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Prevalence and risk factors associated to *Eimeria* spp. infection in unweaned alpacas (*Vicugna pacos*) from Southern Peru

Pablo Díaz[1]*, Rosario Panadero[1], Rosalía López[1], Aida Cordero[2], Ana Pérez-Creo[1], Ceferino M. López[1], Gonzalo Fernández[1], Pablo Díez-Baños[1] and Patrocinio Morrondo[1]

[1]Investigación en Sanidad Animal: Galicia (Grupo INVESAGA), Facultad de Veterinaria, Departamento de Patología Animal: Sanidad Animal, Universidade de Santiago de Compostela, Lugo 27002, Spain; [2]Universidad Nacional Agraria La Molina (UNALM), Lima, Perú

Abstract

A total of 350 faecal samples from unweaned alpacas over 3 months of age were collected from 23 herds in order to determine the prevalence of *Eimeria* spp. in Southern Peru and to identify the risk factors associated to *Eimeria* infection in young alpacas. Samples were examined by a flotation technique and the identification of risk factors was assessed by a logistic regression analysis. Sixty four percent of the examined animals shed *Eimeria* oocysts; herd prevalence was 96%, with an intra-herd prevalence of 60% (range 5.9–100%). Five different *Eimeria* species were identified, being *E. lamae* (91%), *E. alpacae* (87%) and *E. punoensis* (78%) the most prevalent; *E. macusaniensis* (35%) and *E. ivitaensis* (13%) were less common. Mixed-species infections were more frequent (78%) than single infections (22%). *E. lamae* was the most common monospecific infection and *E. lamae/E. alpacae* the most frequent association. The geographical area has a significant effect on *Eimeria* infection rates (74.9% wet Puna *vs* 37.4% dry Puna) as well as the breeding system (65.1% traditional *vs* 63.8% modern). In contrast, the sex of the animals (64.6% males *vs* 64.0% females) showed no influence on the prevalence of infection by *Eimeria*. The high prevalence found at both individual and herd level and the common presence of highly pathogenic *Eimeria* species may lead to important economic losses for alpaca breeders and could require the implementation of suitable control measures.

Keywords

Vicugna pacos, alpaca, *Eimeria*, prevalence, risk factors, Southern Peru

Introduction

Breeding of South American camelids (SACs; alpacas, llamas, vicuñas and guanacos), is an important socioeconomic activity for all the Andean populations of South America (Rodríguez *et al*. 2012). They are source of meat, hides and fibre of considerable commercial value; these animals are also used for transport, and their droppings are employed as fuel and fertilizer. SACs distribution is limited to areas of extreme altitude (3,600–5,400 m) in the Andean cordillera, from southern Ecuador to the extreme north of Chile and the northeast of Argentina, with the highest numbers in Peru and Bolivia (Leguía 1991).

Peru is the major alpaca (*Vicugna pacos*) producing country in the world (90%). According to FAO (2005), Puno department is the largest alpaca producing area in Peru, comprising the 58% of the national production (1,681,919 heads). In this country two alpaca management systems coexist: traditional and modern farms (Cid 2010). Traditional breeding system represents the 80% of these livestock in Peru and is characterized by the lack of separation of animals by age, breed or species such as llamas, sheep and cattle; there is also a high density of animals in pastures, leading to overgrazing. Under those circumstances infectious and parasitic diseases are frequent, causing important economic losses (Leguía and Casas 1999; Cabrera 2008) with high morbidity and mortality levels. In contrast, some management (mechanical shear, rotational grazing, mating control, etc.) and sanitary practices are undertaken in modern farms to improve productive parameters.

*Corresponding author: pablo.diaz@usc.es

International organizations recommend monitoring camelid health as well as determining their susceptibility to diseases affecting domestic livestock (OIE 2010). Parasitic infections are among the factors that limit the productivity of SACs. Pathological effects of parasites lead to reductions in the quality and quantity of meat and wool by reducing appetite and utilization of nutritional resources; low fertility, abortion and death were also reported. It is noteworthy that some bacterial infections (colibacilosis, clostridiosis, etc.), considered major causes of death in crias, are secondary to other intestinal processes, such as coccidiosis (Palacios *et al*. 2005; Cebra *et al*. 2007; Rosadio *et al*. 2010).

Eimeria spp. is one of the parasites of preferential interest during the early life of ruminants, with increasing prevalences beginning 3 weeks after birth and cumulative incidences of up to 100% (Daugschies and Najdrowski 2005). At least 5 species of *Eimeria* have been reported to infect alpacas: *Eimeria lamae*, *Eimeria alpacae*, *Eimeria punoensis* (Guerrero 1967), *Eimeria macusaniensis* (Guerrero *et al*. 1971) and *Eimeria ivitaensis* (Leguía and Casas 1998).

Although coccidiosis is mainly a problem of animals reared in confinement, there are frequent outbreaks of subacute or acute infections in alpacas born late in the breeding season (Fb-Mr) or after weaning (Oc) (Leguía 1991). Such outbreaks seem to be mainly caused by the association of *E. lamae* with *E. macusaniensis*; this coinfection is highly pathogenic since the former species destroys the intestinal epithelium while the latter damages the crypt glands and inhibits regeneration of the epithelium (Leguía 1988). Nevertheless, single infections by *E. lamae* and *E. macusaniensis* are considered pathogenic for alpaca crias (Guerrero *et al*. 1970).

The main purpose of this study was to obtain data on the prevalence of *Eimeria* spp. infecting young alpacas in southern Peru and to determine the effect of some extrinsic (climatic conditions, breeding system) and intrinsic (sex) factors on their prevalence.

Materials and Methods

Area of study

This study was conducted in Puno Department (South-eastern Peru) with a total area of 71,999 km^2 (13°00´66″–17°17´30″S and 71°06´57″–68°48´46″W). This Department includes Altiplano (70%) and forest areas (30%). Considering climatic, geographic and altitude differences, 3 agro-ecologic areas are established in Puno: Circunlacustre, Suni and Puna regions (Tapia 1996). The present study was carried out in the latter. The Puna is a diverse ecosystem that is subdivided in two climatic areas: wet and dry Puna. Wet Puna is characterized by a mean precipitation of 784 mm/year (range 800–1000 mm/year) and a mean annual temperature of 10°C. The dry Puna has a mean precipitation of 549 mm/year (range 540–600) and a mean annual temperature of 4.5°C; in this area, during the driest months, animals graze in small wet Andean meadows called "bofedales" dependent on glacier melt waters.

Samplings

Faecal samples from 350 unweaned alpacas (over 3 month-old) were taken at random from 23 herds during the breeding season (January-March). Individual samples were collected directly from the rectum, placed in plastic tubes and stored in 2.5% potassium dichromate at room temperature. Samplings were performed early in the morning to avoid disturbing routine works in the farm.

Data regarding the characteristics of the farms was recorded, including among other descriptors, sampling location, size of the herd and breeding system.

Parasitological analysis

Oocysts were sporulated as described by Hendrix (1999), using 2.5% potassium dichromate ($K_2Cr_2O_7$) at room temperature for 35 days on a shaker to ensure good aeration and the sporulation of all *Eimeria* species that might be present. Obtained oocysts were covered in 2.5% potassium dichromate solution at 4°C until used. Afterwards, samples were centrifuged at 500 × g for 5 min; the sediment was mixed with Sheather´s sucrose solution (specific gravidity 1.27) and microscopically examined at 1000× magnification. One hundred sporulated oocysts from each sample were identified by means of the morphometric analysis of the oocysts, including individual characteristics of each species (Pellérdy 1974; Leguía and Casas 1999). All oocysts were identified in those samples where less than 100 oocysts were recovered.

Statistical Analysis

Statistical analyses were performed with the R statistical package (R v.3.2.0; R Development Core Team 2015). Positivity was analyzed with a Logistic Regression algorithm. The dependent variable was the positiveness (0–1) to *Eimeria* spp. of the individual animal. Factors indicated previously were introduced in a backward conditional method and removed from the model one by one (on the basis of the highest p-value) until the best model was built. Next, all pairwise interactions that were biologically plausible were evaluated. Odds ratios were computed by raising *e* to the power of the logistic coefficient over the first category of each factor, not over the last.

Results

Sixty four percent (64.3%) of the examined animals shed *Eimeria* oocysts; herd prevalence was 96%, with an intra-herd prevalence of 60% (range 5.9–100%).

Individual prevalence of *Eimeria* spp. in alpaca crias when considering the effect of some extrinsic (climatic conditions,

Table I. Prevalence of *Eimeria* spp in unweaned alpacas in southern Peru

	Sex		Management		Origin area		Total
	Male	Female	Traditional	Modern	Dry Puna	WetPuna	
N# samples	164	186	126	224	99	251	350
Prevalence (%)	64.6	64.0	65.1	63.8	37.4	74.9	64.3

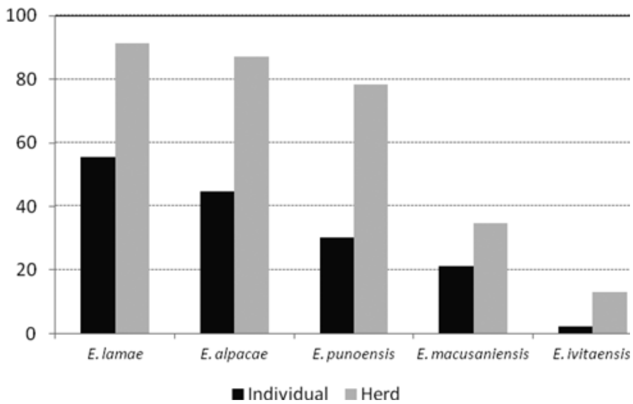

Fig. 1. Individual and herd prevalence by different *Eimeria* spp in unweaned alpacas from Southern Peru

breeding system) and intrinsic (sex) factors is presented in Table I. Logistic regression results indicate that both the geographical area ($P< 0.001$) and the breeding system ($P< 0.001$) were factors associated with the positivity to *Eimeria.* Odds ratio values showed that the animals from the wet Puna had a 12.6-fold probability (95% CI 5.9–26.7) of being positive than those from the dry Puna. In addition, alpacas reared under a traditional management system showed a 4.4-fold probability (95% CI 2.1–9.3) of being infected by the protozoan than those from modern farms.

Figure 1 shows the individual and herd prevalence of *Eimeria* spp. found in this study. In both cases the most prevalent species was *E. lamae*, whereas *E. ivitaensis* was only sporadically found. Table II and Fig. 2 show the measurements (µm) and morphology of oocysts of the *Eimeria* spp. found in alpaca crias.

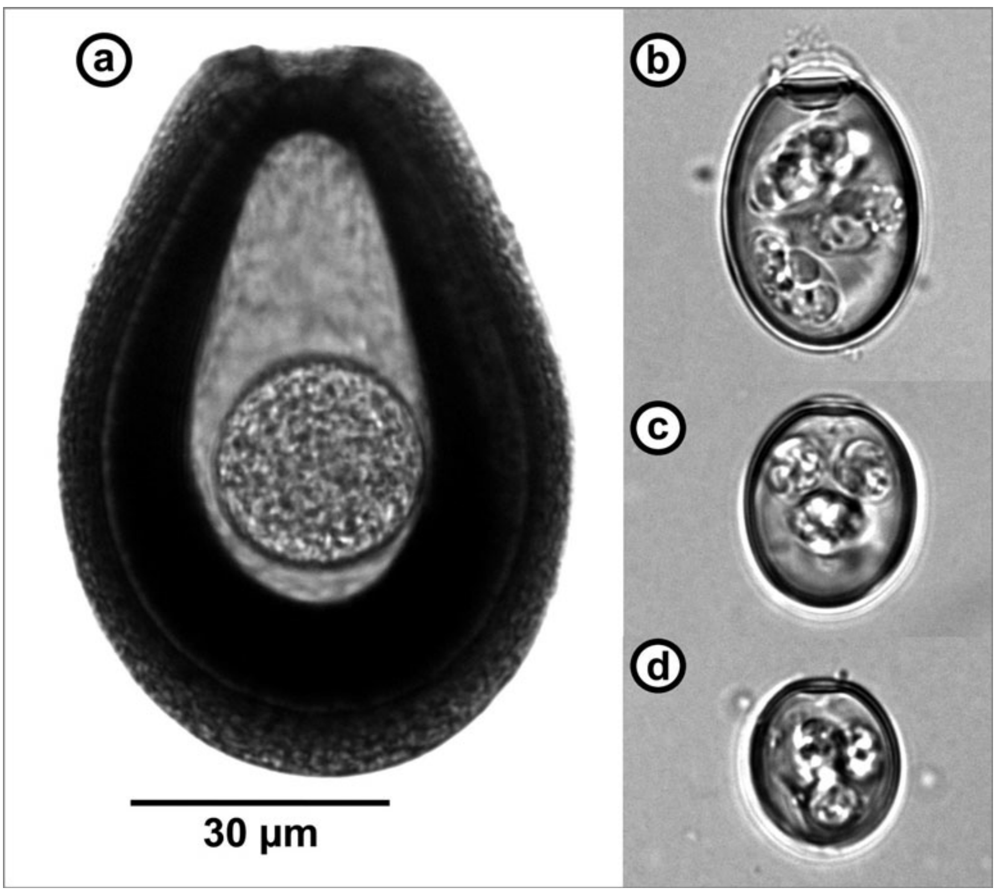

Fig. 2. Scaled microphotographs of the oocysts of the main *Eimeria* species of alpaca: (a) unsporulated oocyst of *Eimeria macusaniensis*; sporulated oocysts of (b) *Eimeria lamae*, (c) *Eimeria alpacae* and (d) *Eimeria punoensis*

Table II. Measurements of sporulated *Eimeria* spp. oocysts found in unweaned alpacas in Southern Peru

Species	Mean ± standard deviation (μm)	Ratio length/width
E. lamae	33.6 ± 2.32 × 24.1 ± 1.48	1.4
E. alpacae	24.7 ± 1.80 × 20.3 ± 1.82	1.2
E. punoensis	19.3 ± 1.23 × 16.8 ± 1.37	1.1
E. macusaniensis	85.8 ± 4.90 × 60.0 ± 3.05	1.4
E. ivitaensis	84.7 ± 4.14 × 53.5 ± 3.65	1.6

Mixed infections by 2 species were the most frequent (35%), being the association *E. lamae/E. alpacae* the most prevalent, followed by triple (25%), monospecific (22%), and infections by 4 (17%) and 5 species (1%). *E. lamae* was the most common monospecific infection (74%).

Discussion

Most of the herds (95.7%) included in this study showed infection by *Eimeria* spp., indicating that this protozoan is widely distributed in alpaca crias from southwestern Peru. The individual prevalence in crias was also significant and higher than those reported in previous studies in Peru by Cordero *et al*. (2011) in alpacas (46.2%) and by Castillo *et al*. (2008) in guanacos (33.3%). The high percentages of infection found in this survey could be attributed to a variety of reasons: (1) the age of the sampled animals; as occur in other species, young alpacas between 2-3 months of age show higher prevalences than older animals (Guerrero 1970; Jarvinen 1999; Cebra *et al*. 2003; Palacios *et al*. 2006; Rodríguez *et al*. 2012). (2) Moreover, crias born at the end of the breeding season (Fb-Mr), as occurred in this study, have a higher risk of acquiring the infection (Leguía, 1991; Leguía and Casas 1999; Rodríguez *et al.* 2012) and finally (3) faecal samples were collected coinciding with the rainy season in Peru (Cid 2010), and it is well known that wet conditions favours oocyst survival and sporulation. *Eimeria* oocysts are detected most often during wetter months, suggesting that crias born during these months may have greater exposure to these pathogens (Cebra *et al*. 2003). According to this author, some Protozoa (*Eimeria*, *Giardia* and *Cryptosporidium*) are most commonly isolated during the fall and winter/wet seasons.

However, it must to be pointed out that the mere presence of *Eimeria* coccidian in a herd is not necessarily related to clinical outbreaks, but due to the high reproductive potential of this protozoan, infected crias may shed millions of oocysts daily during the patence period (Leguía and Casas 1999; Daugschies and Najdrowski 2005). Consequently, the high contamination of the environment with oocysts led to a rapidly increase of the infection pressure in the surroundings of infected crias and also of the individual risk to acquire clinical coccidiosis. Factors that impose stress on the crias e.g. weaning, weather, inadequate feeding or other infectious diseases, may aggravate the condition.

The climatic area was identified by the logistic regression test as a determining factor for *Eimeria* infection in alpaca crias. Our results showed that the prevalence of faecal oocyst shedding was significantly higher in the wet Puna. Different authors (Mamani *et al*. 2009) pointed out that higher precipitation levels in wet Puna and the abundance of wetlands seems to promote a suitable environment for sporulation and survival of sporulated oocysts favouring the infection of the animals. In contrast, Rodríguez *et al.* (2012) did not find a correlation between the presence of bofedales and the *Eimeria* oocyst excretion.

Another factor involved in *Eimeria* infection was the breeding system. Our data reveal that modern breeding systems involved a lower risk of infection by the protozoan since they include the implementation of parasite control programs and the separation of the animals by sex and age, reducing the pressure of infection (Rodríguez *et al.* 2012).

In this study the sex was a factor not significantly associated with prevalence of shedding. Those results coincide with those of Rodríguez *et al*. (2012) in crias, and with Mckenna (2006) in a study including crias and adults.

In this study, all the *Eimeria* species known to infect alpacas have been found. Although is generally assumed the existence of cross-transmission between the different SACs, up to now a sixth species, *E. peruviana*, has been only identified in llamas. *E. lamae* was the most frequent species in this study. According to Guerrero *et al.* (1970) *E. lamae* is pathogenic for alpaca crias in both natural and experimental infections.

The study showed that 88% of positive samples presented mixed infections, caused by 2 to 5 different *Eimeria* species. This aspect has been previously observed by Guerrero *et al.* (1970) and Rodríguez *et al.* (2012) who also reported double and triple infections as the most common. The frequency of multiple infections might show a synergic action, especially between species which destroy the intestinal epithelium (*E. punoensis*, *E. alpacae* and *E. lamae*) with those that damage the crypt glands and inhibit regeneration of the epitelium (*E. macusaniensis* and *E. ivitaensis*). This resulted in complete stripping of the intestinal mucosa and its total loss of function, leaving the intestinal wall exposed to secondary viral or bacterial invasion (Leguía, 1991). The two species most often occurring together in our survey were *E. lamae* and *E. alpacae*, thus there is a clear predominance of species located at the intestinal villi. It must to be pointed out the low prevalence (20%) of *E. macusaniensis* in comparison with the percentages (50.4%) found by other authors in the same area (Rodríguez *et al*. 2012).

The high prevalences found at both individual and herd level, along with the common presence of highly pathogenic *Eimeria* species, may cause important economic losses for alpaca breeders, requiring the implementation of integrated control measures.

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