The diagnostic of gastrointestinal nematodes and coccidiosis in llamas from intensive and extensive agriculture systems in different areas of Argentina

Research Project Veterinary Medicine, University of Utrecht
M.L. van Erp Bsc. studentsnumber 3280489

September 2012 – December 2012

Project tutors:
Dr. J.H. Schapiro, Med. Vet. Área de Enfermedades Parasitarias, Instituto de Patobiología, Centro de Investigación en Ciencias Veterinarias y Agronómicas (CICVyA) INTA Castelar (Buenos Aires, Argentina).
Dra. G. Marcoppido, Vet. PhD. Área de Patología, Instituto de Patobiología, Centro de Investigación en Ciencias Veterinarias y Agronómicas (CICVyA) INTA Castelar (Buenos Aires, Argentina).
Dr. M.C. Pieterse, University of Utrecht, Faculty of Veterinary Science, The Netherlands.

Prefactory note

As part of the curriculum of Veterinary Medicine at the University of Utrecht it is mandatory for all students to complete a research project. This paper is the final report of the research project carried out by M.L. van Erp at the Pathobiologic Department of National Institute of Agricultural Technology (INTA) in Castelar, Buenos Aires (Argentina).

The research was performed to learn more about the presence of parasites in llamas in different regions and housing systems.

All the practical work was done on nematodes and coccidian but also executed upon protozoa of the rumen of llamas. The data obtained for this study can be found in the report of Anna Dekker another student of Veterinary Medicine of the University of Utrecht.

Fig. 1: The frontside of the laboratory Institute Pathobiologica INTA, Castelar

Contents

Abstract

There are four species of South American Camelids (SACs): the domestic alpaca *(Lama pacos*), the domestic llama (*Lama glama*), the wild vicuña (*Lama vicugno*) and the wild guanaco (*Lama guanicoe*). The social and economic importance of the SACs in the population of the high Andes is mainly due to the climatic and high altitudes limitations. The anatomy of the gastrointestinal tract of the llama is different from that of ruminants. The forestomach of llamas is comprised of three compartments, called C1, C2 and C3. Gastrointestinal nematodes are the most numerous and most detrimental of the llama parasites. The most important are: *Haemonchus contortus, Ostertagia ostertagia, Trichostrongylus* and *Lamanema chavezi. Eimeria spp.* is the most important pathogenic protozoa of the llamas. To treat the llamas against gastrointestinal nematods and coccidiosis, antihelmintic and anticoccidial treatment can be used, management changes are even or more important.

The aim of this study was to learn more about gastrointestinal nematode and coccidian in the llamas in intensive and extensive agriculture systems in different regions in Argentina. The provinces sampled were: Jujuy, Buenos Aires and Entre Ríos. Those regions have different climatic measurements. In two farms, the llamas are kept under intensive agriculture systems and in five farms the llamas are kept under extensive agriculture systems. Individual faecal samples were taken directly from the rectum of the llamas and processed by the parasitological techniques; faecal eggs count, faecal oocyst count, coproculture and flotation Willis. The farms in Castelar, Villaguay, La Plata, Carlos Keen and Cieneguillas were positive for gastrointestinal nematodes, eggs per gram ranged from 20 to 1800. The genera identified were *Haemonchus*, *Ostertagia*, *Trichostrongylus,* O*esophagostomun, Nematodirus lamae* and cestode. All the farms were positive for coccidian. The gene identified was *Eimeria lamae*.

There is no significant difference between intensive and extensive agriculture systems in Eggs Per Gram (EPG). The Oocysts Per Gram (OPG) in the intensive agriculture is significantly higher than the OPG in the extensive agriculture. The EPG from the province of Entre Rios (Villaguay) and the provencie of Buenos Aires (Castelar, Carlos Keen and La Plata) have significant more eggs than the province of Jujuy (Pucara de Tilcara, Cieneguillas and Santa Catalina). The province of Entre Rios and the province of Buenos Aires have a lot of rain and a low altitude. There is no significant difference in OPG in the four regions.

2. Introduction

2.1 The South American Camelids

There are four species of the South American Camelids (SACs): the domestic alpaca *(Lama pacos*), the llama (*Lama glama*), the wild vicuñas (*Lama vicugno*) and the guanacos (*Lama guanicoe*)[1]. There are about 160.000 llamas living in Argentina.

The social and economic importance of the SACs in the population of the high Andes is mainly due to the climatic and high altitude limitations. If the altitude is more than 3,000 meter it is not possible to develop agriculture, or even farm cattle and sheep in an efficient and profitable way. As a result, the ecological habitat of the Altiplano or Puna Argentina determines the livestock composition of llamas, and in small number sheep and bovines. The camelids are able to convert shortgrass and hard vegetation into meat, fiber and work for the benefit of the highland population, because of their great adaptation to the ecology vegetation. Parasitism is a limiting factor in the production of fiber and meat in South America and losses reach more than 1.5 million dollar annually [2].

The camelidae have some typical distinctive features, namely:

- Absence of horns;
- True canine teeth, separated from the premolars by a gap called diastema, in both the upper and lower jaws;
- The anatomic structure of the rear limbs allows them to bend their legs beneath the body;
- The presence of a toenail on each phalange (instead of a hoof) and a footpad on each foot.
- Like ruminants, they do not have teeth (incisors) in the upper jaw, which is covered by connective tissue (dental pad) [3].

The anatomy of the gastrointestinal tract of the llama is different than those of ruminants. The forestomach of llamas is comprised of three compartments, called C1, C2 and C3. The largest compartment C1 is predominantly involved in fermentation processes. The contents of all parts are maintained with a pH of 6 to 7 [4].

The gastrointestinal system is termed a pseudo-ruminants system. The forestomach protozoa and bacterial fermentation breaks down plant material to digest nutrients. All three compartments have the ability for secretion and absorption. C1 is functionally similar to the rumen of sheep, it can absorb water and volatile fatty acids. Interestingly, C1 motility waves progress in a cranial to caudal direction [5]. One of the most unique aspects of the camelid foregut are the saccules that are found in the first and second compartments. These thin-walled invaginations of the gut wall are formed by the intersection of primary, secondary, tertiary, and sometimes higher order crests and lie in oblong

regions along the ventral portions of C1 and C2. The saccules of C1 are deeper than in C2 and give the appearance of a distinct, regular pattern of mounds when viewed from outside the chamber. In addition, they also have partial diaphragms that restrict the size of their openings on the inside of C1. With each contraction cycle, the saccules of C1 will partially evert and empty their contents into the lumen of the chamber[6]. The saccules are involved in micro-fermentation and secrete bicarbonate directly into the C1 lumen. It increases the resistance of llamas to grain overload. C2 is believed to mimic the reticulum and omasum in ruminant. C2 allows transit of ingesta only after sufficiently small fiber dimention has been achieved by ruminantion. C3 is considered to be the true stomach because it functions similarly to monogastrics and the abomasum in ruminants by secreting hydrogen and chloride. The proximal 80% of C3 is non-acid secreting and the distal 20% is acid secreting. The junction between these two regions is the typical location for ulcers to develop.

There are some other characteristics of the llama gastrointestinal tract. A prominent duodenal ampulla is located immediately outside of the pylorus. Unlike ruminants, camelids do not have a gall bladder. They do have a spiral colon, the proximal loop is dramatically longer than the spiral colon. The omentum is less robust and prominent, it attaches to C1 along its transverse pilar and does not attach the dorsal body wall [5].

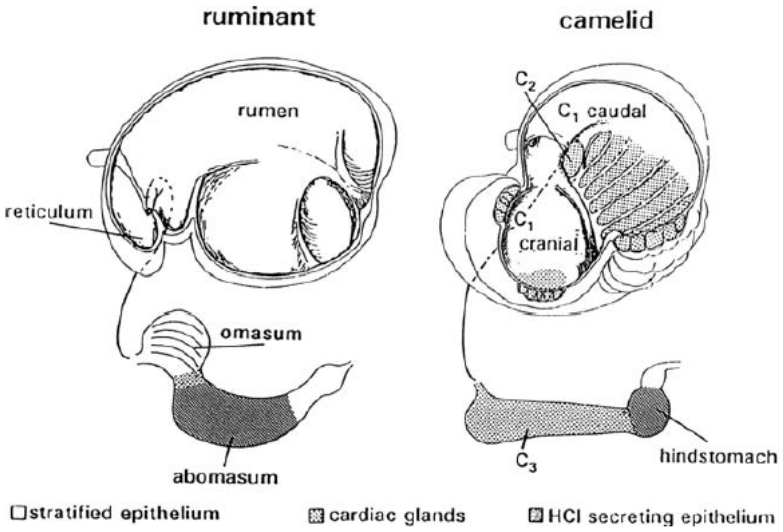

Fig. 2: Anatomy of ruminant and camelid [7]

2.2 Gastrointestinal nematodes

Gastrointestinal nematodes are the most numerous and most detrimental of the llama parasites. Most of them live in the gastrointestinal tract (GI). Many aspects of the GI parasitism are similar, regardless of which species of parasite is involved.

The general direct life cycle for gastrointestinal nematodes is as follows (fig. 2). The female nematodes produces eggs, containing embryos in the morula stage. These eggs are released in the faeces, and the morula matures to become first-stage (L-1) larvae within the egg case. Under ideal conditions, hatching occurs in 1-2 days. The free living L-1 larvae feed on microorganisms in the faeces and molt to L-2 and again to L-3, which is the infective stage, taking 4-6 days. L3 larvae migrate out of the faeces in about 1 week and climb into vegetation. The llama ingests grass containing L-3 larvae, which mature through L-4 and L-5 to become adults in the stomach or the intestine. The prepatent period is about 20 days.

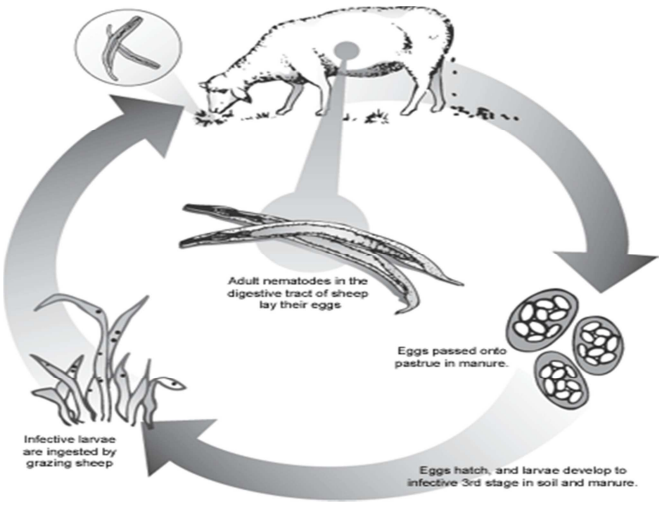

Fig. 3: General direct lifecycle of the gastrointestinal parasites

One of the clinical signs in adult animals infected with GI parasites are less production of milk and a decreased quality of fibre. Most GI parasites produce a hypoalbuminemie, enteritis and will induce changes in the secretory status of the gut. Appetite and utilization of the consumed feed is reduced, depriving the body of vital nutrients. Absorption of calcium and phosphorus is depressed, causing in turn, arrested skeletal development in the young animals. Selenium uptake is also retarded. Young animals are at greatest risk when affected by parasitism because no resistance has been developed to the invading organisms. A cria (a llama between 0 – 1 year old) may be doubly jeopardized because of its own parasite load and lack of nourishment, and as a result of the effect of parasitism on the mother. Emaciation may be seen in longstanding cases, a result of in appetence, leading to

complete anorexic, combined with poor food utilization. In appetence and poor food utilization also inhibits growth en maturation.

Diarrhea is the most prominent sign of enteritis, but diarrhea is not always present in parasitism. Anemia may be seen in heavy infestations, even with parasites that are not bloodsuckers. The cutting mouthparts used for attachment may result in leakage of plasma and cells from capillaries. Death may be caused by overwhelming invasion of an organ of system, but usually parasitism results only in debilitation. The body loses the ability to resist minor infectious agents, and a secondary infection may take the animal's life [2].

Gastrointestinal parasites	Species	Egg size (µm)	Location adults	Location immature
Coccidiose				
Eimeria	*alpacae*	22 - 26 18 - 21	small intestine muscle	
Eimeria	*lamae*	30 - 40 21 - 30	small intestine muscle	
Eimeria	*macusanienis*	81 - 107 61 - 80	small intestine muscle	
Trematode				
Faciola	*hepatica*	130 -150 63 - 90	bilducts	small intestine, peritoneum, liver
Cestode				
Moneizias	*expansa*	56-67	small intestine	small intestine
Nematodes				
Camelostrongylus	*mentulatus*	75 - 85 40-50	C-3	
Haemonchus	*contortus*	70 - 85 41 - 48	C-3	C-1
Graphinema	*aucheniae*	80-90 40-45	C-3	
Lamenema	*chavezi*	150- 170 70-80	small intestine	liver
Oesophagostomum	*columbianium*	73 - 89 34 - 45	small intestine large intestine	
Ostertagi	*ostertagi*	80 - 85 40 - 45	C-3	
Ostertagi	*marshalli*	178- 217 78 - 100	C-3, duodenum	
Nematodirus	*llamae*	160-170 80-90	small intestine	small intestine

Table 1. Most important parasites of the llamas and their characteristics [2,8]

Important gastrointestinal nematodes of llamas

Haemonchus contortus: the predilection site is de C-3 compartment off the stomach. The adults are 2.3-3.0 cm in length. Moreover, the life cycle is direct. The eggs hatch to L_1 on the pasture and may develop to L_3 in a short period (5 days) but development may be delayed for weeks or months under cool conditions. After ingestion, and exsheathment in the C-1 compartment of the stomach, the larvae moult twice in close apposition to the gastric glands. Just before the final moult they develop the piercing lancet which enables them to obtain blood from the mucosal vessels. This is how they can cause an acute haemorrhagic anaemia. This appears two weeks after infection and is characterised by a progressive and dramatic fall in the packed red cell volume. The diagnose is made by faecal cultures for identification or necropsy.

Ostertagia ostertagia: the predilection site is the C-3 compartment of the stomach. The adults are 6.5-7.5 cm in length. There are two types of cycles in this species. The life cycle may vary according to the climate. Type one is typical of a general gastrointestinal direct life cycle. Type two involves developmental arrest in the mucosa of the stomach. Larvae become arrested in early fall and begin development again in winter in northern temperate regions. In southern temperate regions with dry summers and winter rainfall, arrest occurs in late winter and spring, and development begins again in late summer and fall. The cycle in a one area may not be the cycle that will occur in another area and is even less likely to be the cycle in yet a third area or another country. Larvae in the glands of the wall of the stomach stimulate formation of grayish-white nodules. Maturation of previously arrested larvae can cause a build-up of pathogenic adults with heavy egg levels in the faeces and gastritis caused by the adults. The diagnose is made by faecal cultures for identification or necropsy [2].

Trichostrongylus: The predilection side is the stomach and the small intestine. *Trichostrongylus* are small, light, brownish red, hair-like worms and difficult to see with the naked eye. Males measure around 4.0 - 5.5 mm and females 5.5 -7.5 mm in length. The life cycle is direct. Eggs develop to the infective L_3 in about 7 - 10 days under optimal conditions. Following ingestion and exsheathment, larvae penetrate the mucosa of the small intestine and after two moults the fifth stage worms are present under the intestinal epithelium around 2 weeks after initial infection [9]. The diagnose is made by faecal cultures for identification or necropsy. [2]

Lamanema chavezi: This parasite is considered as an important pathogenic nematode present in the small intestine of the SACs. The predilection side is the small intestine. *Lamanema chavezi* is a rather unique trichostrongyloid nematode, because the parasitic third- and fourth-stage larvae undergo an enterohepatic migration. Ingested larvae penetrate the intestinal wall and migrate to the liver and lungs and produces haemorrhages and focal areas of necrosis, resulting in small abscesses that give a

characteristic mottled appearance to the organs. The animals suffer from hepatic and respiratory failure[10]. The diagnose is made by faecal cultures for identification or necropsy[2].

2.3 Pathogenic protozoa of the llama

Coccidia is the most common protozoa of the llama and host specific. The coccidian species identified from llamas are *E. lama*, *E. alpacae* and *E. macusanienis*. *E. lama* and *E. macusanienis* are highly pathogenic. The output of oocysts can decline rapidly in acute infections resulting in clinically affected animals having low oocyst counts. The prepatent period of *Eimeria* spp. in South American Camelids ranges from 10 – 34 days, with *Eimeria macusaniensis* having the longest prepatent period of 33 – 34 days [11].

Species	Size of oocyst (micrometer)	Shape of oocyst
E. alpaca	22 – 26 x 18 - 21	Ellipsoidal
E. lamae	30 – 40 x 21 – 30	Ovoid to ellipsoidal
E. macusaniensis	81 – 107 x 61 – 80	Ovoid

Table 2. Different species *Eimeria* off the llama and their characteristics

Fig. 4 *E. lamae*

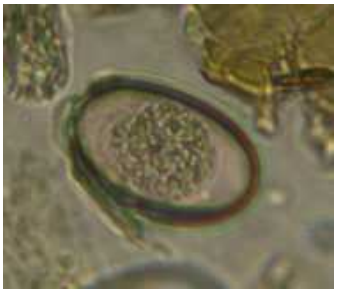

fig.5 *E. alpacae*

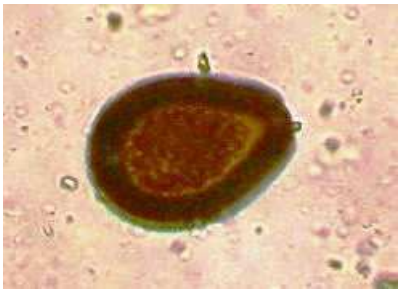

fig.6 *E. macusaniensis*

The life cycle of coccidian includes both sexual and asexual phases. The asexual cycle is called schizogony or merogony. Sporulated oocysts are ingested by the animal and passed along the digestive tract to the small intestine. The oocyst frees sporozoites, which invade the epithelial cells. The sporozoite changes shape and becomes a trophozoite, which, in turn, grows larger and forms a schizont (merzont). Within the schizont, the merozoite form and ultimately rupture the cell and escape to infect other cells. This process may be repeated two or three times. The sexual cycle is called gametogony. The merozoites produced by the last schizogony cycle infect a cell and develop into either male (microgametocyte) or female (microgametocyte) gamonts. The male gamont fertilizes the female gamont while it is still in the cell, producing a zygote. The zygote matures to

become an oocyst, which ruptures from the host cell and is shed in the faeces. The oocyst sporulates in 1 – 2 days to become infective.

Coccidia invade the epithelial mucosa of the small intestine, causing enteritis and diarrhea [2]. Diarrhea is an important cause of morbidity in neonatal llamas and alpacas. The most common pathogens causing diarrhea in neonatal camelids are coronavirus, *Escherichia coli*, *Cryptosporidium spp*., *Giardia spp*. and coccidia. Coccidiosis is typically associated with overcrowding and poor hygiene. The pathogenesis and severity of the clinical signs observed may be associated with the number of coccidian ingested[12].

Pathogen isolated from crias with diarrhea in two different studies

Pathogen	Cebra et al. (2003) ($N=45$)		Data from The Ohio State University (1999–2004) ($N=58$)[a]	
	Cases (%)	Age range (in days)	Cases (%)	Age range (in days)
E. coli	–	–	0	–
Rotavirus	2	210	0	–
Coronavirus	42	10–150	6.9	9–94
Cryptosporidium spp	9	10–45	25.9	7–100
Giardia	18	10–120	32.8	7–120
Coccidia	13	21–60	12.1	21–104
Salmonella	0	–	1.7	45
Nematode ova	2	–	1.7	80
Undetermined cause	–	–	36.2	4–80

[a] This is retrospective data from clinical cases of diarrhea in crias aged less than 4 months at time of diagnosis.

Table 3. Pathogen isolated from crias (llamas till one year) with diarrhea in two different studies[12].

Coccidian infections are generally self-limiting because asexual reproduction is repeated only two or three times. Unless reinfection takes place, only one cycle of development can occur, but in a contaminated environment, reinfection is common and heavy builds up can occur that may kill the host [2]. Immunity to coccidian infection is dependent on exposure. [13].

2.4 Treatment

To treat the animals against gastrointestinal parasites, one can use anti-helmintics such as ivermectin, levamisol and moxidectin. Treatment is only necessary if the EPG is more than 400 eggs. Epidemiologically only 5% of the infection is inside the animal and 95 % is in the paddock. So paddock management changes like rotational grazing are also important [14].

Effective treatment of clinical coccidiosis is achieved using oral sulfadimethoxine at a dose of 15 mg/kg twice daily for 5 days. Amprolium may also be used orally at 10 mg/kg once daily for 5 days. Amprolium inhibits differentiation of merozoites by acting on first generation schizonts in the small intestine. It does not kill the merozoites: these enter the sexual reproductive phase which ultimately produces oocysts. Therefore, an animal treated with amprolium will still excrete viable oocysts.

Clinically affected animals should be isolated and treated. Unaffected animals from the same pen should also be treated since they have been exposed and may be harbouring susceptible coccidian stages. Good management practices and maintenance of hygienic facilities for young animals should be considered as the most important factors in prevention of coccidiosis [12].

2.5 Agriculture systems

Intensive farming or intensive agriculture is an agricultural production system characterized by a high use of inputs such as capital, labour, or heavy use of pesticides and chemical fertilizers relative to land area. Intensive agriculture can involve very large numbers of animals raised on limited land which require large amounts of food, water and medical inputs. Extensive farming or extensive agriculture is an agricultural production system that is the opposite of intensive agriculture characterized by small inputs of labour, fertilizers, and capital. Extensive agriculture can involve very large numbers of animals raised on a very big area of land which require less amounts of food. The difference between animal agriculture systems is complex. They are composed of, and are influenced by, interactions among, biological, climatic, economic, social and cultural factors.[15]

2.6 This study

Many reports have been published of studies on gastrointestinal nematode and coccidiosis but there are fewer reports about llamas. This study describes the presence of nematode and coccidian in llamas *(Lama glama)* from four different geographical regions in Argentina: province of Buenos Aires, province of Entre Ríos, and the valley and the Puna in the province of Jujuy. The study also compares the presence of gastrointestinal parasites, coccidian and difference in species between llamas from extensive and intensive agriculture systems.

3. Materials and methods

This experiment was performed in the laboratory of Parasitology of the Pathobiology Institute of the National Institute of Agricultural Technology (CICVyA INTA Castelar, Buenos Aires, Argentina). The research was done during the period of 1st September 2012 to 30 th November 2012.

3.1 Study locations

The provinces sampled were: Jujuy (Pucara de Tilcara (valley), Cieneguillas (Puna) and Santa Catalina (Puna)), Buenos Aires (Castelar, Carlos Keen and La Plata) and Entre Ríos (Villaguay). In these farms, the llamas are kept under intensive (Castelar and Pucara de Tilcara) and extensive (Villaguay, La Plata, Cieneguillas, Carlos Keen and Santa Catalina) agriculture farming systems.

3.2 Study populations

Castelar (Buenos Aires), intensive housing system. n: 7 animals.

Pucara de Tilcara (Jujuy valley), intensive housing system. n: 9 animals.

Carlos Keen (Buenos Aires), extensive housing system. n: 6 animals

Cieneguillas (Jujuy Puna), extensive housing system. n: 22 animals.

Villaguay (Entre Ríos), extensive housing system. n: 7 animals.

La Plata (Buenos Aires), extensive housing system. n: 17 animals

Santa Catalina (Jujuy Puna), extensive housing system. n: 12 animals.

3.3 Sampling

Individual faecal samples were taken directly from the rectum of the llamas (appendix 9.1) and processed by parasitological techniques as described in appendix 9.2, 9.3 and 9.4.

3.4 Parasitological procedures

- Fecal eggs count per gram (EPG) by the McMaster method to determine quantitative the number of eggs on individual animal (appendix 9.2);
- Oocyst count per gram (OPG) by the McMaster method to determine quantitative the number of oocyst on individual animal (appendix 9.2);
- Coproculture (Corticelli y Lai's Techniques) is a qualitative method for larvae identification to determine which genus is present in the faeces of a group of animals (appendix 9.3). Identification was done by their characteristics and fig. 17;
- Willis is a qualitative flotation method to determine which species of coccidian are present in the faeces of a group of animals (appendix 9.4). Identification was done by using table 2 and fig. 3, 4 and 5.

A farm was called positive when a single positive animal was detected.

3.5 Measurements

The information about the climatic measurements is given by the Climatic and Agrometeorologic Department of INTA Castelar.

3.5.1. Climatic measurements

The weather conditions (see figures 12, 13 and 14) are given for a period of 6 weeks, 6 weeks in advance off the sampling day

Buenos Aires: the province of Buenos Aires has four seasons; in the period of the research it was winter and spring.

Castelar: the altitude is 29 metres above sea level. The rainfall was 138.4 mm. The average temperature at daytime was 19.9 Celcius degrees () (12.7 - 28.4 ºC) and at nighttime it was 9.3 ºC (0.8 - 19.2 ºC).

Carlos Keen: the altitude is 111 metres above sea level. The rainfall was 206.8 mm. The average temperature at daytime was 20.6 ºC (13.4 - 26.8 ºC) and at nighttime it was 9.8 ºC (1.5 - 17.1 ºC).

La Plata: the altitude is 24 metres above sea level. The rainfall was 322.0 mm. The average temperature at daytime was 17.8 ºC (11.0 – 26.0 ºC).

Jujuy: The province of Jujuy has a dry and a wet season; in the period of the research it was the dry season.

Pucara de Tilcara: the altitude is 2500 metres above sea level. The rainfall was 128 mm. The average temperature at daytime was 27.0 ºC (16.0 - 37.0 ºC) and at nighttime it was 14.0 ºC (4.0 - 19.0 ºC).

Cieneguillas: Cieneguillas is located on the Puna. The altitude is 3700 metres above sea level. The rainfall was 75 mm. The average temperature at daytime was 19.0 ºC (17.0 - 24.0 ºC) and at nighttime it was 3.0 ºC (-1.0 - 6.0 ºC).

Santa Catalina: Santa Catalina is located on the Puna. The altitude of Santa Catalina is 4000 metres above sea level. The rainfall was 75 mm. The average temperature at daytime was 19.0 ºC (17.0 - 24.0 ºC) and at nighttime it was 3.0 ºC (-1.0 - 6.0 ºC).

Entre Rios:

Villaguay: The province of Entre Rios has a subtropical area in the north and a four seasons area in the centre and the south. Villaguay is in the centre of Entre Rios. The altitude is 43 metres above sea level. The rainfall was 181,5 mm. The average temperature in the period at daytime was 23.5 ºC (14.9 - 34.0 ºC) and at nighttime it was 10.0 ºC (-1.8 – 18.9 ºC).

3.5.2. Animal measurements

Buenos Aires

Castelar: the herd consists of 7 animals, six of them are more than two years old. They are all males, only one is castrated. The last time they were treated with anti-helmintics was more than a year ago. At the same farm there are cows, sheep's, dogs and a horse.

Carlos Keen: the herd consist of 6 animals. One of them is a cria, the other llamas are more than two years old. They have two intact males, one castrated male and tree female llamas. They have never been treated with anti-helmintics. At the same farm there are also gauzes and dogs.

La Plata: the herd consist of 120 animals from different genders and ages. The last time they were treated with anti-helmintics was in May 2012. In the same farm there is a big herd of cattle, horses and dogs.

Jujuy

Pucara de Tilcara: the herd consist of 12 animals. One is a cria and the rest is more than 2 years old. Two of them are male and the others are female. The last time they were treated with anti-helmintic was more than a year ago. In the paddock next to the llamas lives a castrated male guanaco (7 years old), there are also dogs living in the same area.

Cieneguillas: the herd consist of 800 animals from different genders and ages. The last time they were treated with anti-helmintics was in July 2012. There are also vicuñas, foxs and sheeps living in the same area.

Santa Catalina: the herd consist of 1600 animals from different genders and ages. The last time they were treated with anti-helmintics was in May 2012. There are also wild vicuñas and donkeys living in the same area.

Entre Rios

Villaguay: the herd consist of 80 animals from different genders and ages. The last time they were treated with anti-helmintics was in May 2012.

4. Results

4.1 Faecal egg counts and oocyst per gram in intensive agriculture system

Castelar

#	EPG	OPG
1	40	420
2	320	400
3	380	360
4	0	360
5	40	180
6	60	80
7	80	440
observations	*Nematodirus Lamae*	

Table 4. Results EPG and OPG per gram of faeces

Pucara de Tilcara

#	EPG	OPG
1	0	120
2	0	0
3	0	140
4	0	400
5	0	80
6	0	0
7	0	240
8	0	120
9	0	0

Table 5. Results EPG and OPG per gram of faeces

4.2 Faecal egg counts and oocyst per gram in extensive agriculture system

Carlos Keen

#	EPG	OPG
1	140	240
2	80	140
3	180	60
4	0	0
5	20	80
6	0	0

Table 6. Results EPG and OPG per gram of faeces

Cieneguillas

#	EPG	OPG
1	0	560
2	0	40
3	0	0
4	0	120
5	0	80
6	0	100
7	0	340
8	0	120
9	20	20
10	20	420
11	0	160
12	20	80
13	0	0
14	140	0
15	0	0
16	0	60
17	40	40
18	20	400
19	20	60
20	0	160
21	20	200
22	0	240
Observations	Cestode	

Table 7. Results EPG and OPG per gram of faeces

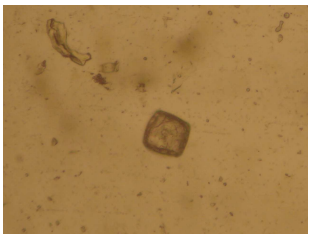

Fig. 7: Cestode in faeces

Villaguay

#	EPG	OPG
1	0	20
2	480	0
3	0	20
4	200	480
5	240	0
6	1800	0
7	80	0

Table 8. Results EPG and OPG per gram of faeces

La Plata

#	EPG	OPG
1	120	120
2	0	60
3	20	60
4	220	0
5	240	0
6	320	0
7	0	100
8	40	80
9	40	120
10	80	60
11	400	0
12	0	0
13	40	80
14	0	20
15	340	0
16	40	60
17	20	20

Table 9. Results EPG and OPG per gram of faeces

Santa Catalina

#	EPG	OPG
1	0	0
2	0	180
3	0	1000
4	0	400
5	0	0
6	0	160
7	0	0
8	0	400
9	0	0
10	0	160
11	0	160
12	0	240
Observations	Cestode	

Table 10. Results from EPG and OPG per gram of faeces

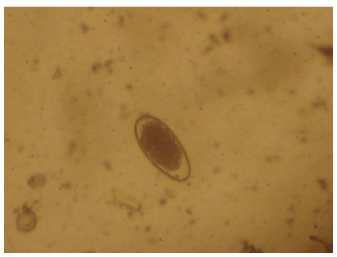

Fig. 8: Gastrointestinal nematode in faeces

4.3 Coproculture

Location	*Haemonchus*	*Ostertagia*	*Trichostronylus*	*Oesophagostomun*
Intensive				
Castelar	*	*	*	*
Pucara de Tilcara	*	*	*	*
Extensive				
Carlos Keen	*	*	*	*
Cienequillas	*	*	*	*
Villaquay	78%	15%	7%	-
La Plata	60%	17%	10%	13%
Santa Catalina	*	*	*	*

Table 11. Species of nematodes in percentages found by coproculture technic on different locations
* No larvae found

4.4 Flotation technic of Willis

Location	Results Willis
Intensive	
Castelar	*Eimeria lamae*
Pucara de Tilcara	*Eimeria lamae*
Extensive	
Carlos Keen	*Eimeria lamae*
Cienequilla	*Eimeria lamae*
Villaquay	*Eimeria lamae*
La Plata	*Eimeria lamae*
Santa Catalina	*Eimeria lamae*

Table 12. Species of *Eimeria* found by Willis technic on different locations

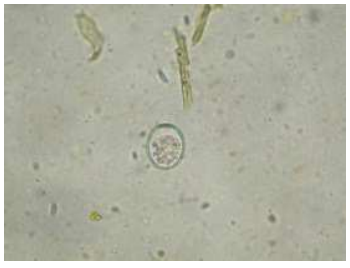

Fig. 9: *E. lamae* in faeces

4.5 Statistic analyse

Statistical significance was considered when $P < 0.05$. All estimations were done by using the Statistix statistical package.

Intensive and extensive agriculture systems:

Group A intensive: Castelar and Pucara de Tilcara N:16

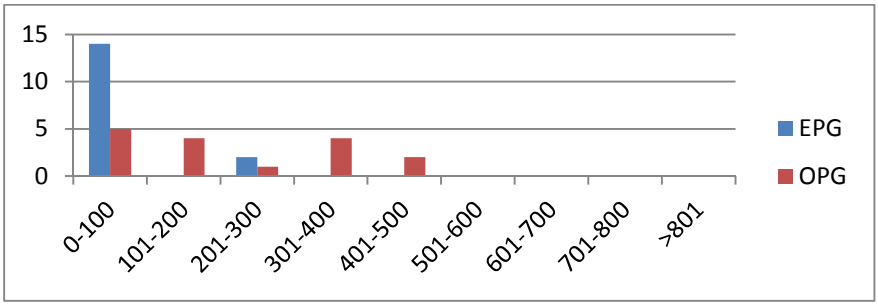

Fig. 10: Number of EPG and OPG in faeces of llamas in intensive agriculture farming systems

Descriptive Statistics for group A N:16,

test	mean	SD	minimum	maximum	median
EPG	57.500	117.50	0.000	380.0	0.000
OPG	208.75	163.95	0.000	440.0	160.00

Table 13. Descriptive statistics for group A (intensive agriculture)

Group B extensive: La Plata, Carlos Keen, Villaguay, Cieneguillas and Santa Catalina N:64

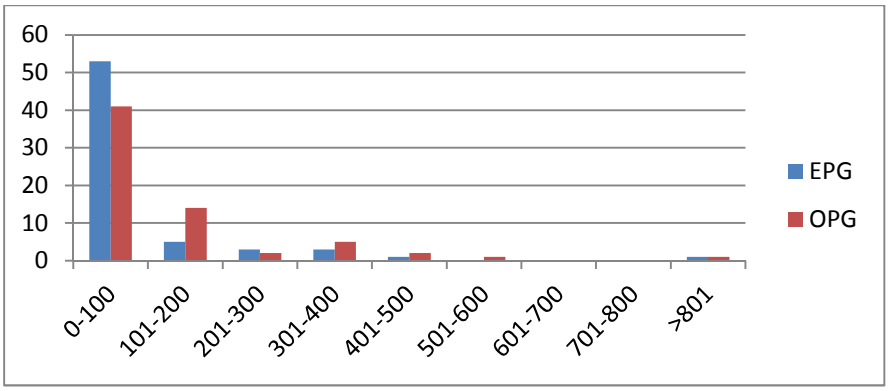

Fig. 11: Number of EPG and OPG in faeces of llamas in extensive agriculture farming systems

Descriptive Statistics for group B N:64

test	mean	SD	minimum	maximum	median
EPG	85.000	242.55	0.00	1800.00	0.00
OPG	120.63	174.46	0.00	1000.00	60.00

Table 14. Descriptive statistics for group A (extensive agriculture)

Difference between intensive agriculture and extensive agriculture in EPG

The EPG is not normally distributed (Shapiro Wilk test, p value=0.000), so a non-parametric test for comparing 2 groups must be applied: Wilcoxon Rank Sum Test.

Wilcoxon Rank Sum Test for EPG by group:

Group	Rank Sum	N	U stat	Mean Rank
A	615.00	16	479.00	38.4
B	2625.0	64	545.00	41.0
total	3240.0	80		

Table 15. Result of Wilcoxon Rank Sum Test for EPG

Normal Approximation with Corrections for Continuity and Ties	0.429
Two-tailed P-value for Normal Approximation	0.6681

This p value means that the EPG in the intensive agriculture is not different from the EPG in the extensive agriculture.

Difference between intensive and extensive agriculture in OPG

The OPG is not normally distributed (Shapiro Wilk test, p value=0.000), so a non-parametric test for comparing 2 groups must be applied: Wilcoxon Rank Sum Test.

Wilcoxon Rank Sum Test for OPG by Group

Group	Rank Sum	N	U stat	Mean Rank
A	829.00	16	693.00	51.8
B	2411.0	64	331.00	37.7
Total	3240.0	80		

Table 16. Result of Wilcoxon Rank Sum Test for OPG

Normal Approximation with Corrections for Continuity and Ties	2.200
Two-tailed P-value for Normal Approximation	0.0278

This p value means that the OPG in the intensive agriculture is different from the extensive agriculture.

Climatic conditions in different regions:

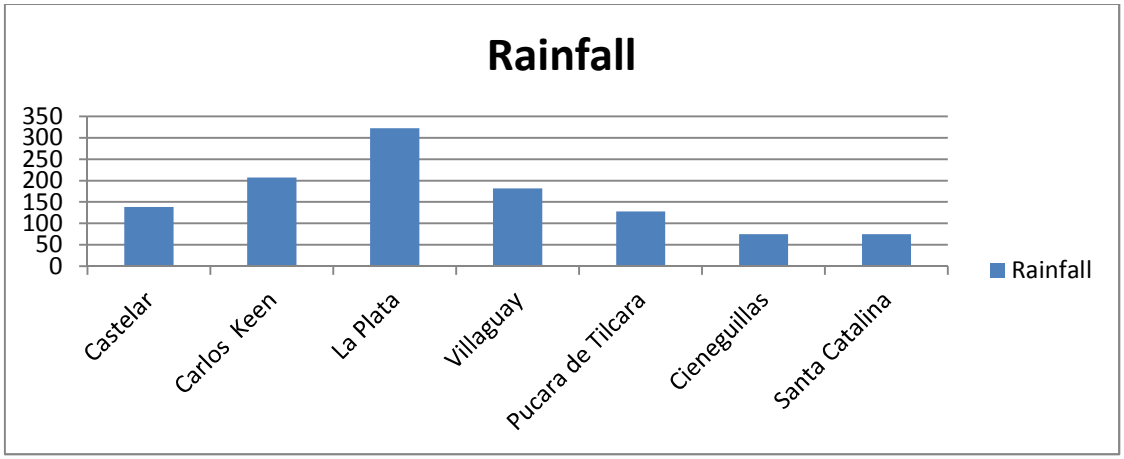

Fig. 12: Total rainfall in mm in a period of 6 weeks in advance of the sampling day on all the sampled farms

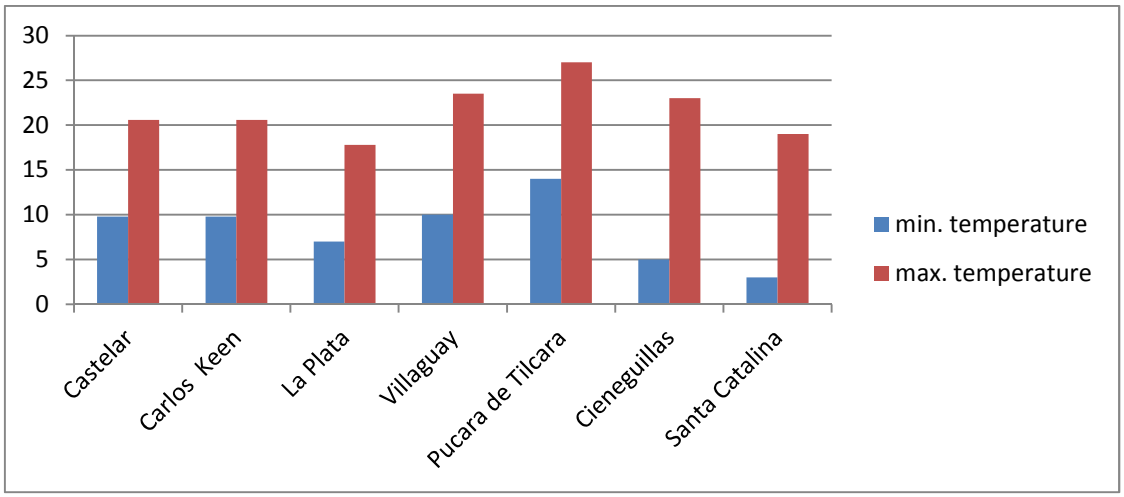

Fig. 13: Minimum and maximum temperatures in degrees Celcius in a period of 6 weeks in advance of the sampling day on all the sampled farms

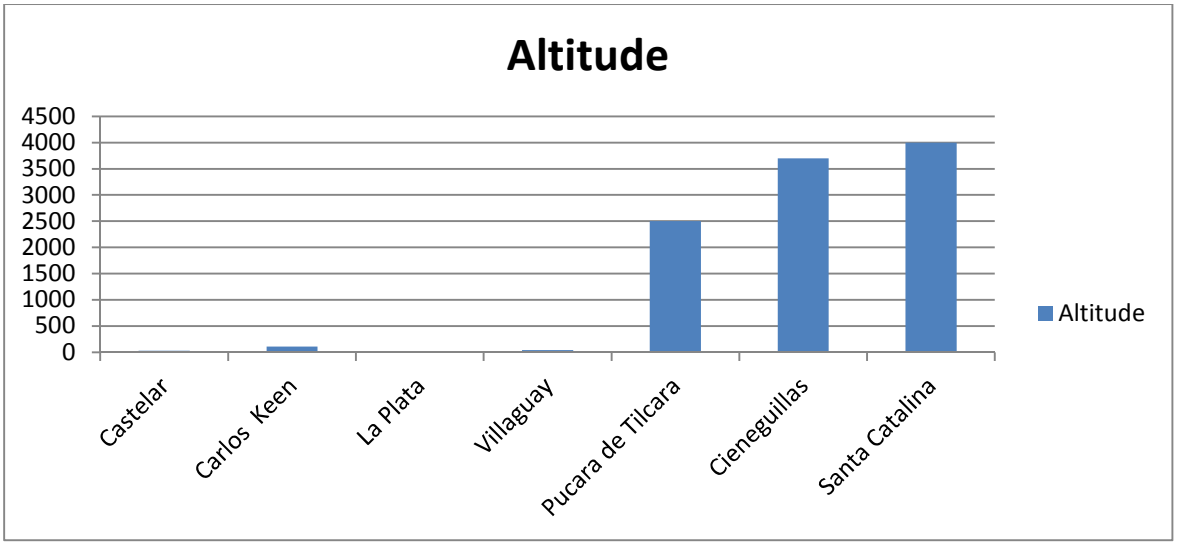

Fig. 14: Altitudes in metres on all the sampled farms

Groups divided by climatic en geographic regions

Group 1: Province of Buenos Aires: Castelar, La Plata and Carlos Keen

Descriptive Statistics for group 1, N:30

test	mean	SD	minimum	maximum	median
EPG	108.67	128.19	0.00	400.00	40.00
OPG	118.00	139.49	0.00	440.00	70.00

Table 17. Descriptive statistics for group 1

Group 2: Province of Entre Rios: Villaguay

Descriptive Statistics for group 2, N:7

test	mean	SD	minimum	maximum	median
EPG	400.00	639.58	0.00	1800.00	200.00
OPG	74.29	179.15	0.00	480.00	0.00

Table 18. Descriptive statistics for group 2

Group 3: Province of Jujuy in the valley: Pucara de Tilcara

Descriptive Statistics for group 3, N:9

test	mean	SD	minimum	maximum	median
EPG	0.00	0.00	0.00	0.00	0.00
OPG	122.22	131.32	0.00	400.00	120.00

Table 19. Descriptive statistics for group 3

Group 4: Province of Jujuy in the Puna: Cieneguillas and Santa Catalina

Descriptive Statistics for group 4, N:34

test	mean	SD	minimum	maximum	median
EPG	8.82	25.20	0.00	140.00	0.00
OPG	173.53	208.88	0.00	1000.00	120.00

Table 20. Descriptive statistics for group 4

The EPG and OPG is not normally distributed (Shapiro Wilk test, p value=0.000), so a non- parametric test for comparing more than 2 groups must be applied: Kruskal Wallis.

Kruskal-Wallis One-Way Nonparametric AOV for EPG by group

Group	Mean Rank	Sample size
1	54.1	30
2	58.6	7
3	22.5	9
4	29.5	34
total	40.5	80

Table 21. Result of Kruskal-Wallis One-Way Nonparametric AOV for EPG

Kruskal-Wallis Statistic	33.1432
P-Value, Using Chi-Squared Approximation	0.0000

This p value means that the EPG is different in at least one region.

Kruskal-Wallis All-Pairwise Comparisons Test of EPG by group

Group	Mean	Homogeneous Groups
2	58.571	A
1	54.133	A
4	29.515	B
3	22.500	B

Table 22. Results of Kruskal-Wallis All-Pairwise Comparison Test for EPG

This p value means, the province of Buenos Aires and Entre Rios are significant different from the both regions in the province of Jujuy.

Kruskal-Wallis One-Way Nonparametric AOV for OPG by group

Group	Mean Rank	Sample Size
1	38.9	30
2	25.4	7
3	40.7	9
4	45.0	34
total	40.5	80

Table 23. Result of Kruskal-Wallis One-Way Nonparametric AOV for OPG

Kruskal-Wallis Statistic	4.4747
P-Value, Using Chi-Squared Approximation	0.2146

This p value means that the OPG is not different between the regions

5. Conclusion

The farms in Castelar, La Plata, Carlos Keen, Villaguay, and Cienequillas were positive for gastrointestinal nematodes, egg per gram (EPG) ranged from 0 to 1800. The genera identified were *Haemonchus*, *Ostertagia*, *Trichostrongylus,* O*esophagostomun, Nematodirus llamae* and cestode. There is no significant difference in EPG between the intensive and extensive agriculture farms. The EPG from the province of Entre Rios (Villaguay) and the province of Buenos Aires (Castelar, Carlos Keen and La Plata) have significant more eggs than the province of Jujuy (Pucara de Tilcara, Cieneguillas and Santa Catalina). The province of Entre Rios and Buenos Aires have a lot of rain and a low altitude.

All the farms were positive for coccidian, oocysts per gram (OPG) ranged from 0 to 1000.The gene identified was *Eimeria lamae*. The OPG in the intensive agriculture is significantly higher than the OPG in the extensive agriculture. There is no significant difference in the four regions in OPG.

6. Discussion

For this study the samples were taken from different farms. These farms used different drugs against nematodes and in different time schedules. This is one of the circumstances that was not equal. For a scientific study it is better to try to get the same anti-helmintic treatment on all farms. If the treatment is more than three months ago, the differences are not due to their anti-helmintic treatment.

The groups had a small number of animals. Because this study only took three months there was not enough time to sample and process more faeces samples. The statistical results will be of more value if there are more samples and the results would be more accurate and reliable.

Cafune M. (2009), found a positive EPG in South American camelids of Northwest Argentina. She has taken the faeces samples in the same region as in this report. In Cafune M. (2009) the samples were taken in December 2004 and May 2009. In this report no eggs were found, this can be because the weather conditions. The samples of this study are taken just after the coldest period of the year. Moyo D.Z. (1996) examined the faeces of cattle on the highfield on 1,200 metres in different seasons in the year in Zimbabwe. The altitude and climate of Zimbabwe is almost equal to the valley and the Puna of Argentina. In Zimbabwe the results showed that the numbers of larvae on pasture increased during the rainy season but decreased at the end of this season. The pasture larval counts decreased to virtually zero levels from July to October. Kaufmann J. (1990) saw the same in Gambia (fig. 15). The samples from Pucara de Tilcara, Cieneguillas and Santa Catalina are almost zero in October. The weather conditions may be an explanation for the difference in EPG between the province of Buenos Aires, Entre Rios and the province of Jujuy. More research must be done in the north of Argentina to know the cause of differences in the EPG count between this report and the results in the article of Cafune M. (2009).

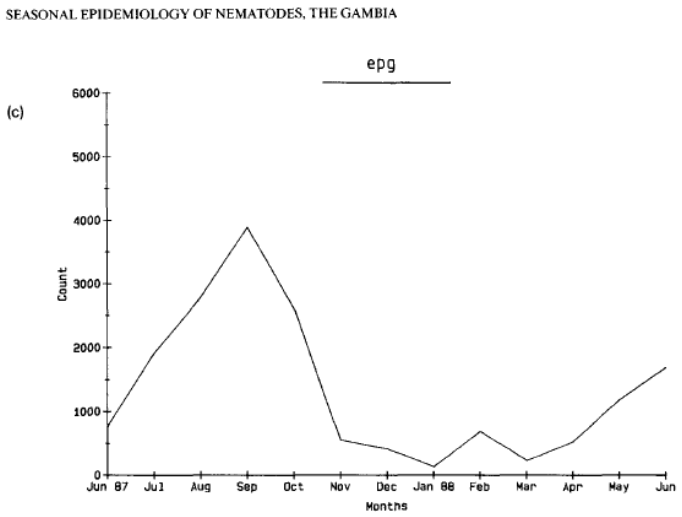

Fig. 15: The EPG results of nematodes in highfield of Gambia [16] .

Testing the faeces by EPG for gastrointestinal eggs, as is done in this study is important to determine the need of anti-helmintic use. It is very important that anti-helmintic drugs are not used if there is no necessity. Where anthelmintic drugs have always been used as the sole means to control the parasitic infections, these drugs are now failing due to the emergence of resistant strains of helminths. Anti-helmintic resistance is now an important issue in most countries of the world. Frequent use of anti-helmintics is probably the greatest predisposing factor and hence resistance has arisen quickest in the warmer countries where parasite turnover is greatest and anti-helmintics needs to be used more often. Wanyangu S. (1996) showed that resistance is now known to exist to all the major classes of broad-spectrum anthelmintics[17]. Gillespie R. (2010) compares three anti-helmintic treatments: ivermectin, moxidectin and levamisol in llamas and alpacas. This study confirms that multiple anti-helmintic resistance is an emerging problem in the United States. Llama gastrointestinal nematodes were highly resistant to drugs from both the avermectin/milbimycin (IVM) and benzimidazole (FBZ) anti-helmintic families, and there was also resistance to moxidectin (MOX) on one of the two llama farms in which this drug was tested. Levamisol, in the imidothiazole/tetrahydropyrimine group, was still effective on the one farm where it was tested [18]. Besides faecal egg count are management systems also very important. Bailey J. (2009) has confirmed the ability of an epidemiologically based grazing management approach (SGSR) and alternate grazing with cattle to significantly reduce pasture infectivity, increase production and reduce anti-helmintic treatment in this environment. Significantly, both methods were most effective in reducing pasture contamination with *H. contortus* and *Trichostrongylus spp*. The same parasites as we saw in this study.

No larvae identification with the Corticelli y Lai technic was possible with the faeces of Pucara de Tilcara, Cieneguillas, Santa Catalina and Carlos Keen because none or fewer eggs were found in the EPG.

Schrey C.(1991) found three species of *Eimeria* in llamas *(lama glama); E. alpacae, E. lamae* and *E. macusaniensis* in Colorado and Wyoming in the USA. In this study only *E. lamae* was found. Cafune M. (2009) found in the north of Argentina (Jujuy) except *E. lamae* also *E. macusaniensis,* using the same flotation technic. She found 88.3% of the llamas (N:626) positive for *E. macusaniensis.* The samples of that report are taken in December and May 2009 on the same locations as this report, this is before the cold season starts. In this study the samples are taken in October, after the coldest period. Although the weather conditions are different in the regions there is no difference in OPG between the regions. This suggests that the oocysts survive in different weather conditions. The

Willis technic should be re-taken during the whole year to find the explanation for the differences in the results between this study and the study of Cafrune M.

On the intensive agriculture farms the OPG is higher than on the extensive agriculture farms. Although the number of oocyst on extensive agriculture farms was not high, this does not mean they can't suffer from coccidiosis. Munashe Chigerwe M. (2003) report that there are several cases of camelids with negative OPG who died of coccidiosis, with the symptoms of anorexie, diarrhea, hypoproteinemia (4.2 g/dl; reference range, 4.7–7.3 g/dl), hypoalbuminemia (1.6 g/dl; reference range, 2.9–5 g/dl5) and an elevated aspartate aminotransferase (AST) activity (634 U/L; reference range, 128–450 U/L5). When post mortem section of ileum and jejunum was preformed, numerous coccidian organisms were found in the lamina propria in various stages of development along with large numbers of eosinophils. The absence of coccidial oocysts on routine faecal flotation examination can be explained because of an incomplete prepatent period or the use of an insensitive faecal flotation technique. The prepatent period of *Eimeria* spp. in South American Camelids ranges from 10 – 34 days, with *Eimeria macusaniensis* having the longest prepatent period of 33 – 34 days [19]. Ruminants develop an immune response to infections with coccidian and are therefore less likely to develop clinical disease in adulthood. It is unknown whether this occurs in South American camelids [2].

No statistical difference between intensive and extensive agriculture in EPG is found. Although Almeria S. (2009) compared the prevalence of *Ostertagia ostertagia* by indirect ELISA in milk samples in cattle on an intensive and extensive agriculture farm in Spain. Almeria S. (2009) found both average individual *O. ostertagi* optical density ratios (ODR) values and bulk tank ODR values were significantly higher in Minorca (extensive agriculture) herds compared to Girona (intensive agriculture) herds ($P < 0.001$ and $P < 0.05$, espectively). The low number of faeces samples from the farms in Argentina and the many zeros influence the statistics. Further research has to be done with more animals in each group.

This research has added new insights on gastrointestinal nematodes and coccidian in llamas in Argentina. Future research has to give more information about:

- The surviving of gastrointestinal eggs and oocysts in Argentina at different altitudes and under extreme weather conditions during the whole year;
- The best invasive technic to diagnose coccidiosis;
- The immune response of llamas against different *Eimeria* species.

7. Acknowledgement

First I want to thank dhr. M. Pieterse for arranging and coordinating the research project from the University Utrecht.

But mostly I want to thank all the people from the Pathobiologic Institute of INTA Castelar for all the help with the project and the warm hospitality during these 3 months. My specials regards are for Gisela Marcoppido, Javier Schapiro, Diego Franco and Maria Ceron. I'm grateful to Gisela for giving us the opportunity to come to Argentina, for all the care, hospitality and opportunities to see so many of Argentina and the South American culture. It was really nice to see how the people in Argentina work and enjoy live. Thanks for all the work and kindness Javier Schapiro gave to me. His effort to make this project a success made that it was very instructive to me. Thanks to Diego Franco for all the hard work with handling the llamas, the friendliness and our visits to "El Campo". Last but not least I want to thank Maria Ceron for all the effort she did for Anna and me.

I had a very nice time working, living and exploring Argentina!!

8. References

1. Cafrune MM, Marín RE, Rigalt FA, Romero SR, Aguirre DH. Lamanema chavezi (nematoda: Molineidae): Epidemiological data of the infection in south american camelids of northwest argentina. Vet Parasitol. 2009;166(3–4):321-325.

2. Fowler, Murray E. Medicine and surgery of camelids. third ed. Iowa: wiley-blackwell; 2010.

3. Lamo DA. South american camelids in argentina, history, use and animal health. jan. 2011.

4. Van Hoogmoed L, Harmon F, Snyder J. Microvascular anatomy of the third compartment of the stomach of llamas. American Journey of Veterinary Research. 2003;March(64 (3)):346-347;348;349;350.

5. Newman KD, Anderson DE. Gastrointestinal surgery in alpacas and llamas. Veterinary Clinics of North America: Food Animal Practice. 2009;25(2):495-506.

6. Rock C. Form and function of the camelid digestive system. The Camelid Quarterly. 2005.

7. Jouany J-. The digestion in camelids ; a comparison to ruminants. Productions Animales. 2000;13(3):165-176. Accessed 20 September 2012.

8. Rehbein RE. Estudio del parasitismo gastrointestinal en llamas (lama glama), en un predio en la IX región de chile. . 1998.

9. Taylor MA, Coop RL, Wall RL, eds. Veterinary parasitology. ; 2007.

10. Cafrune MM, Aguirre DH, Rickard LG. First report of lamanema chavezi (nematoda: Trichostrongyloidea) in llamas (lama glama) from argentina. Vet Parasitol. 2001;97(2):165-168.

11. Schrey CF, Abbott TA, Ann Stewart V, Marquardt WC. Coccidia of the llama, lama glama, in colorado and wyoming. Vet Parasitol. 1991;40(1–2):21-28.

12. Whitehead CE. Neonatal diseases in llamas and alpacas. Veterinary Clinics of North America: Food Animal Practice. 2009;25(2):367-384.

13. Schock A, Bidewell CA, Duff JP, Scholes SF, Higgins RJ. Coccidiosis in british alpacas (vicugna pacos). The Veterinairy Record. 2007;Jun 9(160(23)):805-806.

14. Borgsteede FHM, Sol J, van Uum A, de Haan N, Huyben R, Sampimon O. Management practices and use of anthelmintics on dairy cattle farms in the netherlands: Results of a questionnaire survey. Vet Parasitol. 1998;78(1):23-36.

15. Meek AH, Martin SW, Stone JB, McMillan I, Britney JB, Grive DG. The relationship among current management systems, production, disease and drug usage on ontario dairy farms.. Canadian Journal of Veterinary Research. 1986;50((1)).

16. Kaufmann J, Pfister K. The seasonal epidemiology of gastrointestinal nematodes in N'dama cattle in the gambia. Vet Parasitol. 1990;37(1):45-54.

17. Wanyangu SW, Bain RK, Rugutt MK, Nginyi JM, Mugambi JM. Anthelmintic resistance amongst sheep and goats in kenya. Prev Vet Med. 1996;25(3–4):285-290.

18. Gillespie RM, Williamson LH, Terrill TH, Kaplan RM. Efficacy of anthelmintics on south american camelid (llama and alpaca) farms in georgia. Vet Parasitol. 2010;172(1–2):168-171.

19. Chigerwe M, Middleton JR, Williams F, Tyler JW, Kreeger JM. Atypical coccidiose in south american camelids. Journey of Veterinairy Diagnostic Investigation. 2003;19(1):122--125.

20. Roberts FHS, O'Sullivan PJ. Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. Australian Journal of Agricultural Research. 1950(1):99-102.

21. Niec R. Cultivo e identificación de larvas infectantes de nematodes gastro-intestinales del bovino y ovino. 1968.

9. Appendix

9.1 Sampling

Sampling of faeces in camelids for coproparasitologic diagnostics: EPG, OPG and coprocultures.

a) The faeces have to be extracted from the rectum with gloves and put in a polyetyleen bag;

Quantities: 10 - 20 dung balls of one camelid

b) Put the sample in the plastic bag and remove all the air, close the plastic bag;

c) Write down the identification number of the animal on the plastic bag;

d) Sent the samples refrigerated, it is possible to use a styrofoam box with cooling elements;

DON`T USE CHEMICAL PRESERVATIVE (formaline).

9.2 Technic of faecal egg count and faecal oocyst count

Eggs Per Gram (EPG) and Oocyst Per Gram (OPG) is a technique to count the eggs of gastrointestinal nematode and oocyst in grams in faecal material of individual animals.

Use the McMaster technique.[20]

Camelids:

a) Place 5 g of material faeces in the mortar, mix the sample with 100 ml solucion off NaCL (soluction 1:60, density 1200);

b) Put the mixture in a beaker and put it on a magnetic mixer;

c) Use a Pasteur pipet to take under stirring, some of the contents and fill the counting chambers, do it with precaution to prevent air bubbles in the chambers. There are 4 counting chambers with a capacity of 0.5 ml. each. In total there is a volume of 2 ml. on the McMaster chamber.

d) Leave it for a couple of minutes and go to the microscope to start reading. Count the amount of eggs in the 4 chambers and multiply with factor 10 to express the result in eggs per gram faeces. If you count 2 chambers, you have to multiply the amount of eggs with factor 20 to express the result in eggs per gram faeces.

Fig. 16: filling the McMaster chambers

9.3 Technic of coproculture and larvae identification

Corticelli - Lai is a technique for the larvae identification in faeces of a group animals.

CAMELIDS:

a) Put the faeces in a molter together with vermiculite 1:1 (mineral that contains iron and magnesium and avoids compaction and makes space for the larvae to move freely)
b) Mix the vermiculite and faeces together with the molter
c) The mixture has to be put in a petrie dish of 10 cm.
d) De petri dish with sample has to put open in a petridish of 15 cm.
e) Aqua destila or normal aqua without cloride has to be in de petridish of 15 cm, it has to be filled till 1 cm. then close the big petridish. (veel slordigheid foutjes de appendix zelf nog even doorlezen en zorgen dat je consequent bent.)
f) Put it in the humid camber for 10 days on 27 degrees Celsius
g) Uncover daily the big petridish to get some oxygen to the sample.
h) After 10 days the small petri dish have to be turned, into the water.
i) The petridish has to be in the water??? for 12 hours out of the humid chamber, the cover of the grand petridish has to be taken off to give it oxygen.
j) After 12 hours you can watch the larvae in the water under the microscope. Identification is done with figure 17.

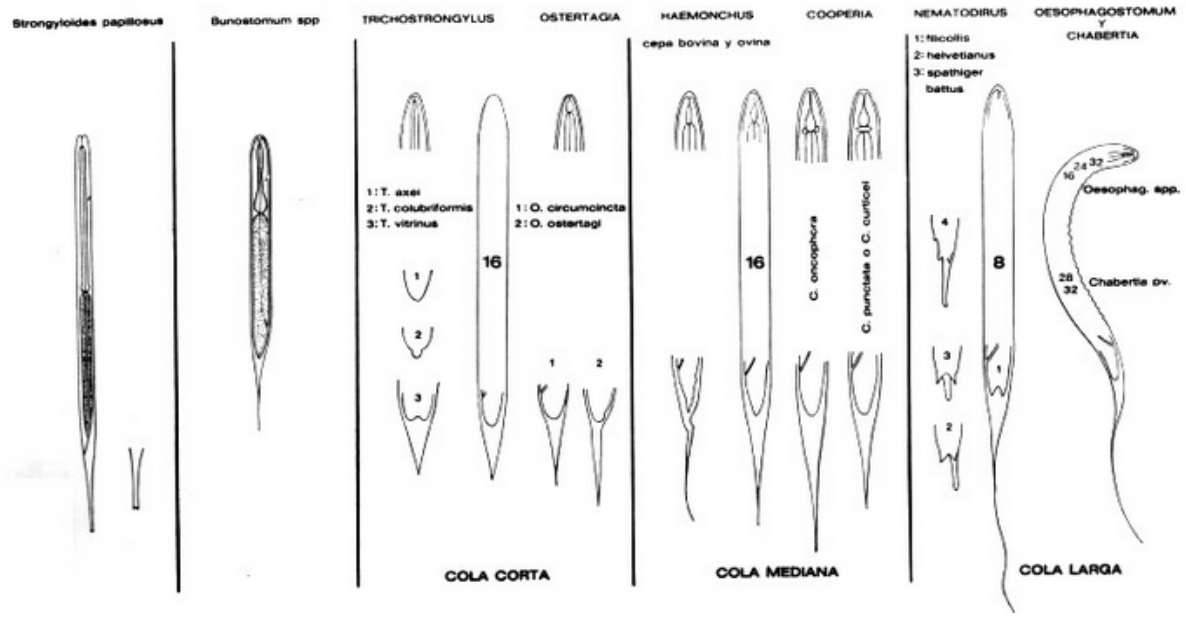

Fig. 17: Characteristics off gastrointestinal nematode larvae[21].

9.4 Appendix Flotation technic Willis

This flotation method is qualitative and is used to diagnose nematodes eggs and oocyst of coccidian.

a) Take 5 g. of faeces to put in a mortar together with un solution of NaCl (1:1);
b) Filter through a common sieve;
c) Put 20 ml in a Willis tube;
d) Put a slide on the Willis tube and let it stand for 30 minutes;
e) Invert the slide and observe it under the microscope.

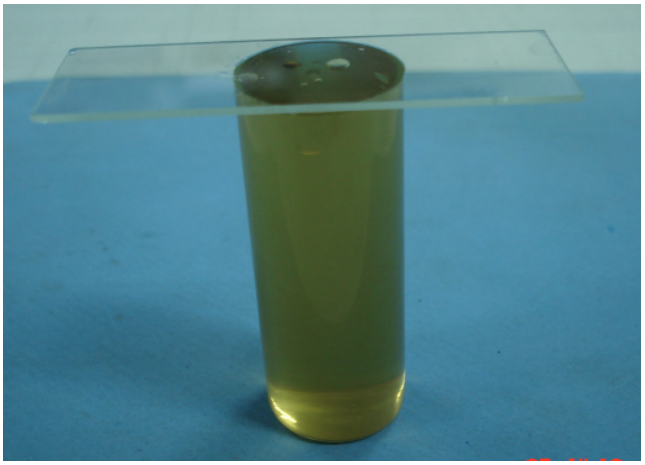

Fig. 18: Willis technic for identification off coccidian