Abosse *et al.*
Surgical and Experimental Pathology (2022) 5:14
https://doi.org/10.1186/s42047-022-00116-8

Surgical and Experimental Pathology

RESEARCH Open Access

Comparative study on pathological changes in sheep and goats experimentally infected with *Haemonchus Contortus*

Jirata Shiferaw Abosse[1*], Getachew Terefe[1] and Bethlehem Mesfin Teshale[2]

Abstract

Background: The parasites causes localized and generalized infections to the host depends on the parasite attachment organs, depth of penetration, site of location and worm burdens.

Methods: The experimental study was done between December, 2019 and April, 2020 in the fly-proof experimental animal facility located in the premise of the College of Veterinary Medicine and Agriculture at Bishoftu, Ethiopia. A total of 14 male goat (G1 and G2) and 14 male sheep (G3 and G4) were allotted in to four equal groups. Single dose of 10,000 of infective larvae of *Haemonchus contortus* (L3) was orally administered to each animal in G1 and G3. Parameters such as body weight, PCV, haemoglobin, worm count, serum total protein, serum albumin, alkaline phosphatase and aspartate aminotransferase were measured. Moreover, histopathological sections were stained and examined for general changes as well as for changes in specific cells such as tissue eosinophilia and parietal cell population.

Results: The findings show that 1) All infected sheep and goats developed the infection with higher mean worm burden in goats (5590) than sheep (2887) and the difference was significant ($P < 0.05$); 2) All infected sheep and goats exhibited a progressive anaemia; the level being more severe in goats than in sheep ($P < 0.05$) with mean PCVs of 13 and 18.6% respectively; 3) While body weight gain was minimal in sheep, goats have lost significant weight compared to pre-infection levels, to control animals or in relation to infected sheep ($P < 0.05$); 4) analysis of biochemical changes revealed marked reduction in serum total protein and albumin which was much more significant in goats than in sheep ($P < 0.05$); 5) the abomasum of infected sheep and goats have shown thickening, nodule development, eosinophilic infiltration and damage to parietal cells. Tissue eosinophilia was more prominent in sheep while parietal cell loss was severe in goats.

Conclusion: In conclusion, goats under experimental infection and similar management condition with sheep develop much more severe infection and associated pathology compared to sheep and hence deserve special attention.

Keywords: Anaemia, Biochemical changes, *H. contortus*, Histopathology, Goat, Sheep

Introduction

Gastrointestinal parasitism is an important problem of livestock in many places of the globe (Vercruysse and Claerebout 2001). Its economic impact due to production losses, mortality and costs of treatment can be huge in susceptible populations (Hawkins 1993; Selemon 2018). Among the most pathogenic and highly prolific gastrointestinal tract (GIT) helminthes is the blood feeding

*Correspondence: jirata474@gmail.com

[1] Department of Pathology and Parasitology, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia
Full list of author information is available at the end of the article

abomasal parasite, *Haemonchus species* affecting ruminants and camels (Urkuhart et al. 1996).

Abomasum is in fact one of the most important sites for nematodes belonging to *Trichostrongylidae* family in small ruminants. It is the site for four species of GI nematodes; *Haemonchus* spp., *Teladorsagia* spp., *Ostertagia* spp. and *Trichostrongylus spp* (Sultan et al. 2010; Vlassoff and McKenna 2010). Although *Haemonchus* infections are reported in climates ranging from tropical to temperate, it has a more significant impact in tropical and subtropical regions of the world (Besier et al. 2016; Selemon 2018). High humidity, at least in microclimate of the faeces and the herbage is essential for larval development and survival.

Surveys in countries around the world have shown that amongst domestic animals, sheep and Goats suffer more frequently from haemonchosis (Maqsood et al. 1996; Nwosu et al. 2007; Tariq et al. 2008). Similar reports have also confirmed that *Haemonchus contortus* is the most important GI parasite in sheep (Fleming et al. 2006; Sharma and Ganguly 2016).

H. contortus sucks about 0.05 ml of blood per day by ingestion (Qamar and Maqbool 2012) and an additional blood is also lost into the gastric lumen because of mucosal irritation and oozing out from when the parasite is detached from the feeding site altogether leading to mild to severe anemia. The pathological consequences of helminth parasitism and the ability of the host to develop immunity and express resistance against them depends on a number of factors; host traits (species, breed, age, physiological status, feeding behavior) and worm characteristics (burden, feeding behavior, lifecycle) (Hoste et al. 2008; Rowe et al. 2008).

For example, Amarante et al. (2007) found an inverse relationship between inflammatory cells and worm burden in sheep infected with *Trichostrongylus colubriformis* and suggested that this condition possibly impaired establishment, development and survival of parasite. Similarly, Terefe et al. (2007a) has demonstrated that resistant breeds of sheep had higher number of circulating eosinophils. In addition, Terefe et al. (2007b) has also demonstrated that in vitro pre-exposure of *Haemonchus contortus* L3 to blood eosinophils reduces their establishment potential in sheep.

Reports from many studies show controversial findings with regards to GIT helminthic profiles between sheep and goat. Although they are kept in the same geographical area (Mushonga et al. 2018), the prevalence of haemonchosis is higher in sheep than in goats. However, whether this difference between host species is due to the inherent factors of the hosts or due to difference in their exposure to the parasite owing to their feeding habit is not clear. On the contrary, Kumsa et al. (2011) has reported an almost equivalent infection rates between sheep and goats in central Oromia of Ethiopia while Tony (2007) described that goats appeared to be more susceptible to helminthes than sheep.

Such variations may be attributed to differences in host breed, animal feeding and management and/or parasite factors (strain and host-adaptation). Obviously, such inconsistency in the host-parasite association could be accompanied by less defined local and systemic pathophysiological changes. As an explanation to such variations and to aid the understanding of the role of host species on the development of gastrointestinal parasitism, it is essential to study the degree and type of pathological consequences occurring in sheep and goats infected with specific parasite, *H. contortus*. We hypothesize that sheep and goats show similar pathophysiological responses if exposed to similar risk of helminthic parasite infection under similar management condition.

This study aimed to demonstrate pathophysiological and histopathological changes and to compare the changes between the two species of hosts experimentally infected with H. countortus using selected indicators.

Materials and methods

Study area

The experimental study was done between December, 2019 and April, 2020 in the fly-proof experimental animal facility located in the premise of the College of Veterinary Medicine and Agriculture at Bishoftu. Bishoftu is located at a distance of about 47 km South East of the capital city of Ethiopia, Addis Ababa. The area is located at an altitude of 1850 m above sea level. It experiences a bimodal pattern of rain fall with a long rainy season from June to October and a short rainy season from March to May and has an average annual rainfall of 800 mm. The area has an average maximum and minimum temperature of 27.7 °C and 12.3 °C respectively (CACC, 2003). The animal house was equipped with different pens containing feeding and watering troughs.

Experimental animals and grouping

All sheep and goats used for the experimental study were purchased from Assela open market. Before, forming experimental groups, the animals were acclimatized for 1 month, during which they were monitored for exposure to helminth eggs by treating them with broad spectrum anthelmintics and sprayed with acaricides. The changes in body weightand general health were also monitored. All animals were housed in four separate boxes with raised concrete based units and a solid partition separated by adjacent pens. Care was taken to avoid contamination of pens with nematode larvae from outside by wearing boots before enter to each pens of the animals.

Two groups of sheep (infected and negative control) and two groups of goats (infected and control) each with seven animals were formed.

Animals were allowed to feed dried hay with sufficient quantities of concentrate feed and water throughout the adaptation and experimental period. Animals were handled and managed according to guiding principles of ethical use and management of experimental animals.

H. contortus isolation and preparation

The adult female *Haemonchus contortus,* were isolated from abomasums of freshly slaughtered goats originating from Borena area. The worms were pooled and homogenized to release the eggs. A fecal material was collected from apparently helminth free animals, tested for absence of eggs and the prepared eggs seeded in it. The mixture was then kept at room temperature for 14 days with frequent mosturization and aeration. Larvae was recovered by the modified Baermann technique and stored for 3 weeks at the temperature of +4 degree centigrade until donor animals were infected for further propagation. Two donor sheep were infected with 10,000 L3 of the prepared larvae. After 3 weeks, following appearance of a good number of fecal eggs, large volumes of fecal materials were collected daily to culture and recover large quantity of L3 for infecting experimental groups of sheep and goats.

Experimental design and animal infection

At the end of the adaptation period animals were weighed and ear tagged for easy identification. A randomized block design was used where goats and sheep were allocated into four groups based on their body weights. Group 1 (G1) was infected goat, Group 2 (G2) non-infected goat, Group 3 (G3) infected sheep and Group 4 (G4) non-infected sheep (Table 1). Each group consisted of seven (7) animals. Group 1 and 3 (G1 and G3) were infected by oral route with 10,000 *H. contortus* L3/animal (all at once) as described in Terefe et al. (2007a).

Data collection

Clinical observations

The experimental animals were thoroughly observed daily for clinical changes with special attention to appetite, general body condition, colour of visible mucous membranes and consistency of the faeces.

Body weight measurement

The animals were weighed weekly for the whole duration of the experiment using spring balance. The body weight of each animal was recorded starting from day0 and all the way through the end of the experiment (D56).

Confirmation of infection

Animals in all groups were monitored for faecal egg by using faecal floatation technique (Urquhart et al. 1996). Once, eggs were detected, animals were periodically tested to confirm that the infection persisted throughout the experiment.

Measurement of anaemia

Blood samples were taken weekly from Day0 to Day56 from each individual sheep and goat in order to determine packed cell volume (PCV) and haemoglobin (Hgb) concentration. About 4 ml of blood was collected into EDTA coated vacutainer tube by puncturing the jugular vein. The packed cell volume was determined using microhaematocrit technique (Jain 1986). For PCV determination fresh blood samples were drawn in capillary tubes and centrifuged in a microhaematocrit centrifuge (Hawksley and sons Ltd. England) at 12000 rpm for 5 min. The PCV percent was read by using a microhaematocrit reader. Haemoglobin concentration was measured by Sahli"s method (acid haematin method) as described by Jain (1986). The method depends on the conversion of haemoglobin to acid haematin by adding a small amount of diluted hydrochloric acid. The resulting brownish-yellow colour was matched with the standard colour of the apparatus. The reading was converted to g/dl.

Serum protein

Blood sample for the evaluation of biochemical parameters was drawn from the jugular vein and transferred to disposable blood collecting tubes (BD vacutainer stubs) and allowed to coagulate at 37°c for 30 min. The serum

Table 1 Experimental groups and sampling schedule

Group	Infection status	Sampling days					
G1	Infected goats (Day0)	D0	D7	D14	D21	D49	D56
G2	Non- Infected goats						
G3	Infected sheep (Day0)						
G4	Non- Infected sheep						

was separated by centrifugation at 3000 rpm for 10 min and transported to the laboratory (Arsho Laboratories PLC-Addis Ababa) in a thermacool box Total serum protein (TSP) and serum albumin were analysed.

Total serum protein and albumin serum determination

The system reagent for the quantitative determination of total protein and albumin in serum were using Beckman Coulter AU analysers (Walker et al. 1990). For determination of total serum protein concentrations using the modification of Weichselbaum method were employed. Cupric ions (Cu^{2+}) in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a blue violet colored complex. For determining albumin concentrations in serum were using the modified the Doumas and Rodkey methods. The albumin reacts with pH 4.2 neutral buffered solution of bromocresol green to form an intense green complex. The results were given the analyser machine by automatically printed out for each sample in g/dl.

Gross examination of the abomasum

At the end of the experiment, 56 days post infection (PI), all sheep and goats were killed humanely. The entire abomasum from each control and infected animal was examined visually for presences gross lesion such as hemorrhages, nodules, ulcerations and the presence of adult worms parasites. The contents of infected abomasum were recovered and preserved in 70% alcohol. The volume of the material was adjusted to 1 l and worms were counted in 10% aliquot as described previously by Terefe et al. (2005).

Histopathology

Representative samples were taken from parasite specific predilection sites such as the fundic and pyloric regions. About $1cm^2$ tissue samples from both infected and control animals were collected and preserved in 10% buffered formalin for observing eosinophil and description of tissue damages. After routine histological processing, 5 µm tick paraffin-embedded tissue sections were prepared. These were then be deparaffinised and stained with haematoxylin and then counterstained with eosin according to the methods described by Winsor (1994). The slides were examined at × 400 magnification in 10 microscopic fields.

Statistical analysis

Data were recorded in Excel spread sheet and summarized with descriptive statistics (means, standard error and percentages) were calculated. Means were compared among groups through analysis of variance (ANOVA) and analysed using STATA version 12.0 (Stata Corporation 2009).

Results

Parasite establishment

Fecal eggs were first detected on day 21 post infection (PI) and persisted throughout the experimental period in all infected animals. No worm eggs were demonstrated in the non-infected sheep and goat at all times. However, the infected groups (G1 (infected goats) and G3 (infected sheep)) were confirmed at postmortem by the presence of numerous adult worms on the surface of the abomasal mucosa. The mean numbers of *H. contortus* recovered at necropsy (Table 2) from abomasum of G1 and G3 animals were respectively (5590 ± 1927.546) and (2887.143 ± 2746.232), the difference between both groups being significant ($P < 0.05$).

Clinical observations

No clinical signs were observed in uninfected control group during the experimental period. Characteristic signs observed in infected groups showed depression, variable degree of inappetance, pale mucous membrane and reduced of body condition (Fig. 1).

Body weight changes

There was no significant variation in the body weights of all the four groups of animals at the beginning of the experiment since they were grouped by blocking body weight. The weight of animals in the negative control groups (G2 and G4) had gradually increased or remained unchanged throughout the experimental period. In response to *H. controtus* infection, goats (G1) and sheep (G3) have responded very differently on their body weight changes (Fig. 2).

Infected goats (G1) have shown progressive decline while, infected sheep (G3) groups displayed mean body weights that more or less maintained their pre-infection values. At the end of the experiment at 56 days of post-infection(D56 PI), G1 had significantly lower mean body weight than animals in G2 with mean values of 19.7 ± 1.3 kg and 24.9 ± 4.9 kg respectively and the

Table 2 Mean worm burden, goats and sheep experimentally infected with *H. contortus*

Worm burden	Group	Mean + SD	95% CIfor Mean	P -value
Adult parasite	G1	5590 ± 1927.546	3807.317–7372.683	< 0.05
	G3	2887.143 ± 2746.232	347.3018–5426.984	

Fig. 1 Development of anemia as expressed by change in the color of the mucousmembrane: Goat with pinkish mucous membrane (left: non-infected), and pale mucous membrane (right; infected)

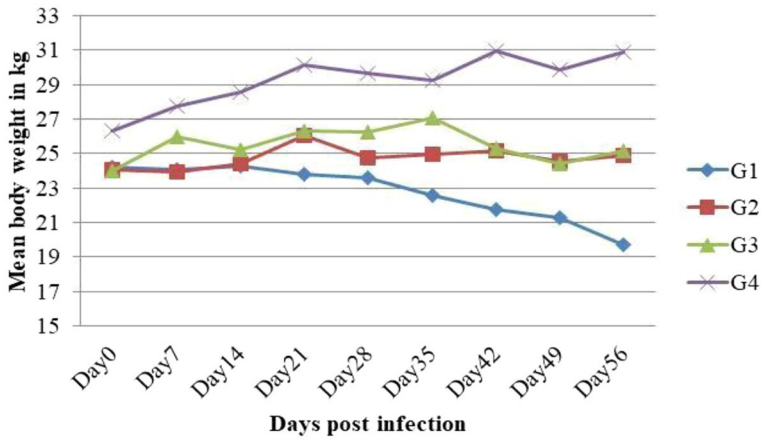

Fig. 2 Mean body weight of infected G1 and G3) and non-infected (G2 and G4) groups

difference was significant ($P<0.05$). On the same measurement point, no significant difference in body weight was observed between G3 and G4 sheep ($P>0.05$). On the other hand, although there was no difference at the start of the experiment, infected goats have lost significantly higher body weight (19.6% loss) than infected sheep (4.75% gain) at the end of the experiment (**$P<0.05$**) compared to day0 values.

Hematological finding

Changes in packed cell volumes (PCV)

At the beginning of the experiment, all four groups had comparable PCV values. While these values remain almost at pre-infection level for the non-infected control groups, PCV progressively declined until the end of the experiment in the infected groups (Fig. 3). Hence, for most part of the experimental period, PCV of infected animals was significantly lower than that of their control counterparts ($P<0.05$); attaining values of $13\pm1.41\%$ and $29.86\pm2.67\%$ in infected and control goats respectively and $18.57\pm5.35\%$ and $29.17\pm2.48\%$ in infected and control sheep respectively at the end of the experiment. When this measurement was compared between infected groups (G1 and G3), it was significantly lower for G1 compared to that of G3 ($P<0.05$) as of D28 PI.

Haemoglobin (Hgb) concentration

A significant reduction from pre-infection level ($P<0.05$) of Hgb concentration was observed as of Day14 and Day28 PI in all infected goats and sheep respectively (Fig. 4). This was also true between infected and control groups. At the end of the experiment, G1 had mean Hgb concentration of 7.23 g/dl while group 3 had mean Hgb of 8.56 g/dl but with no significant difference ($P>0.05$). The change in blood hemoglobin concentration was directly proportional to the decline in PCV ($R=0.6373$, $P<0.05$).

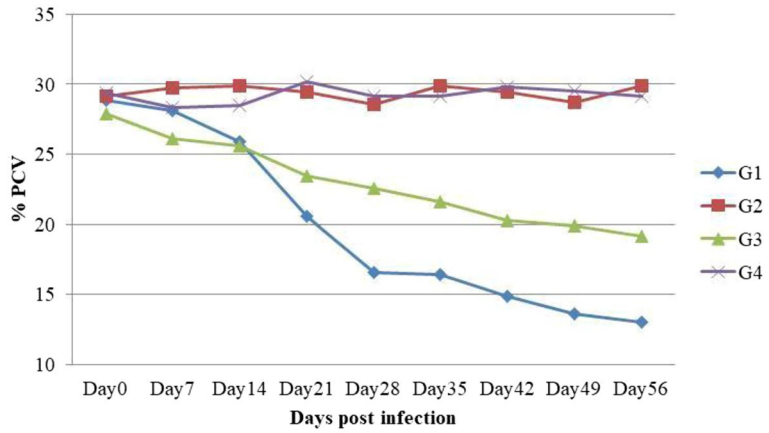

Fig. 3 Comparison of mean PCV values in the four experimental groups

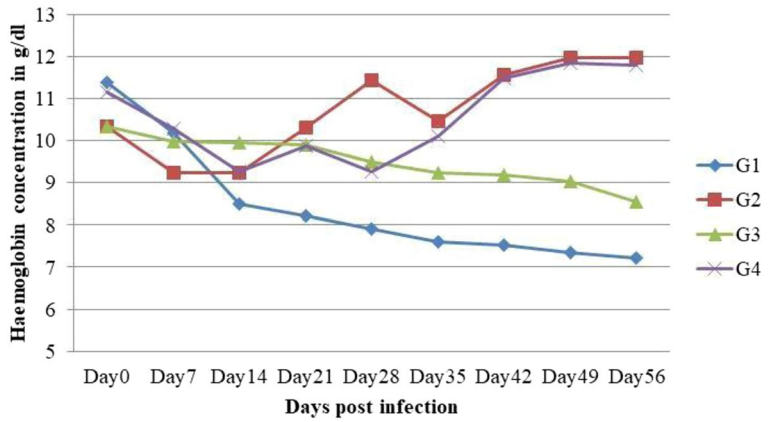

Fig. 4 Mean haemoglobin concentration in infected and control goat and sheep

Serum biochemical changes

Serum total protein and albumin levels

At the beginning of the experiment (D0), the concentration of total serum protein was similar between the four groups (Fig. 5). Significant fall from the initial values was observed in G1 on days 28 and 56 PI ($P<0.05$) respectively. Similarly, when infected (G1) and non-infected (G2) goats were compared, TSP was much lower in G1 than in G2 ($P<0.05$). Comparison of TSP between infected sheep and goats also revealed TSP was significantly lower in G1 than in G3 ($P<0.05$) on day 28 PI. Because there were notable variation between groups at day0 (before infection), albumin concentration in g/dl was transformed into percentage changes in concentration with values at day0 taken as 100%.

With a similar pattern to the observation for TSP, albumin concentration in group G1 has sharply declined on day28 PI compared to its initial value before infection ($P<0.05$). Infected goats have also shown significantly reduced serum albumin level compared to the non-infected control group ($P<0.05$) of goats at 28 and 56 days PI and infected sheep ($P<0.05$) at day 28 PI. Such difference was not detected in sheep when compared to initial readings and between G3 and G4 (Fig. 6). The changes in serum albumin concentration was directly proportional to the decline in total serum protein ($R=0.8683$, $P<0.05$).

Gross and histopathological findings

Macroscopic findings

Harbouring variable number of worms, the abomasum was endowed with gross lesions such as thickening of mucosal folds, petechial haemorrhages and nodule development which were more visible on the fundic region in both infected sheep and goats (Fig. 7). Such lesions were more prominent in goats than in sheep.

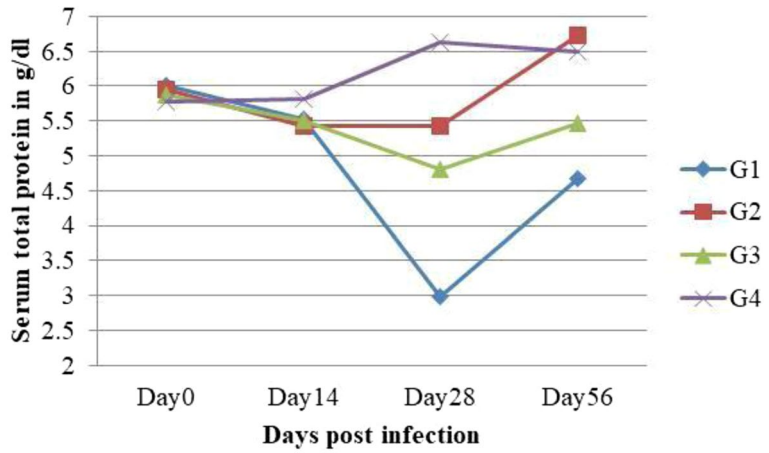

Fig. 5 Mean serum total protein concentration in infected (G1, G3) and non-infected (G2, G4) goat and sheep

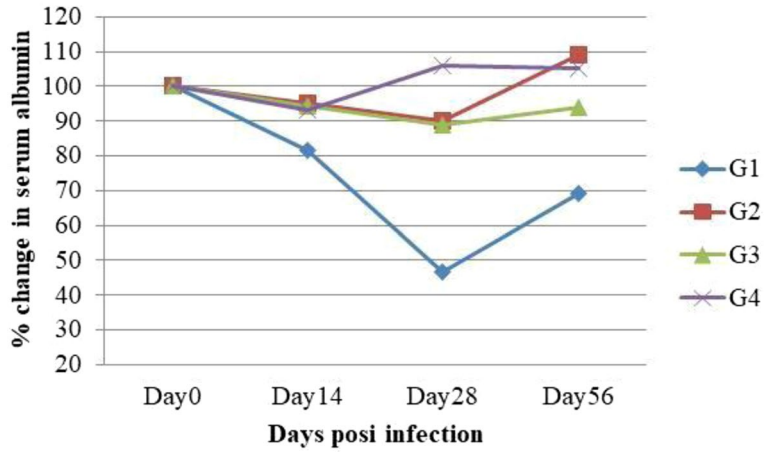

Fig. 6 Percentage changes in mean serum Albumin concentration in infected (G1, G3) and non-infected (G2, G4) goat and sheep

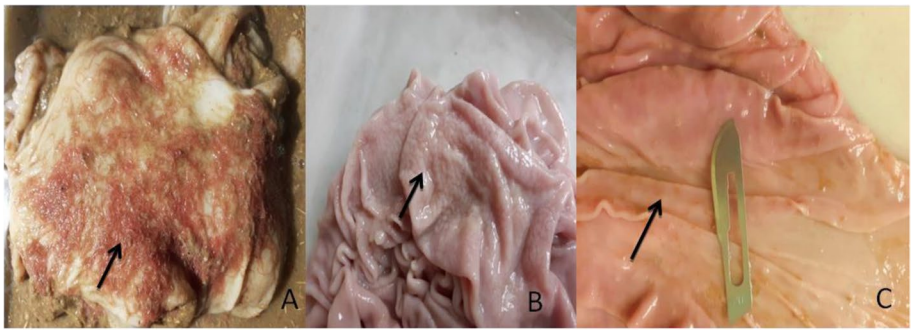

Fig. 7 Abomasum of goat infected with 10,000 L3 of H. controtus: (A) large number of adult worms; (B) thickened and rough mucosal folds (arrow), (C) nodular lesion (blade) and petechial haemorrhages (arrow) in the fundic region

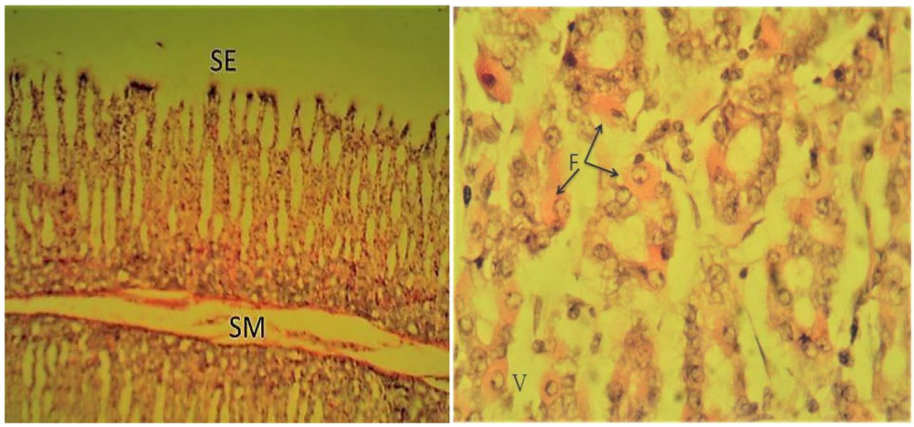

Fig. 8 Histological section of non-infected abomasum of sheep showing intact surface epithelium with goblet cells (SE), the sumbucosa (SM), pink staining normal parietal cells (F) with numberous chief cells nearby. (HE staining, 10x and 40x magnification)

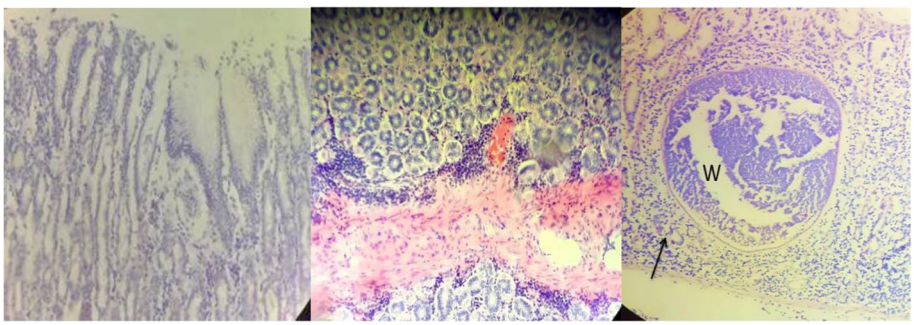

Fig. 9 Histopathological changes in the abomasal fundus of H. contortus infected goat under low magnification (10x): (A) denuded surface epithelium with damaged gastric pits, (B) high cellular infiltration and hemorrhagic submucosa, (C) tissue doweling worm section (W) surrounded by cellular infiltration (arrow); (HE staining); Where A, B and C are from left to right right respectively

Microscopic findings

Histological examination of the fundic region of the non-infected abomasums revealed intact surface epithelium with goblet cells and glandular structure with few leukocyte populations in the lamina propria and submucosa (Fig. 8). On the other hand, denuded surface epithelium, damaged gastric pits, hemorrhagic submucosal layer as well as a cross-section of tissue doweling immature worm were observed at low magnification in infected sheep and goats (Fig. 9). At higher magnification, degenerating chief and parietal cells, as well as very high cellular infiltration dominated by eosinophils were a common characteristics of infected abomasal. Tissue eosinophilia was more extensive in sheep both in terms of distribution along the different layers of the mucosa and the number of cells under a single field of vision (Fig. 10).

Discussion

Both sheep and goats have developed pathological changes due to *H. controtus* infections

The pathology caused by parasites and the host reaction in response to parasitic infection depends on the parasite attachment organs, depth of penetration, site of location and worm burdens (Esmaeilnejad et al. 2012; de Oliveira et al. 2013). In this study, both infected sheep and goats have demonstrated one or more signs of systemic and gastrointestinal disturbances such as depression, reduced appetite and mild to intense pale mucus membrane especially in goats, loss of body condition and body weight during the first few weeks. Similar findings have been reported in other studies (Fox 1997; Saminathan et al. 2015). Such changes in health condition may be related to local pathological changes in the abomasum and/or systemic changes resulting from anemia and gastric secretions such as the hormone gastrin, release of enzymes such as pepsin and the acid HCl (Fox 1997; Lawton 1996; Pérez et al. 2001).

In agreement with the above findings, both infected sheep and goats had reduced PCV and hemoglobin concentration the level of which varied with the number of adult worms (Clark et al. 1962). This supports the report of Rouatbi et al. (2016) in a study on the effect of *H. contortus* on the haematological values of

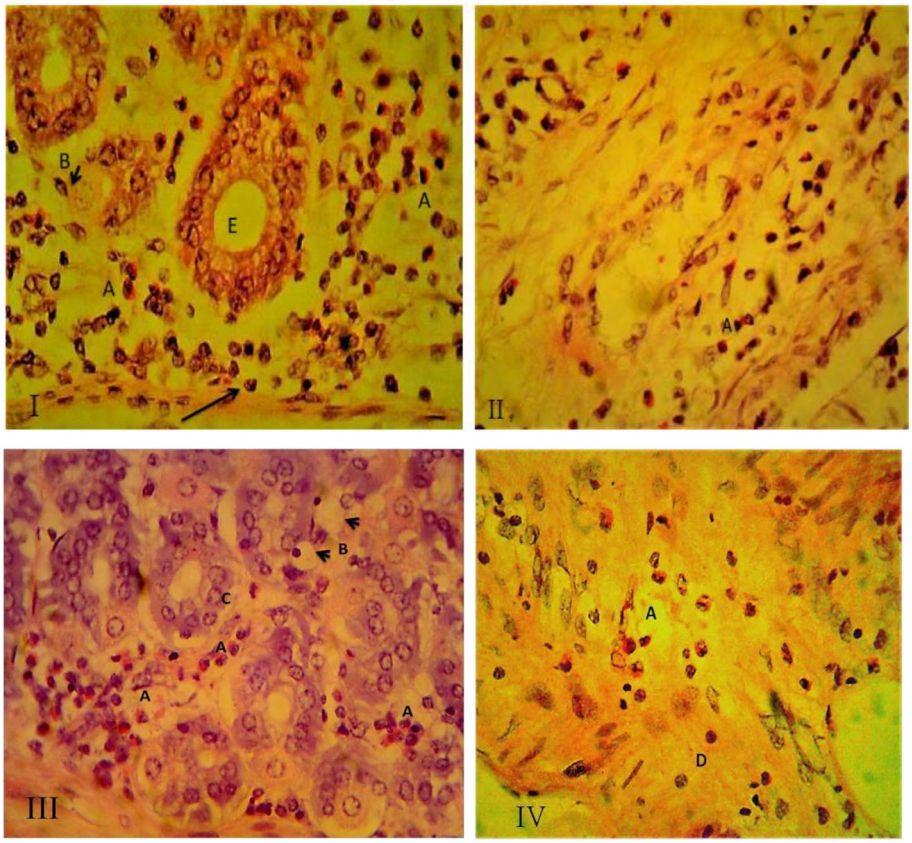

Fig. 10 Histopathological changes in the abomasal fundus of representative goat (I and II) and sheep (III and IV) infected with H. contortus: (A) numerous pink staining eosinophils in the lamina propria and the submucosa, (B) damaged/degenerating parietal cells, (C) intact chief cells, (D) neutrophil and lymphocyte (also arrow in I) in the submucosa, (E) body of abomasal gland; HE staining, 40x magnification

rams in South Africa. Body weight reduction was more marked in goats and can be related to the reduced appetite, loss of serum protein and digestion problems linked to disturbances in gastric secretion (Fox 1997). Similar reduction in body weights have been documented by previous studies in sheep and/goat (Kelkele et al. 2012; Starling et al. 2019).

On the other hand, Rouatbi et al. (2016) reported that live weight was not different between infected and non-infected rams despite infection with 30,000 L3 of *H. contortus*. This difference could be attributed to the level of previous exposure to the infection or genetic resistance of the animals. Infected goats have lost significant amount of serum protein during the infection. This agrees with the reports of Fausto et al. (2014) and Rouatbi et al. (2016). As serum albumin was reduced proportional to the reduction in total serum protein, it is expected that serum globulin concentration, which is a marker of the level of immunoglobulin production (Bisher 1990), also follows the same pattern of reduction.

Gross and histological examinations have revealed that both infected sheep and goats have shown characteristic lesions of various degrees. Such changes have also been reported by previous studies (Fox 1997; Tehrani et al. 2012). These lesions could be responsible for some of the changes in blood protein and systemic signs of reduced appetite and reduction in body weight gain compared to the non-infected control groups.

Goats have affected more than sheep from *H. contortus* infection

Haemonchus controtus worm burden was much higher in infected goats than in sheep suggesting that the former was unable to control the establishment and persistence of the parasite in the abomasum. In this regards, several previous researches have reported conflicting findings. In support of the current finding, Tony (2007) described that goats appeared to be more susceptible to helminthes than sheep. This might be due to the fact that sheep are more resistant to the parasitic infection as they are able to elicit a strong immune response (Watson and Hosking

1989). It might also be possible that, goats as browsing animals are less adapted to the feed (grass hay) provided to them throughout the experimental period which might have consequently reduced their resistance. On the contrary, helminth prevalence was more significant in sheep for Mushonga et al. (2018) and equally important in both species for Kumsa et al. (2011).

Animal genetic and physiological traits, animal management, parasite strain and method of study could be possible factors for such variation. Infected goats had significantly lower body weight, hematocrit and hemoglobin values compared to sheep infected with equivalent number of worms and managed under similar in house condition. The large nematode burden in goats may be responsible not only for these changes but also for the lower serum protein values. The larger the number of *H. contortus* parasites, the more blood it consumes and the more anemic the animal would be (Clark et al. 1962; Hoste et al. 2008). Such large population of the abomasal parasite in goats could also be responsible for greater damage to the abomasal tissue subsequently leading to destruction of parietal cells and hence reduced acid secretion and reduced protein digestion with losses through the GIT and hence the animal becomes unable to gain weight (Fox 1997).

Decline in the total serum proteins might also be attributed to haemodilution, which is a compensatory mechanism for the abomasal haemorrhage caused by the parasite leading to the loss of large quantities of serum protein into the gut (Kelkele et al. 2012). Furthermore, due to abomasal haemorrhage haemodilution occurs which can cause relative hypoproteinemia and hypoalbuminemia (Angulo-Cubillán et al. 2007; Sathis et al. 2017). Weight loss during GIT parasitism could also be ascribed to the reduction in appetite as a result of the heavy infection (Fox 1997). Loss of protein also means that, the animal will be deficient in major ingredient to mount adequate level of protective antibody responses (Bisher 1990).

In agreement with previous reports, (Mannan et al. 2017), marked thickenings of the abomsal mucosa were observed in both infected sheep and goats suggesting that the organs have undergone marked inflammatory processes. Eosinophils are said to be one of a Th2 type effector cells responsible to defend the host against helminthic parasitism (Balic et al. 2006; Terefe et al. 2007a). It has been demonstrated that activated eosinophils in the presence of antibodies and/or complement proteins can effectively kill *H. contortus* larvae in vitro (Rainbird et al. 1998; Terefe et al. 2007a).

The lower resistance of infected goats in this study were explained by the less population of tissue eosinophil observed compared to the situation in infected sheep where the submucosa and the laminapropria as well as interglandular spaces were highly infiltrated. A report by Terefe et al. (2009) has clearly shown that an apparently nematode resistant breed of sheep, the Barbados black belly, had huge blood and tissue eopisophilia as compared to the susceptible INRA 401 breed suggesting that these cells have a potential to limit helminthic development in the host.

Marked increase in the secretion of mucus by mucous cells together with an abundant infiltration of eosinophils, mast cells and globule leukocytes were recorded in the abomasal mucosa especially in the early stages of infection with *H. contortus* (Pérez et al. 2001) suggesting that this cell type may have been involved in rejection of adult nematodes in resistant species as compared to the more susceptible ones. Similar to the findings of our study, other studies have also reported development of numerous nodular lesions with thickening of the fundic mucosa, reduction in the population of parietal cells followed by mucous cell hyperplasia in the fundic mucosae of sheep infected with adult *Ostertagia circumcinta* (Scott et al. 1998).

In conclusion, this study was executed to demonstrate development of pathological features in sheep and goats experimentally infected by *H.contortus* and compared the degree of pathological changes between the two hosts. In addition the study confirmed that both hosts have developed the infection and expressed various clinical, gross and histopathological as well as serum biochemical changes. It was clearly demonstrated that under similar feeding and management conditions and with experimental challenge infection of 10,000 L3 per animal, goats were more susceptible to the infection where significantly larger number of parasites was recovered at the end of the experiment. Accompanying with the difference in worm burden, goats have shown marked anemia, reduced total serum protein, serum albumin, and tissue eosinophilia; all suggesting goats have developed more pathology than sheep. Moreover, in addition to the loss of appetite, the reductions in the number of functional parietal cells seem to have contributed in the reduction in protein utilization and consequently reduction in body weight.

Abbreviations

ANOVA: Analysis of variance; cm: centimeter; CACC: Central Agricultural Census Commission; GIT: Gastro-intestinal tract; g/dl: gram/decileter; Hgb: Hemoglobin; HCl: Hydrochloric acid; km: Kilometer; ml: milileter; mm: milimeter; µm: micrometer; PCV: Packed cell volume; PI: Post-infection; TSP: Total serum protein.

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization and designing of the study were by Getachew Terefe and Jirata Shiferaw. Preparation of materials, follow-up of experimental animals and data collection were performed by Bethlehem Mesfin. The first draft of manuscript

was written by Bethlehem Mesfin and Getachew Terefe. Final paper was edited by Jirata Shiferaw. All authors were contributed to the study in design, analysis, interpretations, reviewed and complemented the manuscript. All authors read and approved the final manuscript for publishing on this journal.

Funding
This study was financed by Addis Ababa University Research and Technology Transfer from thematic research fund entitled "BIOCON" supervised under CVMA-AAU.

Availability of data and materials
The datasets used and/or analysed during the current study are available in the main text and from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All animals were managed following standard ethical principles for use of laboratory animals with permission from the Animal Research Ethics Review Committee of the college of Veterinary Medicine and Agriculture (Certificate Ref. No: VM/ERC/29/01/12/2020).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Pathology and Parasitology, Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia. [2]Semera University, College of Veterinary Medicine, Semera, Ethiopia.

Received: 25 March 2022 Accepted: 22 May 2022
Published online: 20 July 2022

References

Amarante A, Rocha R, Bricarello P. Relationship of intestinal histology with the resistance to *Trichostrongylus colubriformis* infection in three breeds of sheep. Pesq Vet Bras. 2007;27(1):43–8.

Angulo-Cubillán FJ, García-Coiradas L, Cuquerella M, Fuente C, Alunda JM. *Haemonchus contortus*-sheep relationship: A review. Relación *Haemonchus contortus*-ovino: una revisión. Rev Cient, 2007; 17: 577–587.

Balic A, Cunningham CP, Meeusen ENT. Eosinophil interactions with *Haemonchus contortus* larvae in the ovine GIT. Parasite Immunol. 2006;28:107–15.

Besier RB, Kahan LP, Sargison JA. Vanwyk, JA. The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in small ruminants. Adv Parasitol, 2016; 93: 1–49.

CACC. Central agricultural census commission. Addis Ababa Ethiopia, 2003.

Clark CH, Kiesel GK, Goby CH. Measurement of blood loss caused by *Haemonchus contortus*. Am J Vet Med. 1962;23:977–80.

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B. Evaluation of antioxidant status and oxidative stress in sheep naturally infected with *Babesia ovis*. Vet Parasitol. 2012;185(2):124–30.

Fausto GC, Pivoto FL, Costa MM, Lopes dAT, Franca RT, Molento MB, Minervino AHH, da Rocha JBT, do Rego leal ML. Protein profile of lambs experimentally infected with *Haemonchus contortus* and supplemented with selenium copper. Parasit Vectors, 2014; 7:355.

Fleming S, Craig T, Kaplan R, Miller J, Navarre C, Rings M. Anthelmintic resistance of gastrointestinal parasites in small ruminants. J Vet Inter Med. 2006;20(2):435–44.

Fox MT. Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent development. Vet Parasitol. 1997;72:285–308.

Hawkins JA. Economic benefits of parasite control in cattle. Vet Parasito. 1993;l46:159–73.

Hoste H, Torres-Acosta J, Aguilar-Caballero A. Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes. Parasite Immunol. 2008;30:79–88.

Jain N. Schalm"s Veterinary Heamatology. 4 th Edition. Philadelphia. : Leaand Febiger. 1986; Pp. 1221.

Kelkele F, Tolossa Y, Kassa G. Experimental infection of Ethiopian highland sheep by different infective doses of *Haemonchus contortus* (L3): haematological and parasitological parameters, serum protein concentrations and clinical responses. Eth Vet J. 2012;16(1):41–57.

Kumsa B, Tadesse T, Sori T, Duguma RA, Hussein B. Helminths of sheep and goats in Central Oromia (Ethiopia) during the dry season. J Anim Vet Adv. 2011;10(14):1845–9.

Lawton D, Reynolds G, Hodgkinson S, Pomroy W, Simpson H. Infection of sheep with adult and larval *Ostertagia circumcincta*: effects on abomasal pH and serum gastrin and pepsinogen. JVetInter parasitol. 1996;26:1063–74.

Mannan MA, Masuduzzaman M, Rakib TM, Chowdhury S, Hossain MA. Histopathological and haematological changes in haemonchosis caused by *Haemonchus contortus* in small ruminants of Bangladesh. BJVAS. 2017;5(2):17–23.

Maqsood M, Iqbal Z, Chaudhary A. Prevalence and intensity of Haemonchosis with reference to breed, sex and age of sheep and goats. Pak Vet J. 1996;16(1):41–3.

Mushonga B, Habumugisha D, Kandiwa E, Madzingira O, Samkange A, Segwagwe B, Jaja F. Prevalence of *Haemonchus contortus* infections in sheep and goats in Nyagatare District, Rwanda. J Vet Med. 2018;9.

Nwosu C, Madu P, Richards W. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of North-Eastern Nigeria. Vet Parasitol. 2007;144:118–24.

de Oliveira R, Senger M, Vasques L, Gasparotto J, dos Santos J, de Bittencourt Pasquali M, Moreira J, Silva F, Gelain D. *Schistosoma mansoni* infection causes oxidative stress and alters receptor for advanced glycation end-product (RAGE) and tau levels in multiple organs in mice. Int J Parasitol, 2013; 43(5): 371–379.

Pérez J, Garcia PM, Hernandez S, Martinez-Moreno A, de las Mulas JM, Camara S. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*. Vet Res. 2001;32:463–73.

Qamar MF, Maqbool A. Biochemical studies and serodiagnosis of haemonchosis in sheep and goats. J Anim Plant Sci. 2012;22:1.

Rainbird MA, Macmillan D, Meeusen ENT. (): eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. Parasite Immunol, 1998; 20: 93–103.

Rouatbi M, Gharbi M, Rekik M. Effect of the infection with the nematode *Haemonchus contortus* (*Stronglida*: *Trichostrongylidae*) on the haematological, biochemical, clinical and reproductive traits in rams. OJVR, 2016; 83(1): 1–8.

Rowe A, McMaster K, Emery D, Sangster, N. *Haemonchus contortus* infection in sheep parasite fecundity correlates with worm size and host lymphocyte counts. Vet Parasitol, 2008; 153: 285–293.

Saminathan M, Gopalakrishnan A, Latchumikanthan A, Milton A, Aravind M, Dhama K, Singh R. Histopathological and parasitological study of blood-sucking *Haemonchus contortus* infection in sheep. Adv Anim Vet Sci. 2015;3(2):99–108.

Sathis KS, Veerakumari L, Soundarajan. In vivo studies on the anthelmintic efficacy of ethanol extract of *syzygium aromaticum* against *haemonchus contortus*. Life SciJ. 2017;1:563–70.

Scott I, Stear M, Irvine J, Dick A, Wallace D, McKellar Q. Changes in the zymogenic cell populations of the abomasa of sheep infected with *Haemonchus contortus*. Parasitol, 1998; 116(6):569–577.

Selemon M. Review on control of *Haemonchus contortus* in sheep and goat. J Vet Med Res. 2018;5(5):1139.

Sharma R, Ganguly S. Gastrointestinal Nematodiasis in small ruminants and anthelmintic resistance. Review JImmunolImmunopatho. 2016;18(2):100–4.

Starling RZC, de Almeida FA, Viana MVG, de Castilhos AM, do Amarante AFT. Losses caused by gastrointestinal nematode infections in Droper lambs under two nutritional statuses. *Rev.Bras.Parasitol*., 2019; 28(4):1–9.

Sultan K, Desoukey A, Elsiefy M, Elbahy N. An abattoir study on the prevalence of some gastrointestinal helminths of sheep in Gharbia governorate, Egypt *Global Veterinaria,* 2010; 5: 84–87.

Tariq K, Chishti M, Ahmad F, Shawl A. Epidemiology of gastro-intestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley *vet*. Parasitol. 2008;158:138–43.

Tehrani A, Javanbakht J, Jani Sasani MF, Solati A, Rajabian M, Khadivar F, Akbari H, Mohammadian M. Histopathological study of *Haemonchus contortus* in Herrik sheep abomasum. J Bacteriol Parasitol. 2012;3:5–144.
Terefe G, Yacob H, Grisez C, Prevot F, Dumas E, Bergeaud J, Dorchies P, Hoste H, Jacquiet J, *Haemonchus contortus* egg excretion and female length reduction in sheep previously infected with Oestrus ovis (Diptera: Oestridae) larvae. Vet Parasitol 2005; 128: 271–283.
Terefe G, Lacroux C, Andreoletti O, Grisez C, Prévot F, Bergeaud P, Penicaud J, Rouillon V, Gruner L, Brunel J, François D, Bouix J, Dorchies P, Jacquiet P. Immune response to *Haemonchus contortus* infection in susceptible (INRA-401) and resistant (Barbados black belly) breeds of lambs. Parasite Immunol. 2007a;29:415–27.
Terefe G, Christelle G, Françoise P, Jean-Paul B, Philippe D, Jean-Claud B, Dominique F, Isabelle F, Philippe J. In vitro pre-exposure of *Haemonchus contortus* L3 to blood eosinophils reduces their establishment potential in sheep. Vet Res. 2007b;38:647–54.
Terefe G, Lacroux C, Prévot F, Grisez C, Bergeaud JP, Bleuart C, Dorchies P, Foucras G. Eosinophils in Haemonchus contortus-infected resistant and susceptible breeds of sheep: abomasal tissue recruitment and in vitro functional state. Vet Parasitol. 2009;165(1–2):161–4.
Tony W. The Veterniary epidemiology and economics research unit (VEERU). Veterinary parasitology. 2nd ed. UK: Blackwell Science; 2007. p. 307.
Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Veterinary parasitology, vol. Pp. 2nd ed. UK: Blackwell Science Ltd.; 1996. p. 141–205.
Vercruysse J, Claerebout E. Treatment vs. non-treatment of helminth infections in cattle: defining the thresholds. Vet Parasitol. 2001;98:195–214.
Vlassoff A, Mckenna P. Nematode parasites of economic importance in sheep in New Zealand. N Z J Zool. 2010;21:1–8.
Walker HK, Hall WD, Hurst JW. Clinical Methods: The History, Physical, and Laboratory Examinations. 1990; 3rd edition. Boston: Butterworths.
Watson T, Hosking B. Observations on resistance and "self-cure" to nematode parasites exhibited by grazing lambs and Saanen kids. Proceeding of the *New Zealand Soc*. Anim Prod. 1989;49:179–81.
Winsor L. Tissue processing. In woods A and Ellis R eds. *Laboratory histopathology*. New York: Churchill Livingstone, 1994; 1–39.

Publisher's Note