Parasitic Contamination Incidences at Inspection of Harvested Springbok (Antidorcas marsupials) and Gemsbok (Oryx gazelle) in Namibia

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Abstract

Little work has been conducted on parasitic infestation of harvested wild game at game abattoirs in Namibia. The aim of this study was to assess parasitic contaminations that occur in two commonly harvested Namibia game species. We examined springbok (Antidorcas marsupialis) and gemsbok (Oryx gazelle) carcasses for parasite infestation and rectal contents from eviscerated gut for worm egg counts among other routine inspection protocols. A filarial worm, Skrjabinodera kuelzii was found in 104 out of 540 carcasses harvested from separate 3 farms and then submitted for cutting and deboning. These filarial worms were predominantly between the hindquarter muscles, pelvis region and sometimes in renal fat. As a precautionary measure, these carcasses were condemned whole or part depending on the level of infestation. The larvae and filarial worms were only found in springbok but not in gemsbok carcasses. Worm eggs of strongyle, *Strongyloides papillosus*, Toxocara spp, Trichuris spp and coccidia were found in variable numbers in both springbok and gemsbok faeces, indicating a potential risk of transmission to other susceptible species in the ecosystem. The unusual deep muscle contamination of springbok carcasses by S. kuelzii are significant findings which should be considered during routine meat inspection of harvested wildlife.

Keywords: Game; Meat inspection; *Skrjabinodera kuelzii*; Gemsbok; Parasite; Springbok

Introduction

Wildlife farming is increasingly becoming integrated into the mainstream meat industry for food security and income generation in most Southern African countries thus positioning the wildlife industry on a different platform from its traditional past [1]. In recent years, Namibia has witnessed a remarkable recovery and increase in wildlife populations. The number of small communal conservancies, which bring immense benefits to many rural communities, has spiraled significantly in many parts of the country. Surveys indicate that Springbok (*Antidorcas marsupialis*), Oryx (*Oryx gazelle*) and Mountain Zebra (*Equus zebra*) populations increased over 10 times between 1982 and 2000 with about 80% of these located on privately owned farms or freehold conservancies [2]. However, the characteristics of soil, vegetation, spatial distribution of resources and presence of multispecies, such as predators, all influence the dynamics of the host-parasite system [3]. In many parts of the free ranging farms, cattle, sheep, and goats graze together and carnivores such as jackals are common in some areas. Sheep are by far the most numerous livestock species in the southern part of Namibia, and along with goats have the greatest opportunity for livestock-wildlife transmission by grazing remote land frequented. Due to the nature of this important emerging wildlife industry, slaughter practices tend to differ in many aspects from standard procedures applicable in slaughterhouses of livestock species. Small ruminant wildlife are slaughtered by free bullet and immediately bled then eviscerated whilst suspended on mobile line hangers. However, meat inspection procedures conform to standard practices for other food-producing animals and likewise, further investigations are invariably undertaken on all seized carcasses.

The changing ecosystems impact on wildlife populations and their susceptibility to pathogens they carry which underlines an absolute requirement for a systematic and thorough meat inspection process in order to prevent transmission to humans [4,5]. Parasitic infection is not uncommon in springboks [6-12]. In game species examined elsewhere, a numbers of helminth species and new host records for several species of nematodes were reported (http://www.bisoncentre.com). Worm burdens ranging between 2,954 and 71,790 have been reported in South African wildlife [8,9]. Most reviews of animal health monitoring systems mention wildlife disease surveillance only in passing because of the difficulties of establishing population estimates (denominator data) for defining rates, such as disease incidence, or the obstacles to developing systematic surveillance programs coordinating with human disease surveillance [13]. Here, we evaluate the inspection incidence of parasites in springbok (*Antidorcas marsupialis*) and gemsbok (*Oryx gazelle*) carcasses for parasite infestation and rectal contents from eviscerated gut for worm egg counts. These data also provide a baseline for possible future assessments of the distribution of parasites among Namibian ungulates.

Materials and Methods

Pre-slaughter health assessment

Animals from two farms located 100 km apart in different districts of Hardap and Karas regions of Namibia were initially presented for slaughter in 2005. A further 9251 springbok and gemsbok animals sourced from eight different farms were presented for slaughter between

April 2009 and August 2010. Assessment of the general physical and health status of these animals was undertaken by an experienced hunter. This was accomplished by assessing the animal's response to attempted capture. Briefly, this involved a precise estimation of escape distances and flight zones of animals at night. Sick or exhausted animals, generally had shorter escape distances (30-100 m) and flight zones compared to healthy animals which were always greater than 120 m.

Slaughter and meat inspection process

Animals were shot with a free bullet and immediately suspended on to mobile hangers for bleeding and evisceration. Bleeding and evisceration were completed within 30 min of shooting, and carcasses were transported to a field dressing station for an initial health inspection. Carcasses passing initial inspection were transported under chilled conditions to a slaughterhouse for skinning and final inspection as described previously [14,15]. The carcasses underwent a meat inspection process conforming to guidelines stipulated in the European Commission regulation [14]. A detailed schedule of the inspection process is illustrated in South African game regulations [16]. Specific features requiring serious consideration included the following; general body condition, efficiency of bleeding, degree of gross contamination, colour, odour, external parasitic infestation, bruising and injuries. Any condemned whole or part of the carcass was seized and subjected to further detailed examination which included macro- and microscopic examination [14].

Faecal worm egg counts

Approximately 50 grams of faecal contents from the posterior part of the rectum were randomly collected from each of the adult health gemsbok and springbok harvested between April 2009 and August 2010 from different farms. Each pooled sample consisted of five subsamples collected from five animals. Forty five pooled samples from springbok and twelve from gemsbok were collected. The samples were refrigerated at 4°C within 30-60 min after collection until analysis. Parasites eggs were quantified using the Wisconsin double centrifugal sugar flotation method [17]. Briefly, five grams of mashed faecal material with a small grinder was weighed and placed in a 15ml glass beaker. 13ml of water was then added to fill up the glass beaker and stirred thoroughly with a spatula until a mash material was formed. The slurry was then passed through a tea sieve and funnel into a test tube while pressing on the remaining material. The tubes were balanced before centrifugation. The samples were then centrifuged at 1500rpm for 10 minutes. After centrifuging, the supernatant was removed, with care being taken not to lose the fine sediment of the pelleted material. The tube was then filled approximately half way with sugar solution and the pellet was dispersed using a glass rod. The tube was then topped off with sugar solution until a meniscus was formed, and a 22×22 mm cover slip was placed on top. The samples were then centrifuged at 1500 rpm for 10 minutes. Gently, the cover slips were transferred to slides and the entire cover slip was examined. Counts were expressed as per gram of faeces.
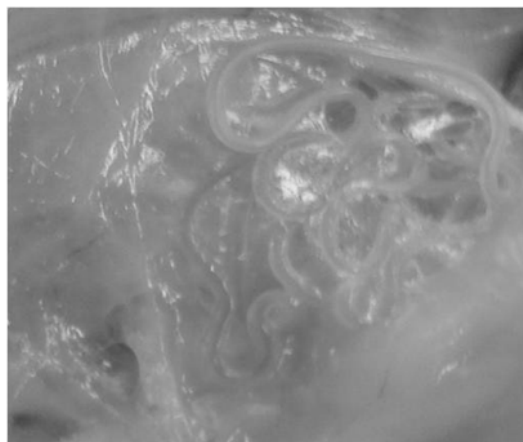
Identification of parasitic larvae

The larvae infested meat samples were preserved in 10% formalin and 70% alcohol before referring them to the Central Veterinary Laboratory in Windhoek. Identification was to the level of genus and where possible to species level, using keys and illustrations based on gross morphological features and microscopic examination using a light microscope. In the identification of *S. kuezii*, the worms were carefully separated from the muscle tissues using dissection equipment

into sterile petri-dishes. The worms were preserved in 70% alcohol. Identification was based on microscopic examination using a light microscope [18].

Results and Discussion

Shooting individual game animals opportunistically on encounter is not an ideal sampling method, and can be prone to biases; for example, towards animals with parasites of high intensity. Therefore, in our study, we assumed that shooting did not result in the selection of thinner animals or those with high intensities of parasites, assuming that group size itself is independent of parasite intensity and based on the post mortem results. During a routine inspection of 279 springbok carcasses, no helminths and or arthropods parasite larvae associated abnormalities or pathological lesions were detected at post mortem inspection although some carcasses, whole or in part, were condemned for aesthetic reasons (bruising, bad bleeding and general gross contamination). Subsequent detailed examination of the passed carcasses during deboning revealed parasite larvae on 39 carcasses more concentrated in the hindquarter muscles (*Musculus biceps femoris, M. gluteus* and *M. semitendinosus*) and seldom in the *Longissimus dorsi* muscles. Thirty-nine out of 279 springbok carcasses were condemned for aesthetic reasons. Notably, the infested carcasses comprised 36 from the 1st farm in Hardap and three were from the 2nd farm in Karas region. Characteristically, the larvae were approximately 7-10 mm long with a few possessing a distinct dark red pigmentation, whilst the majority was creamy in color. Subsequent detailed laboratory examination presumed the larvae to be early or late 1st stage larvae of *Chrysomya* or *Musca* species. We were unable to perform further molecular identification of the larvae to species level and future molecular analysis is recommended for definitive identification. Intriguingly, a further examination of 9251 springbok carcasses from 5 different farming units failed to detect these larvae. Interestingly, it is well-acknowledged that phenotypic identification is best undertaken with third instar larvae [19]. To our knowledge, the deep muscle contamination of carcasses by these parasitic larvae is the first report and hence should be considered during meat inspection of game meat.

Whilst we considered these observations in 39 springbok carcasses to be significant, we could not explain why such larvae were not detected in a further 9251 springbok carcasses from 5 different geographical regions over a four year-period. However, a filarial parasite *S. kuelzii* was detected at a prevalence rate per farm of 19% on one farm and 100% on two farms respectively in 700 springbok carcasses brought in for cutting and deboning. The farms were separated from each other by at least 50km. *S. kuelzii* in 104 springbok carcass, was predominantly located in the pelvic cavity fascia, muscle fascia and renal fat. Figure 1 shows *S. kuelzii* located on pelvic fascia. As a precautionary measure, these carcasses were condemned whole or in part depending on the level of infestation. Subsequent investigations after 3 months confirmed the pelvic region to be the predilection site of these worms. Very little is known about the life cycle of this filarial worm which belongs to the family *Onchocercidae*. An unidentified haematophagous arthropod, most likely a dipteran insect as for other *Onchocercidae*, is believed to be the transmitting vector. It is widely accepted that *Diptera* flies are mechanical and biological vectors of human enteric pathogens and hence should be of great concern from the sanitary and health point of view [20,21]. Occupation of a specific niche within the host to evade immune mechanisms seems to be a paramount survival technique. The significance of these worms is not apparent, they seem not to cause pathological lesions in the peritoneum and no swollen lymph

Figure 1: Skrjabinodera kuelzii adult worms inhabiting the pelvic muscle fascia of the springbok.

Animal species	Parasitic species	Worm egg count per gram of faeces	Number of positive cases expressed as a percentage of the total number examined
Oryx gazella	Strongyle	200-2000	57.14
	Trichuris spp	100-1000	7.14
	Coccidia oocyst	300-1000	28.57
Antidorcus marsupialis	Strongyle	>200	24
	Strongyle papillosus	11-200	7
	Toxocara spp	1-10	2
	Trichuris spp	1-5	5
	Coccidia oocyst	1-1000	44
	Unidentified muscle larvae	N/A	0.4
	Skrjabinodera kuelzii	N/A	77.14

Table 1: Prevalences of parasites eggs detected in farmed Oryx gazelle and Antidorcus marsupialis in Namibia at slaughter.

nodes were detected in the affected animals. However, the numbers and location of worms may vary from farm to farm. On the other hand, absence of *Skrjabinodera kuelzii* could be related to the spatial distribution of *Diptera* flies, which is poorly characterized in Namibia, inclusive of the regions from where the 9251 carcasses originated and furthermore, climatic variation affects the prevalence, geographical distribution and more importantly, the survival of free-living larval stages of parasites [7,22-24]. Typically, in one farm 1-20 worms were found in the pelvic area only while on another farm 15-30 worms were consistently found in the pelvic and inter-muscular connective tissue of hindquarter muscles. Interestingly, the parasite dies within 1 hour after the death of the host suggesting that it probably relies on oxygenated circulating blood or homeostatic body temperature. The parasite was restricted to 3 farms possibly reflecting the distribution of the vector. Undoubtedly, more work is required to define the clinical significance, the biology and epidemiology of this parasite in wildlife in order to inform on safety of meat derived from such sources. Infestation with filarial worms is not uncommon in ruminant wildlife species though. *Skrjabinodera kuelzii* was originally described as *Filaria kuelzii*. *Filaria kuelzii* is now a synonym of *Skrjabinodera kuelzii*. *Filaria kuelzii* was described in three antelopes (*Philantomba maxwelli*), which harboured *S. kuelzii* [25,26]. The parasites were found in the intramuscular connective tissue of the back and pelvis region including subcutaneous tissue and microfilariae could be demonstrated on the blood smears. *Dirofilaria asymmetrica* has been described in the common duiker (*Sylvicapra grimmia*) in South Africa, but the site of infection was not specified. Chabaud and Rousselot synonymised this species with *S. kuelzii*, and described specimens from the intermuscular connective tissue from the black-backed duiker (*Cephalophus dorsalis castaneus*) in the then Belgian Congo [18,27]. *S. tanganyikae* was originally described as *Gazellofilaria tanganyikae* from the peritoneal cavity and mesentery of Thomson's gazelle (*Gazella thomsoni*) and further studies showed that this parasite also occurred in the pelvic cavity of Grant's gazelle (*Nanger-granti*) [28,29].

Rectal contents of both gemsbok and springbok were found to contain variable numbers of different helminth eggs. Whilst the gemsboks harboured eggs of strongyle, *Trichuris* spp and coccidia, the springboks were also infested with *Trichuris spp, Toxocara spp* and *Strongyloides papillosus* in addition those found in gemsboks. Detailed worm egg burdens are given in Table 1. Parasite richness and prevalence in wild animals can be used as indicators of population and ecosystem health and a number of parasitic species from the genus Toxocara and *Trichuris* pose a potential anthropozoonotic transmission risk [30-32]. The significance of the egg counts in this study is not clearly discernible as the carcasses were apparently average body condition but Sub-Sahara Africa is famous for its great mixed variety of distinctive animals with carnivores, or meat-eating animals, include the wild dogs, lion, leopard, cheetah, hyena, jackal, and mongoose [33]. Consistent with this, worm egg loads have been reported to remain constant in healthy animals under normal conditions but increase considerably upon the induction of stressful conditions such as drought, disease and serious injury [34]. In impala, worm egg loads can rise from 20000 to 60000 per animal during drought periods, but the damage to the host largely depends on the composition and dynamics of the worm population [7]. The composition of the worm load is also dependent on the type of vegetation and its condition as determined by seasonal factors [34,35]. A significant difference in the worm burden has been reported between browsers and grazers. Kudus in the Kruger National Park where they predominantly graze had a mean worm egg count of 2251 compared to 339 in those mainly browsing in Etosha National Park in Namibia [34]. However, gastrointestinal nematode infections are known to reduce growth rates in domestic ruminants and decreased body mass and condition have been reported in parasitized animals in a range of wildlife species, including ruminants [36].

Conclusion

Regulatory authorities should consider the possibility of deep muscle contamination by larvae during routine meat inspection of game meat. Furthermore, a better understanding of the ecology and spatial distribution of haematophagous arthropods and other *dipteral* species in Namibia is urgently needed to determine the dynamics of these larvae and filarial worms in free ranging wildlife and domestic species. Lastly, an urgent need exists to define the significance of S. kuelzii in food-producing wildlife.

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