Experimental Infection of Sheep using Infective Larvae (L3) harvested from the Faeces of Naturally Infected Swayne's Hartebeest (*Alcelaphus buselaphus swaynei*) at Senkele Swayne's Hartebeest Sanctuary, Ethiopia

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Abstract

Experimental infection of sheep using nematode larvae recovered from the faeces of naturally infected endangered Swayne's Hartebeest (SHB) was carried out from December/2006 - April/2007 to assess the potential for the inter–species transmission of helminths. Faecal samples were collected from Swayne's Hartebeest without preservatives and cultivated at room temperature for 21 days. Infective larvae were collected overnight by Baermann's Method and identified and counted under a microscope. The sample was divided into eight aliquots of 9400 infective larvae and drenched into eight worm-free sheep kept at zero grazing. After 30 days, faecal samples from infected sheep were examined for ova for further 30 days by the Modified McMaster Method. Adult nematodes were recovered from the infected sheep at post mortem examination and distinguished based on position of barbs, shape and length of spicule, position of cervical papillae and mouth parts. The mean eggs per gram of faeces (EPG) from all infected sheep was 9192 ± 1422. *Haemonchus placei* (86.3%) from abomasums, *Oespophagostomum venulosum* (13.3%) and *Trichuris s*pp (0.3%) from large intestine were identified. No ova and adult parasite were recovered from the control sheep. The study demonstrated that transmission of helminths between Swayne's Hartebeest and sheep is experimentally possible. This is the first study conducted on the potential inter-species transmission of parasites between Swayne's Hartebeest and local sheep and fur-

ther research is recommended to determine the impact of multiple-species habitat use, on pasture contamination and any associated pathological impact.

Key words: Eexperimental infection, helminths, inter-species transmission, local sheep, Swayne's Hartebeest

Introduction

Although some nematodes, particularly the family Trichostrongylidae, show an almost unrestricted range throughout ruminants, others are host specific. However, in general the parasites of ruminants are not host specific (Dunn, 1968). Three species: Haemonchus contortus, Haemonchus placei, and Haemonchus similis are known to occur in both domestic and wild ruminants. They are among the most pathogenic nematodes of sheep, cattle and goats (Lichtenfels *et a*l., 1994). However, domestic livestock is considered more likely to be reservoir hosts of helminth infections than wild species (Cook *et al*., 1979; Richardson and Demarias, 1992). The probability of parasite transmission depends on the distribution and density of animals on pasture. Gregarious grazing and territorial behavior may limit inter-species transfer of parasites to some degree. However the risk of cross species transmission is greatest within ungulate sub-families (Isaza et al, 1990; Milkolon et al., 1994). Dunn (1968) noted that the wild ruminants were the sufferers in communal grazing, acquiring predominantly ovine helminth species from the reservoir in the domesticated ruminants. Careful distinction must be made between the presence of infection and the presence of disease due to that infection (Dunn, 1968). Several factors influence parasite pathogenicity that includes host, age, immune status, diet, sex and intensity of infection (Beaudoin et al., 1970; Kaneene et al., 1985; Kock, 1986; Scullion, 1982; Sloan, 1965; Urquhart et al., 1987). The development and length of worms vary in different host species and worms grow longer in a favorable host than less favorable ones (Fekadu Shiferaw, MSc thesis, 1999, unpublished). The objective of this study was to explore the potential for helminth cross-infection between Swayne's Hartebeest (Alcelaphus buselaphus swaynei) and local sheep.

Materials and Methods

Study area

Senkele Swayne's Hartebeest Sanctuary was established in 1976 to conserve the endangered (IUCN Red List, 2011), endemic Swayne's Hartebeest (Hill-

man, 1993). It is situated south west of Shashamene Town about 320 km south of Addis Ababa. It is located N 7°10' E38°20' at an altitude of between 2000 and 2150 meters, with a total area of 36 km². The sanctuary had an estimated population of about 3500 individuals in 1983 (Yalden *et al.,* 1984), however the population declined to 626 in 1991 and reported to be less than 500 in 2008 **(**Tolera Kumsa and Afework Bekele*,* 2008). The sanctuary is embedded in a transformed human landscape with abundant livestock. Livestock share grazing with SHB in the sanctuary, and the natural vegetation is a grassland of the savanna type with 91 bird species, including other mammals, bohor reed buck (*Redunca redunca*), Oribi (*Ourebia ourebi*), greater kudu (*Tragelaphus strepsiceros*), Spotted hyaena (*Crocuta crocuta*), warthog (*Phacochoerus aethiopicus*), Common jackal (*Canis aureus* and lion (*Panthera leo*).

Study animals

Five female and five male sheep, aged 6-7 months, were brought on December 2/2006 and drenched on December 10/2006 with albendazole (5mg/kg, EXIPTOLä, ERFAR Pharmaceutical Laboratories, Greece); and ivermectin 200µg/kg (Ivomec 1%™, Merial, Brazil) was also administered by subcutaneous injection to clear any remaining parasites. Then both eight experimental and two control sheep were housed without access to grazing. After 10 days, faeces from all sheep examined for helminth ova became negative. During the course of study one female and one male sheep were kept as control adjacent to the infected sheep and were fed on the same food.

Faecal culture and experimental infections

Fresh faecal samples collected from Swayne's Hartebeest in the sanctuary, were broken finely on petri-dish using a stirring device and left at room temperature (≈ 25-28˚C) for 21 days. Infective larvae were then recovered using the Baermann's Technique and identified at 10x10 magnifications by the method as described by Hansen and Perry (1994). Larvae collected were counted and an aliquot of 30 ml containing 9400 larvae was drenched to each of the eight local sheep described above. After 30 days a series of 3 gram of faeces from each sheep were examined every five days over the next 30 days and processed by Modified McMaster Method of Flotation as described by MAFF (1986). During faecal sampling all animals were observed for clinical symptoms. The above work was carried out in the National Animal Health Research Center (NAHRC), Sebeta, (Addis Ababa).

Worm count and identification

Sheep were euthanized and post mortem examination was carried out on all sheep to recover adult parasites by the method described by Hansen and Perry (1994) and preserved using 10% formalin. Parasites were cleared with lactophenol and put on glass slide as a temporary mount. Parasites were counted and identified under the microscope with magnification 15x10 and 15x20 using keys according to Lichtenfels *et al.,* (1994) and Soulsby (1982). Species were differentiated based on the position of barbs, shape and length of spicule, position of cervical papillae and mouth parts. Spicules of *Haemonchus* were measured under 10x20 magnification using a micrometer of 100 divisions (Nikon, Japan) in turn multiplied by 10 to get length in micrometers. The work was carried out in the National Animal Health Research Center (NAHRC), Sebeta, (Addis Ababa) Ethiopia and King Khalid Wildlife Research Center, Riyadh, Saudi Arabia.

Statistical Analysis

The null hypothecs was there is no transmission of helminths between sheep and Swayne's Hartebeest. Data was recorded and summarized using Microsoft Office Excel 2007™ and computed and tested with Student-t Test by GraphPad Instat Software ™ at 5% significance level.

Results

In this study *Haemonchus placei* (Fig.1, 2 & Table 1) was recovered from abomasums of all eight experimentally infected sheep, and *Oesophagostomum venulosum* (Fig. 3, 4 & Table 1) and *Trichuris* species were counteded from large intestine of seven and two sheep respectively. Out of the total worms (N=1384) the proportion of *Haemonchus placei* was higher (86.3%) than *Oesophagostomum venulosum* (13.3%) and *Trichuris* spp (0.3%). Over all 63.8% were female and 36.2% male worms and more worms (73.4%) were observed in male sheep than female sheep (26.6 %). It was not easy to clearly identify *Oesophagostomum* species by counting leaf crowns. Nevertheless as the external leaf crowns were prominent, lateral cervical alae was absent and cervical papillae was not seen before the level of esophagus, it appeared to be *O. venulosum*. *Trichuris* was identified at the genus level with its characteristic filamentous anterior end and a tick posterior tail.

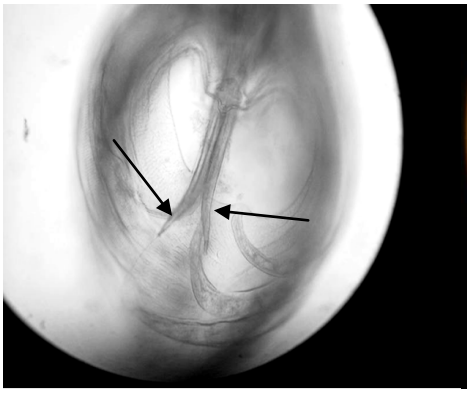

Fig. 1. Position of barb on the spicule of *H. placei* Magnification 300X., King Khalid Wildlife Research Center Laboratory, Saudi Arabia (Fekadu Shiferaw)

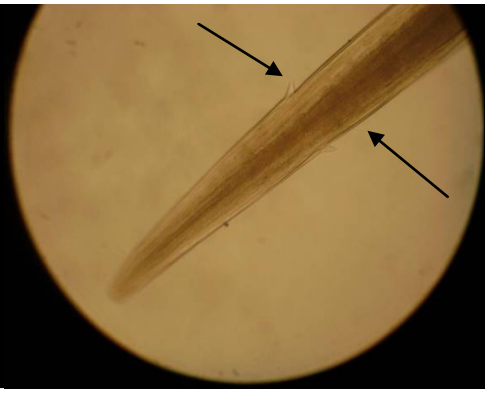

Fig. 2. Cervical papillae of *H. placei* Magnification 300X. King Khalid Wildlife Research Center Laboratory, Saudi Arabia. (Fekadu Shiferaw)

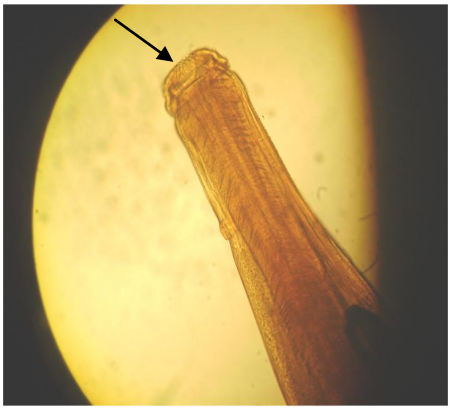

Fig. 3. Mouth part (external leaf crown) of *Oesophagostomum venulosum* Magnification 3000X. King Khalid Wildlife Research Center Laboratory, Saudi Arabia. (Fekadu Shiferaw)

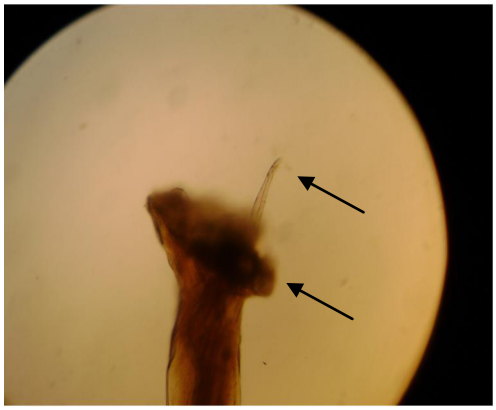

Fig. 4. Spicule and bursa of *O. venulosum* Magnification 300X. King Khalid Wildlife Research Center Laboratory, Saudi Arabia. (Fekadu Shiferaw)

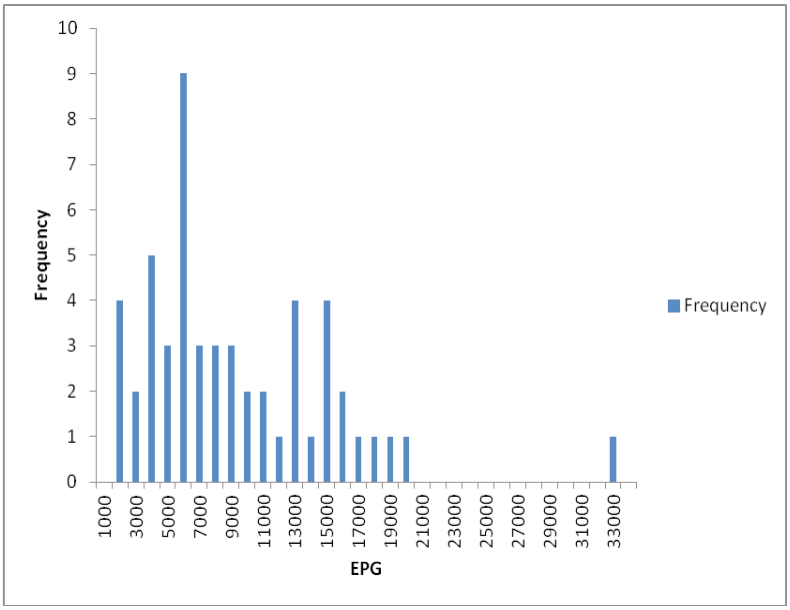

Fig. 5. EPG Kinetics of the Infected Sheep Recovered at Every Fifth day Over a Month

The average EPG of each sheep in the infected group ranges from 3657-16450 (Table 1). Out of all EPG observations more observations (16.9%) lie between 5000-6000. The dynamics of fecal egg count of all the infected sheep over a month is depicted on Fig. 5. There was a positive relationship, Regression statistics, (R^2=0.6096) between number of female worms and EPG burden, and coincidentally there was also a positive relationship, Regression statistics (R^2=0.6696) between total worms and EPG burden. There was no statistically significant difference, (P = 0.2173), between the average eggs per gram of faeces (EPG) of male sheep and female sheep. A total of 25 spicule of male worms of *Haemonchus placei* was measured and the mean spicule length was 477.2 µm, SEM = 6.2 (95% CI) and ranges 430-550 µm. Neither egg nor adult parasites were recovered from the control group.

Table 1. Worms and average EPG recovered from each infected sheep.

Tag	Sheep sex	Mean EPG	Number of worms			Establ-ishment Rate (%)	M/F Ratio (%)	H. placei		O. venulosum		Trichuris spp	
			Male	Female	Total			M	F	M	F	M	F
101	F	10014	90	57	147	1.6	157.8	62	35	27	19	1	3
102	M	16450	110	573	683	7.3	19.2	97	554	13	19	0	0
103	F	10429	77	60	137	1.5	128.3	71	53	6	7	0	0
104	F	3657	40	24	64	0.7	166.7	29	20	11	4	0	0
105	M	6343	54	56	110	1.2	96.4	51	49	3	6	0	1
106	M	10686	59	52	111	1.2	113.5	50	42	9	10	0	0
107	F	5286	16	4	20	0.2	400	16	4	0	0	0	0
108	M	10671	55	57	112	1.2	96.5	21	41	34	16	0	0
109	M	0	0	0	0	0	0	0	0	0	0	0	0
110	F	0	0	0	0	0	0	0	0	0	0	0	0
Total		9192	501	883	1384	1.8	56.7	397	798	103	81	1	4

A male sheep (Table 1: Tag No 102) that died after six weeks of larval infection had the highest (683= 49.3%) worm burden, very high EPG (32600) and high larval patency (7.3%) compared to the group. It showed very pale mucus membrane, severely emaciated, weak, reduced appetite and a rough coat. Interspersed, petechial hemorrhage was observed on the abomasal mucosa at post-mortem examination. Four infected sheep revealed moderate decreased body weight and pale mucus membranes, while this symptom was slight on the remaining three.

Discussion and conclusion

In this study the nematodes*, H. placei, Oesophagostomum venulosum and Trichuris* spp*,* were established experimentally in worm-free sheep after drenching the infective larvae collected from the faecal culture of Swayne's hartebeest. The finding warrants both sheep and SHB could naturally share the above nematodes. Previous studies have also shown evidence in other wild hosts. Mikolon *et al.,* (1994) reported that most strongyle species in *Antilopinae* are capable of infecting sheep and goats. This was further noted by the investigation of Fekadu Shiferaw and Laurenson (2011) in which *H. contortus, T. colubriformis* and *Oesophagostomum* spp were established experimentally in worm-free sheep after drenching the infective larvae collected from the faecal culture of mountain nyala. Preston *et al., (*1979) recovered *H. contortus, Trichostrongylus* and *Cooperia* spp of adult nematodes from post mortem examination of Merino sheep after infection with larvae cultured from Thomson's gazelles' faeces. In this study all larvae drenched sheep demonstrated parasite infection and this might be associated with low resistance. The death of a male sheep might indicate immunity acquired by sheep is of short duration although it was not known if this individual was carrying *H. placei* prior to the treat-

ment. Dunn (1978) documented that sheep do not develop full competence of immune responsiveness to alimentary nematodes until they are two years of age. In the tropical and subtropical areas, Urquhart *et al.,* (1987) pointed out that there is little evidence that sheep from endemic areas develop an effective acquired immunity to *Haemonchus* spp which leads to continuous contamination of grazing land. Given high egg output from the *Haemonchus* species, massive contamination of the grazing land is highly likely to occur frequently in Senkele Hartebeest Sanctuary. Furthermore the infective larvae are relatively resistant to desiccation and may survive for up to 3 months on pasture or faeces. Thus this finding of the presence of *Haemonchus* spp in Senkele Hartebeest Sanctuary is notable and potentially a pathogen to both domestic and wild species present.

In this work the mean spicule length of 25 worms of *H. placei* became 477.2 µm with range 430-550 µm and this corresponds with the work of Lichtenfels *et al.,* (1994) who reported the mean spicule length of 481µm with range 438-511 µm. Lichtenfels *et al.,* (1988) described that, although there was considerable overlap, the spicule lengths *of H. contortus* and *H. placei* were usually distributed below 450 µm for *H. contortus* and above 450 µm for *H. placei.* The spicule length of *H. similis* was recorded shorter (300-400 µm) than the former two species. In this work the spicule length of 84% of the male *H. placei* worms recorded more than ≥ 450 µm. Spicule length provided the quickest and easiest character to use for separating most populations of *H. contortus*, *H. placei* and *H. similis*. Furthermore Lichtenfels *et al.,* (1986) illustrated differences between *H. placei* and *H. contortus* and some of the differential features were based on mean spicule length, the distance between barbs and the tip of the spicule.

Fleming (1988) noted that density dependent factors regulate egg production, parasite numbers and length, and fecundity of populations. In this study despite high doses of infective larvae, low patency/establishment rate of worms in the sheep were recorded and this might be attributed to the large number of larvae drenched resulting in competition for nutrition. On the other hand despite relative few female parasites high EPG was recovered in most sheep and this is not easily explained. However Coadwell and Ward (1982) and Michael and Bundy (1989) reported that the daily faecal egg output is not a measure of the number of individuals present but related to total parasite weight.

The study has demonstrated the possibility of transmission of helminth parasites between livestock and the endangered Swayne's Hartebeest and the potential for pathology in sheep infected with these parasites. Apart from habitat fragmentation and shortage of grazing land, it is possible that pathogenic parasites could be an additional threat to the survival of the species and might compromise integrated wildlife and livestock systems. The sanctuary is heavily grazed by sheep, cattle and goats. *Haemonchus, Oesophagostomum* and some other helminth species affect cattle and sheep (Isaza *et al,* 1990); and both hosts could be a source of infection to the Swayne's Hartebeest in the sanctuary and vice-versa. On the other hand there is some evidence that co-grazing of livestock on wildlife pastures might reduce the overall pasture infection load especially if the livestock are removed at intervals and treated for parasite infection (Soulsby, 1982). It is suggested that this study is followed by examination of the pathogenicity of the parasites present in Senkele on the Swayne's Hartebeest, through epidemiological and pathological studies and assess the cost or benefit of co-grazing on hartebeest health.

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References

Beaudoin, R.L., Samuel, W.M. and Strome, C.P.A., 1970. Comparative study of the parasites in two populations of White–tailed Deer. *J. Wildl. Dis.,* 6, 56-6.

Coadwell, W. J. and Ward, P. F. V., 1982. The use of faecal egg counts for estimating worm burdens in sheep infected with *Haemonchus contortus. Parasitology,* 85, 251-256.

Cook, T.W., Ridgeway, T. and Andrews, R., H., 1979. Gastro-intestinal helminths in white-tailed deer (*Odocoileus verginianus*) of Illinois**.** *J. Wildl. Dis.,* 3, 405-407.

Dunn, A.M., 1968. The wild ruminant as a reservoir host of helminth infection. *Symposium of the Zoological Society of London.,* 24, 221-248.

Dunn, A.M., 1978. Veterinary Helminthology, 2nd edition, Butler & Tanner Ltd, London, UK

Fleming, M.W., 1988. Size of inoculum dose regulates in part worm burdens, fecundity and lengths in Ovine *Haemonchus contortus* infections. *J. Parasitol.,* 6, 975-978.

Hansen, J. and Perry, B., 1994. The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants, ILRAD, Nairobi, Kenya.

Hillman J.C., 1993. A Compendium of wildlife Conservation Information. Vol1. The Wildlife Conservation Society, New York and The Ethiopian Conservation Department, Addis Ababa.

Isaza, R., Courtney, C.H. and Kollias, G.V., 1990. Survey of parasite control programmes used in captive wild animals. *Zoo Biol*., 9, 385-392.

IUCN 2011: SSC Antelope Specialist Group, 2008. *Alcelaphus buselaphus ssp. swaynei*. Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>.

Kaneene, J.B., Tailor, R.F., Sikarskie, J.G., Meyer, T.J. and Richter, N.A., 1985. Disease patterns in the Detroit Zoo: A study of the mammalian population from 1973 through 1983. *J. Am. Vet. Med. Assoc*., 187, 1166-1169.

Kock, R.A., 1986. Enteric nematode infestation in Thomson's gazelles (*Gazella thomsoni*) at Whipsnade Wild Animal Park, The Zoological Society of London. *J. Zoo An. Med*., 17, 61-64.

Kumsa, T. and Bekele. A., 2008. Population status and structure of The endangered Swayne's hartebeest (*Alcelaphus buselaphus swaynei*) in Senkele Swayne's Hartebeest Sanctuary, Ethiopia. *Acta Zool. sin*., 4, 569-575.

Lichtenfels, J.R., P.A. Pilitt. and Le Jambre. L.F., 1986. Cuticular ridge patterns of *Haemonchus contortus* and *Haemonchus placei* (Nematoda: Trichostrongyloidea). *Proc. Helminthol. Soc. Wash*., 53, 94-101.

Lichtenfels, J.R., P.A. Pilitt. and Le Jambre. L.F., 1988. Spicule lengths of the ruminant stomach nematodes *Haemonchus contortus*, *Haemonchus placei*, and their hybrids. *Proc. Helminthol. Soc. Wash*., 55, 97-100.

Lichtenfels, J.R., Pilitty. P.A. and Hobergz, E.P., 1994. New Morphological Characters for Identifying Individual Specimens of *Haemonchus* spp. (Nematoda: Trichostrongyloidea) and a Key to Species in Ruminants of North America. *J. Parasitol,*. 1, 107-119.

MAFF, 1986. Manual of Veterinary Parasitological Techniques Reference book, No.418, Ministry of Agriculture, Fisheries and Food Mannual of Veterinary, Her Majesty's Stationary Office, London.

Michael, E. and Bundy, D. A. P., 1989. Density dependence in establishment, growth and worm fecundity in intestinal helminthiasis: the population biology of *Trichuris muris (Nematoda)* infection in CBA/Ca mice. *Parasitology,* 98, 451-458.

Mikolon, A.B., Boyce, W.M., Allen, J. L., Gardener, I.A. and Elliot, L.F., 1994. Epidemiology and control of nematode in a collection of captive exotic ungulates. *J. Zoo Wildl. Med.*, 13, 156-160.

Preston, J.M., Karstad, L., Woodford, M.H. and Allonby, E.W., 1979. Experimental transmission of gastrointestinal nematodes between sheep (Ovis aris) and Thomson's gazelles (Gazella thomsoni). *J. Wildl. Dis*., 15, 399-404.

Richardson, M.L. and Demarias, S., 1992. Parasites and condition of coexisting populations of White-tailed and exotic deer in South-central Texas. *J. Wildl. Dis*., 28, 485-489.

Scullion, F.T., 1982. An investigation into the parasite burden available to Grant's Zebra (Hippotigris burchelli) and Bacterian camels (Camelius bacterius) in the Dublin Zoo. *J. Wildl. Dis*., 13, 156-160.

Shiferaw, F. and Laurenson, M, K., 2011. Risk of disease transmission between domestic livestock and wild ungulate in the Bale Mountains National Park, Ethiopia. *Walia-Special Edition on the Bale Mountains*, 1, 269-281.

Sloan, J.E.N., 1965. Helminthiasis in ungulates. *International Zoo Year Book*. 5, 24-28

Soulsby, E.J.L., 1982. Helminths, Arthropods and Protozoa of Domesticated Animals, Baillie´re Tindall, London.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W., 1987. Veterinary Parasitology, Longman Scientific & Technical, England.

Yalden, D.W., Largen, M.J. and Kock, D., 1984. Catalogue of the Mammals of Ethiopia 5. Artiodactyla. Moni. *Zool. Ital. (NS) Supl*., 19, 67-221.