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Full Length Article

## Gross and histopathologic findings in sheep with plastic bags in the rumen

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## ABSTRACT

The objective of this study was to evaluate the gross and histo-pathological lesions caused by plastic bags in the rumen of sheep. Sixteen (16) castrated, one-year old Dorper sheep were used for the study. The animals were divided into 4 groups each consisting of 4 sheep. Three of the groups had 129 g, 258 g and 387 g of plastic bags, respectively, introduced into the rumen through rumenotomy, while the fourth group, without implants served as control. All the animals were observed daily for 6 weeks following implantation. All animals were euthanized on day 42 and subjected to gross and histopathological examination. Gross changes observed at post mortem included; atrophy of the muscle and body fat, atrophy and fibrosis of the spleen, liver, kidneys and hydropericardium. Gross lesions in the rumen included stunting, atrophy, thinning and loss of ruminal papillae, erosion, ulcerations and nodular formation on the ruminal mucosa. Histopathological examination revealed atrophy, ulceration, erosion and disruptions of the stratified epithelial layer of the papillae. Other changes included parakeratosis, hyperkeratosis, prominent rete pegs, oedema and severe hydropic degeneration of different parts of the mucosal layer. There was increased mononuclear cell infiltration, increase in the number of lymphatic vessels and lymphangiectasis in the submucosa and oedema in the muscularis and serosal layers. The pathological changes observed contributed to clinical signs and may interfere with the absorption of nutrients resulting in poor condition of the animal, and loss of production and productivity.

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## 1. Introduction

Sheep allowed to roam and scavenge for food ingest various indigestible foreign bodies [1]. These materials, particularly, used plastic bags pollute the grazing fields due to indiscriminate disposal of waste [2,3]. The risk factors that predispose sheep to ingest these non-food materials include: deficiency of minerals such as calcium, phosphorus and other micronutrients [4], lack of feed [5], pica [6], lack of owner care, poverty among animal owners hence inability to provide feed, and increased number of animals per given land space [7].

Presence of indigestible foreign bodies in the digestive tract may cause complications in the rumen as well as other organs of the body [8]. The complications caused by the ingested foreign

materials vary with the type of material, duration in the body, location and the degree of interference with flow of ingesta [9]. Some of the gross and histopathological lesions in tissues of sheep with indigestible rumen foreign materials observed at slaughter or post mortem include, sloughed and hyperplastic epithelia; atrophy and loss of ruminal papillae; rumenitis; erosion, ulceration and scarred ruminal pillars and epithelia [10–12]. However, the types of foreign bodies, quantities of those materials, the duration of their presence in the rumen, as well as the degree of obstruction that produced the reported effects in the sheep are unknown, though those of goats have been evaluated [8].

Plastic bags are the most predominant indigestible materials found in the rumen of sheep at slaughter and at necropsy [1,13–15]. However, gross and histopathological changes in sheep due specifically to plastic bags in the rumen have not been previously evaluated as has been done for goats. The current study was aimed at evaluating gross and histopathological changes in sheep experimentally implanted with specific quantities of plastic bags in the rumen over a period of 6 weeks.

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## 2. Material and methods

### 2.1. Ethical approval

Animal use was approved by the Biosafety, Animal use and Ethics Committee (BAEC), of the Faculty of Veterinary Medicine, University of Nairobi, Kenya, according to international standards of animal use in research; clearance certificate number: 11250313.

### 2.2. Experimental animals

Sixteen Dorper sheep with a mean body weight of  $26.7 \pm 1.6$  kg and a body condition score of  $3.5 \pm 0.5$  (on a scale of 1–5) were used. The animals were housed in 4 groups in the stalls at the Large Animal Unit of the Department of Clinical Studies, University of Nairobi, for the whole period of the experiment. The animals were allowed 6 weeks to acclimatize to the environment and the feed. They were fed on chopped Rhodes grass hay and supplemented with commercially produced small stock concentrate meal (UNGA AFYA Meal, UNGA Farm Care Ltd, Nairobi, Kenya). Feed and drinking water were provided *ad libitum*. The sheep were treated against endo-parasites with 2.5% Albendazole (Alfabas<sup>®</sup> Norbrook, Kenya) administered at a dose rate of 4 mL/kg of body weight, P/O. They were also treated against ecto-parasites with Ivermectin (Supermec<sup>®</sup>, Assia Pharmaceuticals, Kenya) at a dose rate of 1 mL/50 kg of body weight, S/C. All the animals were administered 20% injectable Oxytetracycline HCl (Alamycin LA 20<sup>®</sup>, Norbrook, Ireland) at a dose rate of 20 mg/10 kg of body weight, I/M as a prophylactic measure against stress induced by transportation and any bacterial infections. The animals were subjected to routine physical examination over the acclimatization period.

The sheep were assigned into 4 groups of 4 animals each and designated SE1, SE2, SE3 and SC4. Three groups of sheep (SE1, SE2 and SE3) were implanted with 4831 mg/kg live weight (129 g), 9663 mg/kg live weight (258 g) and 14,494 mg/kg live weight (387 g) respectively, of soft plastic bags into the rumen through rumenotomy as previously described by Hendrickson [16]. The plastic bags implanted were the non-perforated small soft polythene bags (KEBS Industries Ltd, Nairobi, Kenya). Each poly bag measured 167 mm  $\times$  290 mm in size, 30  $\mu$ m thick and a packet of 100 pieces weighed 129 g. These were the most common type of plastic bags found in the rumen of sheep and goats slaughtered at two abattoirs in Nairobi [1]. Sheep in the fourth group (SC4) served as control on which rumenotomy was done but with no plastic bags implanted. All animals were routinely monitored for a period of 6 weeks.

### 2.3. Euthanasia of experimental animals

Six weeks post-implantation of plastic bags in the rumen, all the surviving animals were euthanized for postmortem examination. Euthanasia was carried out humanely by sedation using Xylazine hydrochloride at a dosage of 0.2 mg/kg body weight, followed by stunning with a captive bolt pistol after which the animals were exsanguinated.

### 2.4. Postmortem examination

Postmortem examination was done through inspection of individual carcasses and organs of both the control and experimental animals. The carcasses were weighed, then flayed to examine the state of the musculature and to note any abnormalities. The carcasses were then opened and the gastrointestinal tract was examined for gross pathological lesions. The rumen was examined while intact with the contents, and then incised to inspect the contents

and the nature of the implanted plastic bags as previously reported [8]. The contents were removed and the rumen thoroughly washed for better inspection of the wall, mucosa, the papillae and the pillars for any abnormalities. Findings in all tissues were recorded on data collection sheets. Photographs of carcasses and tissues of both experimental and control animals were taken using an iPad 4 with retina display application (Apple Computers Inc, USA).

### 2.5. Histopathological examination

Rumen tissues of 1–2 cm thickness were collected. Twenty (20) samples were collected from each group of sheep from areas with gross pathological lesions as well as areas with no visible pathological lesions. The samples were immediately preserved in 10% buffered formalin and allowed to fix before processing. The formalin fixed tissue specimens were processed for histological examination as previously described by Smith and Bruton [17]. They were then stained with haematoxylin and eosin (H&E) and examined under the light microscope using  $\times 4$ ,  $\times 10$ ,  $\times 40$  and  $\times 100$  objective lenses. The results were recorded and where necessary, photomicrographs were taken using the photomicroscope (Olympus CXSF1, Olympus Corporation, Tokyo, Japan).

## 3. Results

### 3.1. Gross pathological findings

Gross pathological findings observed in carcasses and gastrointestinal tract in sheep implanted with plastic bags for 6 weeks are presented in Table 1. Two out of four sheep implanted with 258 g and 387 g of plastic bags had ascites and generalized oedema and emphysema of the subcutaneous tissues throughout the carcass. All sheep implanted with plastic bags had atrophy of the muscles and body fat which increased in severity with the weight of implanted plastic bags. Sheep implanted with 387 g of plastic bags had atrophy and degeneration of the omental and mesenteric fat.

The rumen had variable lesions including congestion, haemorrhages as well as stunting, bending, atrophy and thinning of the papillae and the pillars (ruminal folds). Areas of erosion and ulcerations were also observed in the rumen of all the sheep implanted with 258 g and 387 g of plastic bags. Scars and nodular lesions were present particularly on the ruminal pillars in all sheep implanted with 258 g and 387 g of plastic bags. The rumen wall was thinner than normal in all sheep implanted with plastic bags, with the thinnest in those with 387 g. The reticular and omasal mucosa was congested and haemorrhagic and the papillae were stunted and thin. In some cases were absent. Abomasal mucosa was congested and haemorrhagic. Overall, pathological changes were more severe in sheep implanted with 387 g of plastic bags than those with 258 g. Two sheep died before the end of 6 weeks, one in each of the groups implanted with 258 g and 387 g of plastic bags respectively. These animals had similar pathological changes as described for the rest. Apart from a slight mottled appearance of the pillars and compressed papillae of the rumen observed in 2 of the sheep implanted with 129 g of plastic bags, the remaining 2 and the control sheep did not show gross pathology at the end of the 6 weeks. The gastrointestinal tract distal to the reticulum was empty of any ingesta in all the sheep implanted with 258 g and 387 g of plastic bags.

### 3.2. Histopathological findings

Histopathological findings of rumen tissues from sheep in both control and experimental groups are presented in Figs. 1–4. In the control sheep all the papillae were present, well developed and

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