccidiosis, particularly if due to *E. macusaniensis*, can be involved, among other causes, in worsening the health status of crias.

Furthermore, BCS condition of individuals seems to be also linked with coccidian burden. Animals with lower BCS demonstrated to have higher prevalence value for *Eimeria* spp. and both higher prevalence and oocysts output values for *E. macusaniensis*. It is always difficult to understand if the poor body condition is facilitating the presence of parasites or if it is vice versa, but a relationship between nutritional status, immunological conditions and parasitic load was already described [15]. It is worth of note that the present study was implemented during the initial phase of the critical period for energy supply (dry season) and this aspect could explain the poor body condition of high energy demanding animals, such as lactating and pregnant females (average BCS: 2.37). Higher cria mortality rates can be also due to poor nutritional status of adult lactating females. Due to a deficient diet, the volume of colostrum and milk produced by thin females can decline or completely cease [4, 7] and failure of passive antibody transfer is one of the principal factors predisposing crias to infectious and parasitic diseases [7, 8].

Low values of BCS can be due to different causes, but our results suggested that they can be also associated to coccidian infection, hence it could be used as a monitoring system, when laboratory facilities and skilled personnel are not available in the area. The finding of BCS rates under expected values in many animals of the herd, or in specific physiological classes, should alarm the farmer and suggest the need for an appropriate intervention, including the OPG quantification of coccidian burden. The problem can be also probably prevented by an improvement of the diet (e.g. supplementary feeding), but farmers have to be instructed on how and when to apply specific treatments for Coccidia, since it seems from the questionnaire survey that they are not used to perform this kind of treatment.

In conclusion, the present study found that coccidian infections are the parasitic group with higher prevalence and output in the investigated populations of alpacas. Coccidia mostly affected young animals (in particular crias), and higher burdens were found in subjects with lower BCS. Targeted treatments and diet improvement would be beneficial in order to prevent infestation of crias with *Eimeria* species and therefore reduce cria mortality rate. Monitoring BCS in the different physiological classes, can integrate the more qualified diagnostic services, such as the quali-quantitative copromicroscopic examination, which is not easily available in remote areas of Peruvian Andes.

In general, BCS is a relevant monitoring method in the Peruvian pastoral context, as it can allow farmers to perform a periodical check of the nutritional status of their animals. This can guide farmers to the correct action to be taken (e.g. identification of the cause of the problem through appropriate diagnostic test, followed by treatment or supplementary feeding of selected groups of animals, if available), therefore helping to improve offspring survival and herd production. Obviously, to meet this objective, farmers should be encouraged and trained by local authorities and Non Governmental Organizations, especially in those remote areas where veterinary services are not available.

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