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Coprological Assessment of Enteric Parasites in Argali Sheep (*Ovis ammon*), Siberian Ibex (*Capra sibirica*), and Domestic Sheep and Goats at the Ikh Nart Nature Reserve in Mongolia

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Abstract

In the spring of 2009 (April/May) the Denver Zoological Foundation in collaboration with the Mongolian Academy of Sciences conducted a field coprological assessment feasibility study at the Ikh Nart Nature Reserve in southeastern Mongolia. Our initial effort was directed at finding simple methodologies that would work consistently in the field for identifying some of the enteric parasites for argali sheep (*Ovis ammon*) and Siberian ibex (*Capra sibirica*), and then to compare these to samples from local nomad domestic fat-tailed sheep (*Ovis aries*) and cashmere goats (*Capra hircus*). Direct fecal examination yielded less eggs than the flotation techniques, but was still felt to be useful as a quick screening tool. From the flotation techniques we settled on using sugar because it appeared to yield the most eggs and sugar is readily available in Mongolia. We successfully recovered *Entamoeba* sp., *Eimeria* spp., trichostrongyles, large trichostrongyle species, *Trichuris ovis* and *Strongyloides papillosus*. We are using the digital images we captured to create a field guide for common enteric parasites found in wildlife and domestics ungulates found in the reserve. In the future, we plan to use the field guide and the quantitative modified McMaster technique to compare parasite egg-type numbers in both wild and domestic ungulates during different seasons.

Key words: *Ovis ammon*, *Capra sibirica*, domestic sheep and goats, coprological evaluation, Ikh Nart Nature Reserve

Introduction

Since 2000, the Denver Zoological Foundation, in collaboration with the Mongolian Academy of Sciences, has been conducting ecological research on a variety of species at the Ikh Nart Nature Reserve (Ikh Nart) in southeastern Mongolia. Ikh Nart was established in 1996 to protect 66,000 ha of open valleys and large maze-like rocky outcroppings in northwestern Dornogobi Aimag (Myagmarsuren, 2000; Reading et al.; 2006). The region is high upland (altitude ~1,200 m) covered by semi-arid steppe vegetation. Permanent cold-water springs are available in some of the several, shallow valleys draining the reserve. The climate is strongly continental and arid, characterized by cold winters (low ~43ºC), dry, windy springs, and relatively wet, hot summers (high ~35ºC). Precipitation is low and seasonal, with most occurring in the summer.

The two free-ranging ungulate species we have been studying using radio-collars are argali sheep (*Ovis ammon*) and Siberian ibex (*Capra sibirica*). Argali are listed as Appendix II by the Convention on the international Trade in Endangered Species. Nomadic pastoralists intensely graze domestic fat-tailed sheep (*Ovis aries*) and cashmere goats (*Capra hircus*) in the reserve. Periodic droughts combined with global warming also create regular poor-foraging conditions in the reserve, further stressing exotic ungulates. There is not only a strong potential for interspecific competition for nutritional resources, but also the potential for disease transmission and exchange of parasites due to shared pastures.

In the spring of 2009 (April/May), as part of our ecological research, we collected fresh fecal samples from argali, ibex, and domestic sheep and goats for coprological evaluation, in order to characterize some of the enteric pathogens associated with these ungulates. In addition to identifying the enteric parasites in both exotic and domestic sheep and goats, we wanted to create a field guide for enteric parasitic eggs to be used by

project staff for future projects.

Materials and Methods

All coprological examinations were performed at our permanent research ger camp in the Ikh Nart (N45.72318 E108.64502). Fresh fecal material was obtained by hiking in the reserve each day and looking for resting argali and ibex. When they were located, we waited until they moved off, and then went to the spot and searched for fresh feces, indicated by intense fly activity. We collected 40 to 50 gm of pelleted feces in a Whirl-Pak® bag (Nasco International, Fort Atkinson, Wisconsin 53538, USA). To our knowledge, no samples came from animals with symptomology consistent with clinical parasitism.

Microscopy was performed using a Swift M7000 Series microscope (Swift Optical Instruments, Inc., San Jose, California 95126, USA) and a 1280 x 1024 pixel digital camera that attaches to the objective eyepiece (Swiftcam 2, Swift Optical Microscope). The camera and software also allow the microscopist to make measurements of the eggs. Fecal samples were weighed on a digital scale (PS121, Ohaus Corp., Pine Brook, New Jersey, 07058, USA). We did not have a variable-speed centrifuge in the field, so we could not perform any centrifugal-sedimentation parasite recovery techniques.

We performed a direct examination on each sample by adding 10 gm of feces to a paper cup and mixing it with 30 ml of saline (Sloss *et al*., 1994). One to two drops of the fecal solution were dropped on a glass slide and then covered with a cover slip. Direct samples were examined immediately. All samples were evaluated at magnifications 10x and 40x.

We also evaluated three fecal flotation solutions for use in field conditions; Sheather's sugar flotation solution, magnesium sulfate solution, and 33% zinc sulfate solution (Sloss *et al*., 1994; Foryet, 1997). Sheather's sugar flotation was prepared by combining 454 gm of granulated sugar with 355 ml of tap water and allowing this solution to sit for 1 hour (Sloss *et al*. 1994). The specific gravity (SpG) should be 1.2 to 1.3. Magnesium sulfate solution was prepared by mixing 453 gm of Epsom salt with 1294 ml boiled water (Williams & Zajac, 1980). The resulting SpG should be 1.28. The 33% zinc sulfate solution was prepared by adding 330 gm of anhydrous $ZnSO_4$ to 1000 ml of distilled water, producing a SpG of 1.18 (Sloss *et al*., 1994). We validated the SpG using a hydrometer (Nalgene Plain-Form Hydrometer, Fisher Scientific HealthCare, Houston, Texas 77038, USA) in the US prior to leaving for Mongolia for each solution. Many parasite eggs will float in a solution with a SpG between 1.2 to 1.3 (Foreyt, 1997). A SpG much higher may cause plasmolysis, osmosis, and egg rupture (Foreyt, 1997). Flotation solutions will not recover the eggs of flukes and thorny-headed worms, while there is a difference of opinion on recovery for tapeworm eggs (Sloss, 1994; Georgi, 1999).

We mixed 30 ml of each flotation solution with 10 gm of feces in a cup and then poured the mixture through cheese cloth or gauze into a 3 ml plastic tube until it formed a meniscus. A cover slip was then applied to the meniscus. Slides prepared from sugar solution can be kept for more than 24 hours prior to evaluation, while those in salt solutions must be read in 30 to 40 minutes or the eggs will become distorted and crystals will form (Foreyt, 1997). The issue with not promptly evaluating samples is that the eggs will continue to develop, possibly confounding identification.

Finally, we evaluated a quantitative fecal egg count technique, the modified McMaster test, for use in the field (Sloss *et al*., 1994). The solution we used was magnesium sulfate, as described previously. We mixed 4 gm of feces with 56 ml of the magnesium sulfate solution. Large particulates in the solution were strained with gauze or cheesecloth. A pipette was used to fill the double chambered McMaster counting slide (Chalex Corporation, P.O. Box 187, Wallowa, Oregon 97885, USA). Each type of parasite (eggs and oocysts) was counted separately in each lane from both chambers. The results were multiplied by 100 and divided by 2 to find eggs per gram (epg) of feces.

Results

We performed coprological evaluations on 14 argali sheep (7 male, 6 female, 1 unknown), 12 ibex (10 male and 2 female), 2 fat-tailed sheep (sexes unknown) and 2 cashmere goats (sexes unknown). Unfortunately, no comments were made by the microscopist for 1 ibex direct sample and 2 ibex sugar flotation samples, so they were excluded from the analysis.

Direct samples were useful for identifying the protozoans (*Entamoeba ovis* and *Eimeria* spp.), but were a poor technique for identifying the nematode eggs (Table 1). The sugar, zinc, and magnesium flotation techniques all yielded a greater diversity of parasite eggs than direct samples for recovering both the protozoan and nematode eggs. But we found that after searching for samples in the reserve all day, there simply was not enough time to do a direct exam and all three different flotation techniques for every sample. Since sugar is readily available in most field situations we decided to concentrate our efforts on using that methodology.

We obtained fresh feces from two domestic sheep and two domestic goats owned by a local nomad and performed direct, flotation, and the McMaster quantitative test. The results for the McMaster technique are listed in Table 2.

Table 1. Results from direct and sugar flotation techniques. The number and percent indicate the number positive and the percentage positive for the samples evaluated out of the total samples evaluated for each egg type. Negative results are also reported.

Direct	Coccidia	Trichostrongyle	Lg Trichostrongyle	*T. ovis*	*S. papillosus*	*Entamoeba*	Negative
Argali *n* = 14	7 (50%)	0	2 (14.3%)	1 (7.1%)	0	8 (57.1%)	2 (14.3%)
Ibex *n* = 11	3 (27.3%)	0	0	0	0	10 (90.1%)	1 (0.9%)
D. sheep *n* = 2	2 (100%)	0	0	0	0	2 (100%)	0
D. goat *n* = 2	0	0	0	0	0	2 (100%)	0
Sugar	Coccidia	Trichostrongyle	Lg Trichostrongyle	*T. ovis*	*S. papillosus*	Entamoeba	Negative
Argali *n* = 14	11 (78.5%)	5 (35.7%)	9 (64.3%)	3 (21.4%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
Ibex *n* = 10	9 (90%)	9 (90%)	6 (60%)	0	1 (10%)	0	0
D. Sheep *n* = 2	2 (100%)	2 (100%)	2 (100%)	0	0	0	0
D. goat *n* = 2	2 (100%)	2 (100%)	1 (50%)	0	0	0	0

We found that direct, flotation, and McMaster tests for enteric pathogens and fecal egg counts are feasible and reproducible under our field ger camp conditions.

Discussion

Internal parasitism is a serious concern, causing significant morbidity and mortality for intensively reared domestic sheep and goats (Aitken, 2007). Although fatalities are possible it is the loss of weight, condition, and anemia from *Haemonchus contortus* that cause serious economic hardships for herders (Smith & Sherman, 1994; Hambidge, 2008). Sheep and goats in Mongolia are moved periodically following the availability of grasses. They share the range in Ikh Nart with smaller numbers of wild ungulates, potentially exposing them to high numbers of parasitic eggs. This project was undertaken in order to describe the enteric parasites that might be shared by domestic sheep and goats and free-ranging argali sheep and Siberian ibex in Ikh Nart.

The most common parasite we found using the direct technique in all four ungulate species

Table 2. Results from the quantitative McMaster test for two domestic sheep and two domestic goats from a local nomadic pastoralist grazing within the Ikh Nart Nature Reserve.

Species	Small Trichostrongyle	Large Trichostrongyle	Coccidia
D. sheep	100/gm	100/gm	800/gm
D. sheep	100/gm	100/gm	800/gm
D. goat	100/gm	None	1900/gm
D. goat	100/gm	None	1900/gm

(Table 1**;** argali [57.1%], ibex [90.1%], fat-tailed sheep [100%], and cashmere goats [100%]) were amoeba in the genera *Entamoeba.* These are the smallest parasites we observed. The trophozoites contain a large endosome in the nucleus and several granules, typically measuring 13 to 14 x 11 to 12 µm, with uninucleate cysts measuring 4 to 13 µm (Soulsby, 1982; Levine, 1985). *Entamoeba ovis* is for all practical purposes indistinguishable (may even be the same species) from *E. bovis* (Soulsby, 1982). We only found this parasite once with the sugar flotation technique in an argali. *Entamoeba* is regarded as a non-pathogenic enteric commensal organism of sheep and goats (Levine, 1985).

The most commonly recovered parasite group, both by direct examination and sugar flotation, was the protozoa or coccidians in the genus *Eimeria* (Table 1). *Eimeria* are 10 to 60 µm, and are found worldwide in the small and large intestines of ruminants (Zajac & Conboy, 2006; Aitken, 2007; Scott 2007). There are several species of *Eimeria* described in sheep and goats, and each host has host specific *Eimeria* spp. (Levine, 1985; Smith & Sherman, 1994; Hendrix & Robinson, 2006; Aitken, 2007). They are speciated by structural and biochemical characteristics (Levine, 1985; Hendrix & Robinson, 2006). Mixed *Eimeria* infections are quite common in domestic animals (Levine, 1985). We observed two different sized (50 µm and 23 µm, Figure 1) unsporulated oocysts, presumably from two different species of *Eimeria*. Clinical coccidiosis is seen under

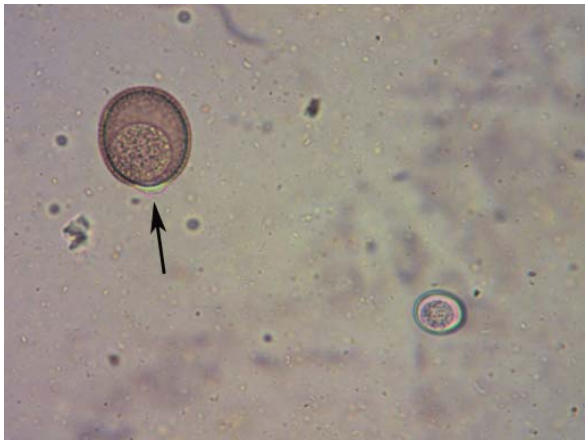

Figure 1. A large (50 x 38.4 µm, LxW) and smaller (23 x 20.9 µm, LxW) unsporulated coccidian oocyst presumably from two different species of *Eimeria* from an argali ewe (40x). One of the oocyst has a cap at the end (arrow).

Figure 2. A trichostrongyle egg (81.1µm x 42.9 µm, LxW) from a male ibex (40x). All of the trichostrongyles have similarly appearing eggs.

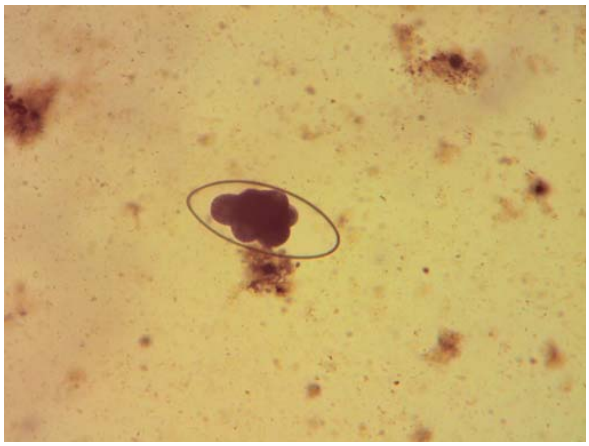

Figure 3. The large trichostrongyle egg *Nematodirus* sp. (256.5 µm x 120.4 µm, LxW) from an argali ewe (10x). Note the 4 to 8 cells in the morula, and the tapering ends characteristic for this species.

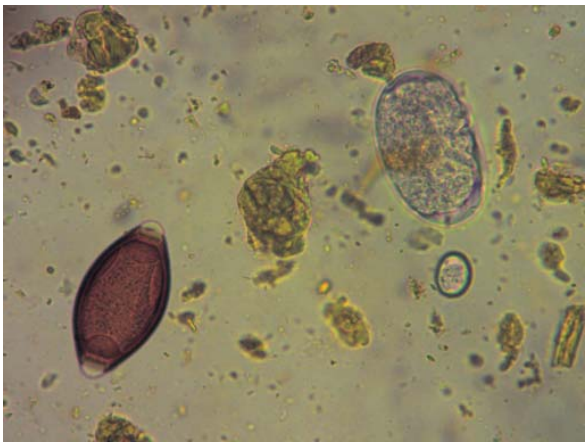

Figure 4. Trichuris ovis (74.4 µm x 37.1 µm, LxW), trichostrongyle sp. (71.0 µm x 44.2 µm, LxW), and an *Eimeria* sp. (21.4 x 16.0 µm, LxW) from a male ibex.

intensive management situations and is the most common cause for diarrhea in domestic kids. However, high numbers can also be present in clinically normal animals (Smith & Sherman, 1994; Aitken, 2007; Scott, 2007; Bowman, 2009). There can be significant mortality (10%) for feedlot domestic lambs (Levine, 1985). Domestic animals receive a degree of immunity following coccidiosis from subsequent clinical disease, but not from reinfection (Levine, 1985). There have been many *Eimeria* spp. reported to infect bighorn sheep (*Ovis canadensis*) in North America (Levine, 1985).

The helminth parasites typically receive the greatest attention worldwide in domestic ruminants, due to the significant impacts on sheep and goat morbidity and mortality (Aitken, 2007b; Scott 2007b). Many of the trichostrongyles and large trichostrongyles have been reported, both in domestic sheep and goats, and the free-ranging argali and ibex in all Mongolian provinces (Sharhuu, 2004). There are many genera of nematodes that have strongyle-type eggs (65-120 µm x 34-50 µm, LxW) which infect the abomasum, small and large intestine, and are often termed collectively as trichostrongyles (Georgi, 1999; Hendrix & Robinson, 2006; Zajac & Conboy, 2006; Hambidge, 2008). The eggs are oval and thin-shelled, with four or more cells forming the morula (Georgi, 1999; Hendrix & Robinson, 2006; Zajac & Conboy, 2006). Helminth species cannot be diagnosed from the appearance of the egg. For definitive species identification, feces must be cultured to recover the characteristic third-stage larva (Zajac & Conboy, 2006). We did not recover any trichostrongyle eggs by direct examination, but recovered trichostrongyle eggs in all four species (argali, ibex, and domestic sheep and goats) using sugar flotation (Table 1, Figure 2). Many of the trichostrongyles that affect domestic sheep and goats also affect bighorn sheep and Spanish (*Capra pyrenaeca*) and Siberian ibex (Becklund & Senger, 1967; Lavin, 1997; Samuel, 2001; Sharkhuu, 2001). We also recovered two large trichostrongyle eggs; *Nematodirus* spp. (150 to 230 µm x 80 to 107 µm) (Figure 3), which are elongated with tapered ends and 4 to 8 cells in the morula, and *Marshallagia* sp. (160 to 200 µm x 75 to 100 µm) which have parallel sides, rounded ends, and a 16 to 32 cell morula (Ivens, 1978; Hendrix & Robinson, 2006). *Nematodirus* is not usually associated with clinical disease but in North America, *N. battus* can cause a severe spring time diarrhea, associated with 30% mortality in lambs (Bowman, 2009). Both *Nematodirus* and *Marshallagia* are commonly found in North American bighorn sheep (Samuel, 2001).

We also recovered the very distinctive *Trichuris ovis* (commonly known as whipworms) egg with bipolar plugs measuring 75 µm x 35 µm (Figure 4) in a single direct and three (21.4%) of the sugar flotations in argali only. The adult worms attach to the cecum and colon of ruminants (Foreyt, 1997; Hambidge, 2008). With the sugar technique we recovered *Strongyloides papillosus* egg (commonly known as the intestinal thread worm of cattle, sheep, and goats) in one argali and one ibex (Hendrix, 2006). The eggs are thin-walled, ellipsoidal, and larvated, measuring 40 to 64 µm x 20 to 25 µm (Ivens, 1978).

The modified McMaster Test is used to quantify the numbers of an egg type per gram of feces (epg). For domestic animals, this is used as a guide in determining whether treatment may be required or if the parasites are developing resistance to the anthelmintic (Sargison, 2008). We evaluated the McMaster technique to determine whether it could be performed at our research ger camp and used in the future to compare egg counts between exotic and domestic ungulates during different seasons. We hoped to obtain samples from several different nomadic herds, but due to poor grass conditions in 2008 and in the spring of 2009, most nomadic pastoralists had moved their herds out of Ikh Nart in search of better pasture. We were able to locate one nomad with a herd within the park. We sampled two domestic sheep and two domestic goats (Table 2). As a general guideline, epg $\leq$ 250 indicates low worm burden, 250 to 800 indicates moderate worm burden, and $\geq$ 800 to 1000 indicates severe worm burden for trichostrongyles (Kaufmann, 1996; Sargison, 2008). Low counts may not accurately reflect infection rates, due to prepatency period or developing host immunity (Kaufmann, 1996). The trichostrongyles were present in low numbers ($\leq$ 250 epg). The coccidian were present in comparatively higher numbers, but epg and clinical disease have not been characterized for coccidia.

In conclusion from the results of this study, we intend to construct a field guide for the ungulate parasites found in Ikh Nart. With continued intensive grazing of domestic ungulates which shed large numbers of parasitic eggs stressed

wild ungulates may be increasingly susceptible to clinical parasitism. In future field seasons, we hope to utilize this information to do more quantitative comparisons, using the McMaster test to contrast the worm burdens found in domestic sheep and goats with free-ranging argali sheep and Siberian ibex.

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Хураангуй

Монгол орны зүүн өмнөд хэсэгт байрлах Их Нартын Байгалийн Цогцолборт газарт АНУ-ын Денверийн Амьтан Судлалын Сан, Монгол улсын ШУА-тай хамтран 2009 оны хаврын улиралд (4 ба 5-р саруудад) копрологийн анализын судалгааг гүйцэтгэв. Бидний судалгаа аргаль (*Ovis ammon*) ба янгирын (*Capra sibirica*) ялгадасны анализд тулгуурлан тэдгээрийн дотоод шимэгчдийг илрүүлэхэд зориулагдсан энгийн аргыг боловсруулахад чиглэгдсэн бөгөөд энэхүү судалгааны дүнг нутгийн бог мал болох хонь (*Ovis aries*) ба ямааны (*Capra hircus*) ялгадасны анализын дүнтэй харьцуулан авч үзэв. Ялгадаснаас шимэгч хорхойн өндгийг шууд ажиглалтаар илрүүлэх нь уусмалд хөвүүлж илрүүлэх аргаас бага үр дүнтэй байсан боловч энэ нь богино хугацаанд ажиглалтыг явуулдагаараа ач холбогдолтой байв. Судалгаагаар *Entamoeba* sp., *Eimeria* spp. зэрэг шимэгч эгэл биетнүүд, *Trichuris ovis*, *Strongyloides papillosus* зэрэг дугариг хорхойг илрүүлэв. Түүнчлэн тус цогцолборт газарт тохиолдох зэрлэг ба гэрийн тэжээмэл туруутан амьтдын дотоод шимэгчдийг тодорхойлоход шаардагдах дижитал зургуудыг бэлтгэсэн ба цаашид шимэгчид ба тэдгээрийн өндөгийг янз бүрийн улиралд тодорхойлох McMaster аргын хувилбарыг боловсруулж байна.

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