Spatial and Temporal Variations in Gastrointestinal Parasitism in the Critically Endangered Hirola and Livestock in Southern Kenya

Justin M. Njeru1  Paul M.Wanjala2  Geoffrey M. Wahungu3  Samwel A. Andanje4
1. Department of Wildlife Management, School of Natural Resource Management, University of Eldoret, P. O. Box 1125-30100, Eldoret
2. Department of Biological Sciences, School of Science, University of Eldoret, P. O. Box 1125-30100, Eldoret
3. National Environmental Management Authority, PO BOX 67839-00200, Nairobi
4. Department of Ecological Monitoring, Bio-prospecting and Biodiversity Information Management, Kenya Wildlife Service, P. O. Box 40241-00100, Nairobi

Abstract
Seasonal and environmental differences can influence host-parasite dynamics. In this study we investigated the influence of changes in season on prevalence and intensity of gastrointestinal parasites of Hirola or Hunter’s Antelope (Beatragus hunteri) and livestock in two different areas in Southern Kenya using noninvasive coprological procedures. Faecal samples were collected from both Hirola and livestock in Tsavo East National Park and Ishaqbini Hirola Conservancy and analyzed using a combination of sedimentation and McMaster faecal floatation techniques. The results showed that both Hirola and livestock were infected by a wide variety of strongyles, trematodes, cestodes and coccidia. Season was found to be the major factor influencing infection patterns in both Hirola and livestock with significantly higher prevalence and intensity being recorded during the wet as compared to the dry season. Apparently, differences in gastrointestinal parasite prevalence and intensity in Ishaqbini and Tsavo were not statistically significant. This was an indication that the environmental differences between the two areas influenced the host-parasite dynamics in Hirola and livestock only to a small extent.

Keywords: Faecal samples, strongyles, parasite intensity, parasite prevalence

INTRODUCTION
Hirola is one of the world’s most endangered genera of large mammals and perhaps the world’s rarest and most endangered antelope. It is the sole survivor of a formerly diverse group, and is sometimes referred to as a living fossil. Historically, the antelope was common throughout Eastern Africa but has suffered a devastating decline in the last three decades, with numbers plummeting from around 14, 000 in the 1970s to an estimated 600 today (Andanje and Ottichilo, 1999; Butynski, 1999; Dahiy and Aman, 2002). Few, if any, today exist in the Republic of Somalia though in 1979 they were approximately 2,000 (Butynski, 1999). Together with factors such as predation, drought, habitat loss and degradation, competition for resources with livestock and poaching, diseases have been implicated in this sudden decline (Butynski, 1999; Andanje and Ottichilo, 1999; Dahiy and Aman, 2002, Njeru et al., 2014).

In order for this rare antelope to survive, intensive conservation efforts are needed. Among such efforts has been the need to address the issues of Hirola-livestock interactions such as competition for forage and transmission of diseases. So far, there has been limited work that has focused on the implications of this interface on the survival of the critically endangered antelope. However, the concern for the outcomes of the Hirola-livestock interface is currently high especially in the view of the resurgence of some wildlife and domestic animal diseases in the Hirola range that were previously under control. Such resurgence has involved the incursion of the rinderpest virus that is associated with cattle in the Somalia ecosystem, (Wambwa, 2002). In light of this, a species based action plan, targeting diseases, pathogens and parasite management is needed (IUCN SSC Antelope Specialist Group, 2008). However, among other diseases, little is known of parasitosis in Hirola and more so in regard to the habitat that they share intensely with livestock.

The epidemiology of gastrointestinal parasite infections has been known to depend on several factors. According to Urquhart et al; (1988), the ultimate occurrence of parasitic infections is as a result of host susceptibility, introduction of infective stages, alteration in host susceptibility, introduction of susceptible stock and the introduction of infection. Host factors include conditions such as nutritional status, physiological status, age, sex, breed and levels of acquired innate resistant (Tariq et al., 2008, Kanyari et al., 2009). In this study, the main focus was on the influence of variations in season on prevalence and intensity of gastrointestinal parasites of Hirola and livestock in two different locations in Southern Kenya.
MATERIALS AND METHODS

Study Areas
The present study was carried out in Ishaqbini Hirola Conservancy and Tsavo East National Park. Ishaqbini Hirola Conservancy is located in Masalani Division of Ijara District (Garissa County) (01° 55’32.4” S, 040° 10’ 17.6” E) in North Eastern Province of Kenya. The conservancy was registered in 2007 and covers approximately 72 km². A survey conducted in July 2008 in the conservancy recorded 152 Hirola.

Tsavo East National Park on the other hand is located 2° 45’ S and 38° 34’ E and is Kenya’s largest National Park covering an area of approximately 11,700 km². The Hirola were first introduced in the park in 1963 and a second group in 1996. During the translocations Hirola were released on Irima and Dika Plains respectively (Adanje, 2002), approximately 2 km south of River Voi, at about 200 km south-east of the south-eastern limit of the species’ known natural range. The translocated population in the park numbered approximately 105 individual by the year 1999 (Andanje and Ottichilo, 1999).

In both Ishaqbini Conservancy and Tsavo East National Park ecosystems, rainfall is distributed bimodally, with long rain from April through June and short rains from November through December. Distinct dry seasons occur between the rains, particularly during January-March. The mean annual rainfall ranges from 350 mm in the northern extreme of the range to 700 mm on the southern edge of the range. At average, rainfall in the area occupied by Hirola in Tsavo is slightly higher than in Ishaqbini. Annual daily temperature ranges between 21° C and 30° C.

Vegetation types within the natural range of the Hirola vary from lush savannah grassland in the south to open bush grassland in the center, to dry thorn bush in the north. The natural range is bordered to the south by a humid coastal forest-savannah mosaic and to the west by a narrow band of riparian forest along River Tana. The region immediately to the west of River Tana is also arid and extremely over-grazed, with the result that it is today largely an area of dense bush and little grass, and appears to be a poor habitat for Hirola (Butynski, 1999). In Tsavo East National Park, the vegetation cover varies a lot but studies have shown that Hirola in Tsavo have habitat preferences similar to their natural range, (Andanje and Ottichilo, 1999). Here, Hirola use fairly open, short, green grassland habitats where grass heights average about 17 cm. More shrubby areas are used during the dry season and more open areas during the wet season (Andanje and Ottichilo, 1999).

Apart from Hirola, Ishaqbini Conservancy holds several other animal species of conservation interest including the reticulated giraffe, cheetah, African wild dog, desert warthog, Somali bush baby, buffaloes, lion, leopard, lesser kudu, bush-buck, Harvey’s duiker, Beisa oryx, topi, Tana River Red Colobus, and Tana Mangabey. Tsavo East National Park has a vast abundance of large mammals including great herds of elephant, antelope, hippo, black rhino, eland, lions and giraffe plus a host of avifauna. Waterbucks, kudus and dik-diks are common along the banks of the River Galana.

Collection of Fecal Samples
Fecal samples were collected from late September 2009 to the end of March 2010. During this period, Hirola were searched for in the specific areas where they were known to occur.

After locating a group, it was monitored until one or more of the individuals in the group defecated. For each defecation, the age and sex of the defaecator was recorded along with the position of the fecal sample. To ensure adequate sampling for each group, at least 6 samples were collected from groups with >10 individuals and at least 3 samples from groups with <10 individuals. For groups with less than five individuals it was possible at times to take all samples. Samples from livestock were collected from sheep, goats and cattle found grazing within the study sites. They were also monitored and samples collected using the same procedure as for Hirola and preserved in 70% ethanol.

Parasitological Analysis
Fecal samples collected between 7:00am and 1:00pm were evaluated for gastrointestinal parasites using a combination of sedimentation and floatation techniques. Fecal floatation techniques are best for recovery and quantification of nematode and cestode eggs and protozoan cysts from faeces, but fail to recover trematode eggs or nematode larvae (Bowman, 2003, Turner and Getz, 2010). Sedimentation technique was used for qualitative analysis of fecal samples (MAFF, 1980) with the objective of identifying and evaluating the presence or absence (prevalence) of the parasites in the samples.

With the sedimentation technique, 3g of feces was measured into a 250ml beaker. Then 46 ml of tap water was added and stirred thoroughly using a fork. Once a homogenous solution was obtained, the fecal suspension was filtered through a strainer into another 250ml beaker. The filtered material was poured into a test tube and allowed to sediment for 5 minutes. The supernatant was removed carefully by decantation and the sediment resuspended in 5 ml of water. This was again allowed to sediment for a further 5 minutes and the supernatant discarded by decantation. Using a teat pipette, a drop of the sediment was transferred onto 2mm x 2mm microslide and covered with a coverslip. All observations made were recorded under serial slide 1 (sub sample one). The sediment in the 250 ml was agitated once more to prepare sub sample two, and the procedure repeated to prepare sub sample three.
To quantify ova and oocyst output in fecal material, a modification of the McMaster fecal egg counting technique with saturated Sodium chloride as the flotation solution (MAFF, 1980, Ezenwa 2004) was used. For each sample, 4g of faeces was carefully weighed out and put into a labeled vial. The sample was homogenized in 56 ml of NaCl (specific gravity 1.2) after which it was sieved to remove large debris using a strainer into a tube. After agitation, an aliquot was taken from the tube and pipetted into a single chamber of the McMaster slide. The tube was further agitated to fill a second chamber. The chamber was then allowed to stand for 5 minutes. The two chambers were examined under x10 objective of a light microscope to identify and count all parasite eggs, larvae, and cysts. For each sample, all eggs and oocysts in the two chambers of the McMaster slide were counted and the total multiplied by 50 to determine the number of eggs and oocysts per gram of feces. This was recorded as slide 1 after which two more McMaster slides were prepared and examined using the same procedure such that from the same sample, three slides were examined.

Data Analysis
In data analysis “prevalence” was taken as the proportion of individuals examined that were shedding parasite propagules in feces and “intensity” as the estimated number of parasite propagules shed per gram of feces by infected individuals. To determine whether prevalence of the parasitic infections was independent of the host, area and season, Chi-square test was used. In the case of parasite intensity, the quantitative data on fecal egg counts (FEC) and fecal oocysts counts (FOC) was normalized using log _10 (x +1) transformation and then analysis of variance (ANOVA) was used to test for differences in intensity of infection as inferred from mean eggs per gram (EPG) and oocysts per gram (OPG) of fecal material based on the risk factors. The results were thereafter back-transformed using antilog (minus 1) and represented as the geometric fecal egg counts (GFE) for strongyles and geometric fecal oocysts counts (GFOC) in case of coccidia. Statistical significance for all analyses was determined at 5% alpha level.

RESULTS
A total of 2460 serial samples (sub samples) were analysed for gastrointestinal parasites from 410 animals which included 141 Hirolas, 120 cattle, 77 sheep and 72 goats.

Overall Prevalence of Gastrointestinal Parasites
Results revealed that both Hirola and livestock were infected by a wide variety of gastrointestinal parasites. However, eggs and oocysts of the parasites were conservatively assigned to broad taxonomic groups to avoid identification errors especially in view of the fact that gastrointestinal parasites of Hirola have not been described earlier (Njeru et al., 2014). Thus infection patterns were analyzed based on four broad groups of parasites, namely: strongyle nematodes, trematodes (flukes), cestodes (tapeworms) and coccidia (protozoans).

Overall, 327(79.67%) of the samples were positive for at least one group of gastrointestinal parasites (Fig 1). As indicated in Fig 2, with a prevalence of 65(83.98%), the highest prevalence of infections was observed in goats, whereas the lowest number of positive cases were observed in Hirola which had a prevalence of 103(72.82%). However, these differences in prevalence between the four hosts were not statistically significant (χ^2 = 1.025; df = 3, p = 0.795). This strongly suggested co-infection with the parasites among the study species. This view is also supported by data by Njeru et al., (2014) that showed that patterns of gastrointestinal aggregation among Hirola, goats, sheep and cattle in the same study areas were not statistically significant (p<0.05). The scenario in which goats had such high cases of infections with gastrointestinal parasites could be due to slow development of immunity against gastrointestinal parasites. Cattle and sheep are believed to have faced prolonged challenge by parasites over generations, but in goats, the decline of sufficient browsing area and expansion of crop husbandry has forced them to graze alongside cattle and sheep that had already developed good resistance (Regassa et al., 2006). Similar observations were made by Kanyari et al., (2009) and (Ghanem et al., 2009) in studies of gastrointestinal prevalence in livestock. However, the result contradicted observations made by Wairuri et al., (1995). Perhaps the most plausible explanation for the high prevalence in sheep is their feeding habit. Sheep tend to graze very close to the ground and this ultimately predisposes them to high chances of picking the infective larval stages.

Among the four hosts, Hirola had the lowest prevalence of gastrointestinal parasites. Like sheep and cattle, Hirola are grazers suggesting that they are potentially exposed to the infective larval stages in contaminated forage. Since species dependent factors result in certain species having higher prevalence levels than others, it is likely that Hirola have a higher genetic resistance to the parasites than livestock, or in better ways than livestock, they avoid feeding on contaminated plants. Also feeding on plants containing high levels of anthelmintic substances such as tannins, perhaps not taken by livestock could lead to such low prevalence levels. In addition, given that Hirola and livestock have for decades shared the same environment (Burnesdon, 1985), and that co-infection with the parasites between Hirola and livestock exists, then it is likely that Hirola have acquired better immunity against the parasites studied as compared to livestock. However, such a conclusion would warrant further investigation.
Out of the 327 (79.67%) of the individuals that were found to be infected, 45.37% (168) were multiple infections while 141 (34.30%) were single infections. As shown in Fig. 2, goats had the highest cases of multiple infections (55.84%; n = 43) while cattle had the highest cases of single infections (41.11%; n = 49). These differences in infection were statistically significant for sheep ($\chi^2 = 17.600; \text{df} = 2, p < 0.001$); cattle ($\chi^2 = 11.282; \text{df} = 2, p = 0.004$) and goats ($\chi^2 = 24.999; \text{df} = 2, p < 0.001$) but not for Hirola ($\chi^2 = 2.670; \text{df} = 2, p = 0.263$).

In natural host-parasite interactions, multiple infections are commonplace (Read and Taylor, 2001). Numerous theoretical studies show that they are an important determinant of virulence evolution (Taylor et al., 1998; Davies et al., 2002; Massey et al., 2004). Indeed, López-Villavicencio et al., (2010) asserted that the optimal virulence of a parasite under multiple infections was often different from that under conditions of single infection. Studies have also shown that when parasites of different genotypes or strains compete for limiting resources within a host, virulence per genotype is predicted to increase with lethal effects (van Baalen and Sabelis, 1995; Frank, 1996; Brown et al., 2002).

In this study goats were observed to have the highest prevalence of multiple infections with 43 (55.84%), while Hirola had the lowest, 57 (40.43%). Going by this observation, it would mean that the threat posed by multiple infections in Hirola was lower than it was the case in livestock. However, it was difficult to predict immediately the implications of these infection patterns in Hirola based on the available data. Furthermore, some authors have reported no effect or even lower virulence in multiple infections (Hood, 2003; Hughes et al., 2004) often when the competition is such that only one group or genotype wins.

### Variations in prevalence based on the two study areas

Table 1 shows the comparison between prevalence of gastrointestinal parasites in Tsavo East National Park and Ishaqbini Hirola Community Conservancy. As shown in the figure, generally the parasites were more prevalent in Tsavo than Ishaqbini but in none of the cases were the differences between the two areas statistically significant (p>0.05). Of much interest was the observation that Hirola in Ishaqbini had higher prevalence of strongyles and trematodes than those in Tsavo East National Park.
Prevalence of gastrointestinal parasites has been shown to vary considerably due to differences in environmental conditions such as humidity, temperature, rainfall and management practices (Magona and Musisi, 2002; Regassa et al., 2006). In Tsavo National Park, the amount of rainfall, humidity, temperature and elevation are at average slightly higher than in Ishaqbini and the rest of the natural range of Hirola (Butynski, 1999). These differences could possibly have accounted for the higher prevalence of the parasites in Tsavo. Furthermore any slight variation in the environmental conditions can have significant effect on survival and development of free living stages of the parasite.

However, it was interesting to note that on the basis of locality, the parasites exhibited different prevalence patterns across the hosts from what was observed for the overall prevalence. For instance, both Hirola and cattle had slightly more cases of strongyles in Ishaqbini than in Tsavo while Hirola and goats had slightly more cases of trematodes in Ishaqbini than in Tsavo. In case of cattle, the most plausible explanation for this unexpected pattern was the difference in management practices. There were occasions when cattle from neighboring ranches were encountered within the hirola range in Tsavo National Park and were sampled alongside other livestock. Most of those cattle tested negative for nematodes leading to the observed low prevalence. It is likely that this was due to routine use of anthelmintics and the good plane of nutrition that they had been subjected to. In the case of goats and Hirola, the cause for the unexpected pattern could not be immediately ascertained.

Table 2 indicates the prevalence of gastrointestinal parasites based on seasons. We observed that comparatively more animals had been infected with the parasites in the wet season than in the dry season. All the hosts showed statistical differences in the prevalence of strongyles in the two seasons with the differences being more pronounced inHirola and goats and least in sheep. Coccidia occurred significantly more in all the hosts during the wet season (sheep, \( \chi^2 = 15.327; df = 1, p < 0.001 \); cattle; \( \chi^2 = 10.227; df = 1, p < 0.001 \); Hirola; \( \chi^2 = 3.840; df = 1, p = 0.050 \) and goats; \( \chi^2 = 20.198; df = 1, p < 0.001 \)). We also observed season-related prevalence patterns of trematodes in all the hosts. However, unlike sheep and Hirola, cattle and goats showed no statistical difference in the occurrence of trematodes in both seasons. Moisture is known to be a major factor enhancing the development, survival and transmission of infective stages of gastrointestinal parasites (Dunn, 1978; Armour, 1980), and hence the direct relationship between prevalence with humidity and temperature.

Spatial and Temporal Variations in the Intensity of Gastrointestinal Parasites

Based on faecal egg counts (FEC), the mean eggs per gram (EPG) differed significantly across the hosts (ANOVA, \( F = 3,407 = 9.376; P = 0.001 \)). This is shown in Figure 3a in which eggs are represented as geometric faecal egg counts (GFEC). Goats had the highest mean eggs per gram (with 386.75 ± 43.55 SE) whereas Hirola (with 262.24 ± 33.42 SE) had the lowest. Also based on faecal oocysts counts (FOC), it was observed that the

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Table 1: Prevalence of gastrointestinal parasites in Hirola and livestock based study location

<table>
<thead>
<tr>
<th>Parasite group and study location</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Hirola</th>
<th>Goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsavo</td>
<td>8</td>
<td>7 (8.75)</td>
<td>51</td>
<td>7 (10.13)</td>
</tr>
<tr>
<td>Ishaqbini</td>
<td>64</td>
<td>49 (76.56)</td>
<td>0.026</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>56 (77.78)</td>
<td>120</td>
<td>74 (61.94)</td>
</tr>
<tr>
<td>Trematodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsavo</td>
<td>8</td>
<td>3 (37.5)</td>
<td>51</td>
<td>21 (40.52)</td>
</tr>
<tr>
<td>Ishaqbini</td>
<td>64</td>
<td>23 (35.42)</td>
<td>0.755</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>26 (35.65)</td>
<td>120</td>
<td>47 (38.89)</td>
</tr>
<tr>
<td>Coccidia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsavo</td>
<td>8</td>
<td>3 (37.5)</td>
<td>51</td>
<td>19 (36.6)</td>
</tr>
<tr>
<td>Ishaqbini</td>
<td>64</td>
<td>18 (28.13)</td>
<td>0.120</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>21 (29.17)</td>
<td>120</td>
<td>40 (33.06)</td>
</tr>
</tbody>
</table>

P indicates the Chi-square test for differences of level of prevalence between Tsvo and Ishaqbini

* Indicates that the differences in level of parasite prevalence between the two areas are statistically significant (at 0.05)
mean oocysts per gram (OPG) also differed significantly across the hosts ($F_{3, 407} = 5.91; P = 0.000$). These results are depicted in Figure 3b as geometric faecal oocysts counts (GFOC). It was noted that just like in the case of strongyles, goats recorded the highest mean oocysts per gram (i.e. with $537.78 \pm 135.59$ SE) and Hirola the lowest ($259.08 \pm 63.47$ SE). Generally, these values were low indicating light levels of infection.

According to Hansen and Perry (1994), the degree of infection is considered light if the EPG values are between 800 in livestock. However due to the potential of rapid build-up of worms on pasture, Torres-Acosta and Hoste (2008) suggested that deworming of animals was necessary when the average FEC was between 100 and 200 eggs per gram. In addition, the overall intensity of infection in livestock was low compared to what had been previously reported by Maichomo et al., (2004) but agreed with a report by Ghanem et al., (2009) on livestock from Somalia. In the case of Hirola the significantly lower intensity of infection in relation to livestock was surprising. Though currently there is no data on the species for use to benchmark these findings as either low or high, the observed levels were low as compared to reports from a number of other African bovids (Woodford, 1976; Boomker, 1987). The results contradicted observations by Apio et al., (2006) in a study on bushbuck (in Uganda) which had a much higher intensity of coccidia, but a much lower intensity of strongyles.

Results for the analysis of fecal egg and oocyst output based on site showed that unlike livestock, Hirola had higher mean EPG and OPG in Ishaqbini than in Tsavo (Fig 3a and Fig 3b). In cattle the differences were statistically significant for the intensity of eggs per gram ($F_{1, 119} = 14.584; p < 0.001$) and also oocysts ($F_{1, 119} = 5.420; p = 0.020$).

Overall, animals in Ishaqbini conservancy had lower intensities of gastrointestinal parasites than those in the Tsavo area. This suggested that environmental differences between the two areas could have influenced the observed differences in intensities of infection of gastrointestinal parasites in these animals. However, though the intensities of coccidia in Hirola were higher in Tsavo than their counterparts in Ishaqbini, the intensity of infection of strongyles showed contrary results. The reasons for this disparity could not be immediately ascertained. However, further research into parasite diversity and host ecology in the two areas would help shed more light on this matter.
As shown in Fig 4a, for all hosts, higher FEC was recorded during the wet season than in the dry period. These differences were statistically significant for sheep ($F_{1, 71} = 9.36; p = 0.004$); cattle ($F_{1, 119} = 12.68; p < 0.001$) and Hirola ($F_{1, 140} = 9.23; p = 0.003$) but not for goats ($F_{1, 79} = 3.08; p = 0.082$).

Similarly, we observed higher intensity of infection with coccidia in the wet season than in the dry period (Fig. 4b). Among Hirola and cattle, the effect of season on coccidia intensity was higher than it was among sheep and goats. For cattle and Hirola the differences were statistically significant ($F_{1, 119} = 5.972; p = 0.016$, and $F_{1, 140} = 25.82; p < 0.001$ respectively) whereas for sheep and goats they were not ($P>0.05$).

Significantly higher intensities of infection of both strongyles and coccidia were recorded during the wet than dry season. This pattern was the same for all the animals sampled and it signified the role of environmental factors in determining the intensity of infection with gastrointestinal parasites. Armour (1980) observed that adult female worms released their eggs preferentially when the environmental conditions were optimal for the successful survival and development of their infective larvae. In the environment humidity is required for successful development and survival of parasite stages and movement of larval nematodes (Nielsen, 2007).

The seasonal patterns in parasitism indicated that the long dry season may have limited the development and survival of parasite stages in the environment and their transmission to hosts. A study by Jacquet et al (1995) in the semi-arid area of Mauritania found that young goats born during the dry season were free from gastrointestinal nematode infections until the following rainy season. This indicated a lack of transmission during the dry season. Thus, increased parasitism in the wet season may have been due to a resumption of transmission, or parasite activity in the case of arrested strongyle larvae. In addition, the pulse of new born naïve hosts during the wet season increased the number of susceptible hosts especially in the case of hirola which...
mostly calf during the rainy season between October and November.

CONCLUSION AND RECOMMENDATIONS

The main focus on this study was on the prevalence and intensity of gastrointestinal parasites of the Hirola and livestock in Southern Kenya and how these were influenced by season and area. To this end, we found that there were seasonal and area differences in the patterns of gastrointestinal parasite infections in the host populations with more parasite occurrences and intensity being found in the wet season. There was no significant relationship between prevalence and intensity of the parasites with area. Unexpectedly, Hirola in Ishaqbini were found to have had higher prevalence and intensity than their counterparts in Tsavo. Further research into parasite diversity and host ecology in the two areas would help shed more light on this matter.

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**BIO-DATA OF MAIN AUTHOR**

Justin Mugendi Njeru holds a Master of Science degree in Wildlife Management from the University of Eldoret. He is involved in research in the field of wildlife ecology and wildlife management. Currently, he is a part-time lecturer at the University of Eldoret, Kenya