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COMPARATIVE STUDY OF THE EFFECTS OF GASTROINTESTINAL PARASITES ON DIFFERENTIAL LEUKOCYTE PROFILE OF DJALLONKÉ SHEEP KEPT UNDER EXTENSIVE AND SEMI-INTENSIVE MANAGEMENT SYSTEMS IN NORTHERN GHANA

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SUMMARY

The effects of gastrointestinal parasites on the differential leukocyte profile of Djallonké sheep managed under farmer-conditions in the extensive system or on-station under the semi-intensive system of management were investigated for six months during the dry season (November, 2006-April, 2007). A total of 461 faecal and blood samples each were collected from 40 sheep stratified into young (6-9 months) and adults (>9 months). The system of management significantly affected the faecal egg count for *Strongyloides* spp. and oocyst counts of *Eimeria* spp. with higher loads in sheep managed extensively than those managed semi-intensively($P<0.05$). The count of faecal eggs for *Strongyle* spp. in both management systems was however similar. The levels of lymphocytes and eosinophils in sheep managed extensively were also significantly ($P<0.05$) higher than those observed in the extensive system. The number of neutrophils, monocytes and basophils did not differ significantly ($P>0.05$) in the two management systems. Eosinophil counts in both management systems was positive and linearly correlated with eggs/oocysts of all the three intestinal parasites and became significant with the eggs of *Strongyloides* spp. ($r=0.32$; $P<0.05$) and *Strongyle* spp. ($r=0.54$; $P<0.05$) under the extensive and again with *Strongyle* spp. ($r=0.40$; $P<0.05$) under the semi-intensive system. The relationship between the count of eosinophils, and the egg and oocyst count of *Strongyloides* spp. ($r = 0.13$; $P>0.05$) and *Eimeria* spp. ($r=0.25$; $P>0.05$), respectively was also positive but not significant under the semi-intensive system of management. Younger animals in the extensive system were found to have significantly higher ($P<0.05$) egg and oocysts counts of *Strongyloides* spp. and *Eimeria* spp. respectively than those raised under semi-intensive management. *Strongyle* spp. showed resistance to anthelmintic use while extensive eosinophilia was consistently associated with gastrointestinal parasitism.

KEYWORDS: *Eimeria* spp., Eosinophils, Gastrointestinal parasitism, Management system, *Strongyle spp.*

INTRODUCTION

The Djallonké also called West African Dwarf (WAD) sheep is widely distributed throughout the West and Central zones of Africa. It is believed to have evolved from the ancient Egyptian sheep *Ovis longipes palaeoaegypticus* (Yapi-Gnaore' *et al.*, 1997). It is generally smaller in size but physically and sexually vigorous with a high reproductive potential. It is also stress and disease resistant especially to unfavourable climate and trypanosomosis. Climate is therefore not likely to have any significant effects on its performance although variations in forage supply could influence physical and physiological maturation. The coat colour varies from spotted black and white to solid black, white or brown. The presence of a mane or neck ruff on males is a typical characteristic of the breed. Males are horned while females are polled. Apart from serving as a source of meat and income, its most obvious role is in religious ceremonies where its commercial value is placed far beyond its value in terms of carcass weight (Charray *et al.*, 1992).

It is estimated that 97% of all small ruminants in Africa are carriers of parasites of the digestive system (Charry *et al.*, 1992) which cause mortality and morbidity each estimated at US$2 billion annually (Putt and Hanks, 1993). In Ghana, gastrointestinal parasitism account for 27% of all deaths and constitute the probable limitation to the multiplication of sheep (Buadu and Osafo, 1994). The effects of morbidity on sheep production include expunge weight loss, poor growth rate, infertility, abortions, lower milk yield, increased cost of drugs and vaccines, and extended age in the attainment of puberty. These are however often overlooked because their effects are not apparent, but the economic effects of morbidity on production are estimated to be higher than those of mortality since they increase the cost of production and also lower productivity (Charry *et al.*, 1992). Gastrointestinal parasites therefore cause serious economic losses and are considered the most serious health problem of sheep and goats (Perry *et al.*, 2005).

The extensive system of rearing sheep commonly practised by rural farmers predisposes them to parasites of the gut, especially in the dry season when they graze stubble very close to the ground, thereby ingesting encysted larvae. The semi-intensive and intensive systems are practised by only a few large-scale farmers, research and higher educational institutions. Gastrointestinal parasites build up and become endemic in these systems if the pen is not routinely cleaned, disinfected and the animals dewormed, and even when all these practices are observed, the animals are still liable to becoming rapidly reinfested because deworming often unarms the natural immunity of the host subsequently rendering them more susceptible to higher infestation (von Kaumann and Fitzhugh, 1993; Skyes, 1994).

A strong positive correlation between egg/oocyst counts and the prevalence of the parasites themselves in livestock has already been established with 100% of the eggs of *Strongyle* spp. and *Strongyloides* spp. developing into viable infective larvae (Ibrahim *et al.*, 2006). The count of eggs/oocysts can therefore be best used to estimate the prevalence of parasitic infestation. Gastrointestinal parasites act as antigens and their presence often evokes immune responses that cause changes in the normal physiology of the animal. The presence and degree of infestation of gastrointestinal parasites in sheep can therefore cause changes in the normal leukocyte differential. This study investigated the relationship between egg/oocyst counts as a measure of gastrointestinal load and leukocyte profile of Djallonké sheep kept extensively or semi-intensively.

MATERIALS AND METHODS

Study area

The study was conducted at Nyankpala in the Tolon-Kumbungu district of the Northern region of Ghana. It is located on latitude 9.5° N. The area is in the guinea savannah zone characterised by a unimodal rainfall pattern.

Rains begin in April, rising to a peak in August - September and ending in October or November. The dry season spans from November to March. Rainfall averages 1060 mm and temperatures usually range from 15°C in January when the weather is under the influence of the North easterly wind, to 42°C towards the end of the dry season in March (SARI, 2006).

Animal management and experimental design

A total of 40 Djallonké sheep, 20 from the extensive system managed by farmers in Dundo, a rural community, and 20 from the semi-intensive system managed by the Department of Animal Science of the University for Development Studies (UDS), Ghana, were used for the study. Those managed by the UDS were allowed to graze on natural pasture during the day but confined in the evening in a pen. The pen was routinely cleaned and disinfected while the animals were dewormed with Albendazole® (7 mg/kg), medicated (Oxytetracycline®) and supplemented with whole cotton seed. Those managed by farmers under the extensive system were also provided with pens which were hardly cleaned and/or disinfected. They were not given any medication and often slept outside the pens. The animals in each management system were stratified by age into young (6-9 months) and adults (9 months and above). Age estimation was based on dentition (Charry *et al.,* 1992). Each management system consisted of 10 young ones and 10 adults. Data was collected for a period of 6 months (November, 2006-April, 2007). The general male: female sex ratio was 2:3 and 3:7 in the semi-intensive and extensive systems, respectively.

Faecal worm egg and oocyst counts

A total of 461 faecal and blood samples were collected fortnightly from the experimental animals for analysis. Three lambs died during the course of the study. The McMaster floatation technique of faecal egg count was used. About 3g of the faecal material obtained from the rectum of each animal was macerated in a mortar with about 15 mls of distilled water. The content was then poured into a plastic test tube and centrifuged at 1,233 gm. The supernatant fluid was poured off and distilled tap water added. The process was repeated and saturated sodium chloride (NaCl) solution added and centrifuged again at the same speed and time. The saturated NaCl solution enables the eggs to float (except eggs of flukes). The fluid was then drawn from the surface of the mixture of NaCl and faeces and the McMaster counting chamber filled. The counting chamber was then mounted on a microscope and examined under 40 (x40) objective lens. Under the microscope, eggs of *Strongyle* spp. are oval (rugby ball-like) with a thin shell and a dark content. A major distinction between the eggs of *Strongyle* spp. and *Strongyloides* spp. is that larval (L_1) movements are typically observed in the eggs of freshly collected faeces in the latter but the egg content of the former consists of intact dark cells. The number of eggs were counted and multiplied by a scale factor of 100 (Adu *et al.*, 2006) to obtain total eggs per gram (epg). Oocyst counts were determined through the modified McMaster technique by methods described by MAFF (1977).

Blood sampling and leukocyte count analysis

A total of 461 peripheral blood samples were similarly collected at the time of sampling the faeces. Smears were made from samples taken from the ear vein of each animal and fixed with absolute ethanol, and stained with Giemsa stain. The different types of leukocytes (neutrophils, basophils, eosinophils, lymphocytes and monocytes) were identified and counted with a microscope with 100/1.25 oil immersion objective lens and a haemocytometer using the straight edge method (Addah *et al.,* 2007).

Data analysis

Data from the two management systems were compared using t-test and the relationship between the worm egg/oocyst and the differential leukocyte count determined by linear correlation matrix using GenStat (version 6.0) software.

RESULTS

The effects of management system and age of host on faecal egg/oocyst and differential leukocyte counts are shown in Table I. Regardless of the management system, faecal egg/oocyst counts were highest for *Strongyle* spp. (64%), lower for *Eimeria* spp. (27%) and lowest for *Strongyloides* spp. (9%). The infestation of *Eimeria* spp. and *Strongyloides* spp. in the extensive system was significantly higher ($P<0.01$) than in the semi-intensive system, however, the prevalence of *Strongyle* spp. between the two management systems was similar (*P*>0.05) (Table I) but significantly higher than the other parasites within each management system (*P*<0.01) (Fig.1).

The level of faecal worm eggs and oocysts in younger animals did not differ significantly from adult ones (*P*>0.05) though both younger and adult animals in the extensive system had higher counts than their counterparts in the semi-intensive system (*P*<0.05). All losses due to mortality (7.5%) during the study occurred in the extensively managed flock. Faecal samples recovered from the dead lambs showed mixed and heavy infestations of *Eimeria* spp. (1,412 opg), *Strongyle* spp. (1,902epg) and *Strongyloides* spp. (623 epg).

Eosinophils counts were significantly higher (*P*<0.01) in extensively managed sheep while lymphocyte counts were higher in those kept semi-intensively but the system of management did not significantly affect (*P*>0.05) the counts of neutrophils, monocytes and basophils. In either age groups, strongyloidiosis and coccidiosis induced eosinophilia in both management systems and lymphopenia in only semi-intensively managed sheep (*P*<0.05). As indicated in Table II, the leukocyte differential, especially eosinophils, of sheep in either age group of the extensively managed flock varied greatly from normal.

The matrix correlation coefficients between the three gastrointestinal parasites and differential leukocyte counts are shown in Tables III and IV. The level of eosinophils in the two management systems was positively correlated with all the three intestinal parasites under consideration and became significant with strongyloidiosis (*r* = 0.32; *P*<0.05) and strongylosis (*r* = 0.54; *P*<0.05) in the extensive system and also with strongylosis (*r* = 0.40; *P*<0.05) only in the semi-intensive system. The relationships between the counts of eosinophils, and the egg and oocyst counts of *Strongyloides* spp. (*r* = 0.13; *P*>0.05) and *Eimeria* spp. (*r* = 0.25; *P*>0.05) respectively were also positive but not significant under the semi-intensive system of management.

TABLE I: Effects of type of management system and age of animals on faecal egg/oocyst count and leukocyte profile (mean ± S.E) of Djallonké sheep.

	Faecal worm egg count			Leukocyte profile (%)				
	Strongyle spp. (epg)	*Strongyloides* spp. (epg)	*Eimeria* spp. (opg)	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Type of mgt. system								
Extensive	1460 ± 194.92	307.2 ± 61.36	799.5 ± 177.71	39.03 ± 0.38	51.87±0.39	7.32 ±0.21	1.11 ± 0.08	0.45 ± 0.04
Semi-intensive	1192 ± 325.73	71.33 ± 21.91	310.4 ± 60.24	38.62 ± 0.39	54.02±0.31	5.98 ±0.20	1.03 ± 0.06	0.45 ± 0.04
Significance	Ns	**	**	Ns	**	**	Ns	ns
Effects of Age								
Young(6-9 mo)								
Extensive	1717 ± 283.3	414.9 ± 115.1	1036 ± 291	39.28 ± 0.6	51.17 ± 0.7	7.55 ± 0.3	1.16 ± 0.1	0.48 ± 0.6
Semi-intensive	1243 ± 267	216.6 ± 57.4	600.8 ± 216.6	38.82 ± 0.5	53.17 ± 0.4	6.35 ± 0.3	1.12 ± 0.1	0.53 ± 0.1
Significance	Ns	*	*	Ns	**	**	Ns	ns
Adult(>9 mo)								
Extensive	143.0± 267.8	216.6 ± 57.4	600.8 ± 216.6	38.82 ± 0.5	52.46 ± 0.4	7.13 ± 0.3	1.07 ± 0.1	0.44 ± 0.1
Semi-intensive	675.80 ± 140.4	46.83 ± 21.2	255.00 ± 74.6	38.79 ± 0.6	54.33 ± 0.4	5.62 ± 0.3	0.95 ± 0.1	0.35± 0.1
Significance	Ns	**	ns	Ns	**	**	Ns	ns
Ext. + Semi-int. ***(over all)***								
Young(6-9 mo)	1713 ± 367.07	241.6 ± 57.45	671.9 ± 143.97	38.82 ± 0.37	52.55 ± 0.39	6.90 ± 0.21	1.14 ± 0.08	0.46± 0.04
Adult(>9 mo)	959.6 ± 151.99	131.7 ± 31.00	427.9 ± 114.85	38.81 ± 0.39	53.40 ± 0.31	6.38± 0.21	1.00 ± 0.06	0.45± 0.04
Significance	Ns	ns	ns	Ns	Ns	ns	Ns	ns

**** = P<0.01; * = *P*<0.05; ns = not significant**

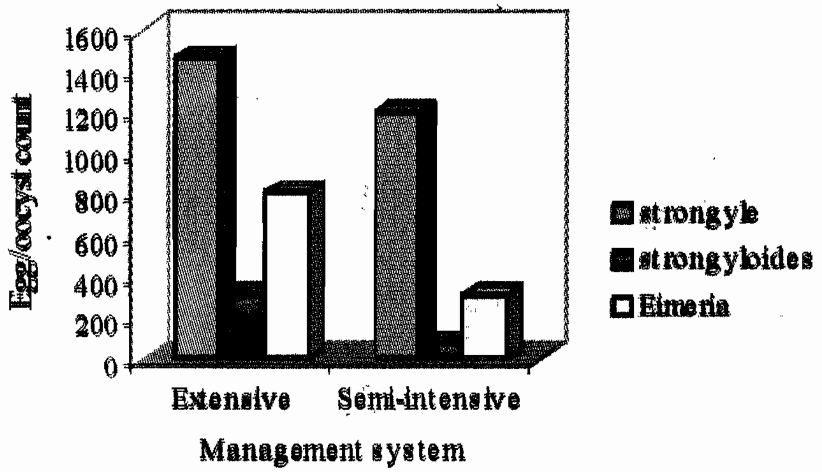

FIGURE 1: Effects of type of management on egg and oocyst counts

TABLE II: Effects of gastrointestinal parasites on percentage variation in the leukocyte from normal count

	Management system		*Normal Leukocyte count	Variation from normal	
	Extensive (%)	Semi-int. (%)	(%)	Extensive (%)	Semi- int. (%)
Young (6-9 months)					
Neutrophils	39.28	38.44	42.45	7.5 Lp	9.4 Lc
Lymphocytes	51.17	53.71	50.1	2.0 Lc	7.2 Lc
Monocytes	1.16	1.12	1.85	37 Lc	39.5 Lp
Eosinophils	7.55	6.35	3.25	132.3 Lc	95.4 Lc
Basophils	0.48	0.48	2.45	80 Lp	80.4 Lp
Adults(> 9 months)					
Neutrophils	38.82	38.79	39.6	1.96 Lp	25 Lp
Lymphocytes	52.46	54.33	55.9	6.2 Lp	2.8 Lp
Monocytes	1.07	0.95	2.45	56.3 Lp	61.2 Lp
Eosinophils	7.13	5.62	1.8	296.1 Lc	212.2 Lc
Basophils	0.44	0.53	0.25	76 Lc	112 Lc

**Normal leukocyte count of healthy Djallonké sheep screened of endoparasites with anthelmintics (Ahunu and Assuoku, 1987); Lc: Leukocytosis; Lp: Leukopenia*

TABLE III: Matrix correlation co-efficient of egg/oocyst and differential leukocyte counts (extensive)

Parasites	Neutrophils	Monocytes	Lymphocytes	Eosinophils	Basophils
Strongyle spp.	0.061	0.014	- 0.378	0.537	0.030
Strongyloides spp.	- 0.009	0.062	- 0.113	0.315	0.092
Eimeria spp.	- 0.138	0.088	0.003	0.223	-0.070

TABLE IV: Matrix correlation co-efficient of egg/oocyst and differential leukocyte counts (semi-intensive)

Parasites	Neutrophils	Monocytes	Lymphocytes	Eosinophils	Basophils
Strongyle spp.	0.007	- 0.072	- 0.252	0.404	0.130
Strongyloides spp.	- 0.026	- 0.009	- 0.045	0.130	-0.073
Eimeria spp.	- 0.095	0.066	- 0.073	0.253	0.073

DISCUSSION

Faecal egg count of *Strongyle* spp. predominated over *Strongyloides* spp. and oocyst of *Eimeria* spp. in both management systems throughout the study period. *Strongyle* spp. (cyathostomes) are highly prolific, persistent and efficient in reproduction; about 99% of *Strongyle* eggs are passed out in faeces and they take 12 24 hours to hatch into infective larvae which do not require an intermediate host. The cyst can remain up to 2 years burrowed in the gut wall; hence their predominance. Another reason for a relative dominance of *Strongyle* eggs is that, unlike *Eimeria* spp. which are species-specific and cross-infestation, for example between goats and sheep is usually not possible, *Strongyle* spp. are not species-specific and cross-infestation is common (Lindsay and Todd, 1993). Similar studies in sheep and goats in Kenya show that *Strongyle* nematodes were the most prevalent gastrointestinal parasites in both species (51%) with higher infestations in sheep than goats (Waruiru *et al.*, 2005).

Sheep under the extensive system in Ghana are usually kept in smaller household flocks, often less than 10, and of varying ages, scavenging in the dry season or foraging extensively or tethered each day, on fresh pasture in the rainy season. It was expected that this practice, unlike that of the semi-intensive system where the flock is normally kept in relatively large numbers on paddocks, often with little rotation, would have resulted in lower infestation but that was not observed. The routine deworming exercise practised in the semi-intensive system could have accounted for the lower egg counts of *Strongyloides* and oocyst of *Eimeria* than in the extensive system in this study. In intensive and semi-intensive management systems, careful routine management of grazing pastures can minimize the prevalence of L_3 of most worms but this is rarely achievable in extensive communal pastoral/grazing systems and control of gastrointestinal parasites in such management systems is entirely dependent on the use of anthelmintics (Hunter, 1996) which unfortunately are economically inaccessible to pro-poor rural farmers and ineffective in preventing reinfestation (Restrepo and Preston, 1989). In contrast to these findings, very high faecal egg and oocyst counts of *Strongyloides* spp. (1,697 epg) and *Eimeria* spp. (21,929 opg), respectively have been observed between November and December in lambs raised semi-intensively under established pasture in the same region (Agyei, *et al.*, 2005). This may be explained by differences in management practices, especially with regard to deworming schedules.

The predominance of *Strongyle* spp. over *Eimeria* spp. and *Strongyloides* spp. in the semi-intensive system despite routine deworming (Fig. 1) may be due to their proficiency in reproduction and/or possible resistance to Albendazole®. High counts of *Strongyle* eggs found in semi-intensively managed goats during the dry season despite Oxyclozanite + Levamisole (Nilzan®) use have severally been attributed to resistance to anthelmintics (Shavulimo, 1989 ; Ahunu and Assoku, 1987). The results of the present study suggest that routine management practices such as regular deworming, cleaning and disinfection of pens, usually carried out by research and higher educational institutions under the semi-intensive system could be effective in reducing the infestation of *Strongyloides* spp. and *Eimeria* spp. but not *Strongyle* spp.

The prevalence of gastrointestinal parasites in scavenging sheep also has public health implications, especially in rural households in Africa. This is because animal keepers in many rural communities maintain close association with their animals and in some communities, people share houses (living in the same house) with small ruminants, calves and even adult cattle where they are few, for fear of theft (Kambarage *et al.*, 2004). Under such conditions, scavenging animals pose a threat of zoonosis. *Strongyloides stercoralis* is typically a common species of *Strongyloides* that is highly zoonotic (LT, 2003).

The relationship between the prevalence of *Strongyle* spp. and *Eimeria* spp. in this study was generally indefinite and inconsistent in kids however, *Strongyle* spp. predominate only after 53 days of age when the dominance of *Eimeria* spp.† (On day 39) has lowered host immunity and receded (Agyei *et al.*, 2004). Younger animals therefore tend to be more susceptible to coccidiosis leading to deaths while adults develop chronic *Strongyle* infestations that serve as a reservoir for (re)infestation of the general flock, especially in intensively managed systems with higher stocking densities (Charry *et al.*, 19921). Though ovine *Strongyles* (*Cabertia ovina, Oesophagostomum* spp. *and Nematodirus* spp.) are more prolific, they do not migrate through the blood vessels and hence may not cause as much damage to the host as *Eimeria* spp. and other nematodes. *E. ovinoidalis*, a dominant and pathogenic species is responsible for the mortality of most lambs in Ghana since diarrhoeic conditions in lambs have always been misconstrued as a sign of only helminth infestation and often treated with only dewormers without considering the use of coccidiocides (Agyei *et al.*, 2005).

The level of eosinophils in each management systems was positively correlated with strongylosis, strongyloidiosis and coccidiosis. Rising levels of eosinophils are associated with the introduction of multicellular parasites into the body via the lungs and/or gastrointestinal tract in which they phagotosize antigen-antibody complexes while an increase in the level of neutrophils, monocytes, lymphocytes and basophils is an indication of the presence of unicellular invaders such as bacteria, fungi and viruses in the body (Thibodeau, 1987). The high egg/oocyst count which is indicative of the prevalence of the parasites could have accounted for the higher counts of eosinophils observed in the extensively managed flock.

The differential leukocyte profile reported in the present study for sheep under the semi-intensive management system also varied greatly from those observed for the same breed of similar age range managed under similar conditions at the University of Ghana's Agricultural Research Station in the coastal region of Ghana (Ahunu and Assoku, 1987). Ovine *Strongyle* nematodes do not traverse through the blood vessels but they burrow into the gut wall and may cause irritations, diarrhoea and colic thereby eliciting a rise in the production of eosinophils. The correlation between eosinophils, and *Strongyloides* spp. and *Eimeria* spp. were not significant under the semi-intensive system of management even though these two parasites have been incriminated in 20% of lamb losses in Ghana (Agyei *et al.*, 2005).

A consistent eosinophilia with increasing gastrointestinal parasitism was observed in both management systems and age groups. The level of eosinophils in younger animals in the extensive system was 17 times (132 %) higher than the normal count for sheep in that age group compared to a 15 fold rise (95 %) in the semi-intensive system. A similar trend of leukocytosis in response to gastrointestinal parasitism was observed in adult animals under the extensive system where the percentage variation in eosinophil count reached 296% compared to 212% in the semi-intensive (Table II). In healthy animals devoid of endoparasites, the normal percentage variation in eosinophil count ranges from 1015 % (Ahunu and Assoku, 1987).

A drastic deviation in leukocyte levels from normal as observed in this study has experimentally been shown to stimulate increased adrenal secretion of hydrocortisone and the physiological implications of such a surge include decreased utilization of glucose, decreased amino acid transportation to muscle cells and increased rate of glucogenesis (Chandrawathani, 2004). Studies by Coop and Holmes (1996) have further shown that gastrointestinal nematodes reduce voluntary feed intake and efficiency of feed utilization, by increasing endogenous loss of protein into the gastrointestinal tract. There is also a re-allocation of protein from productive processes into repair of the gastrointestinal tract, synthesis of plasma proteins and mucoprotein production. The extended effects of these processes are anaemia, general unthriftness and poor growth performance especially in young animals. The percent deviation in the counts of the rest of the other leukocytes from normal was however generally inconsistent between the two management systems and age groups except lymphocytes which decreased (lymphopenia) with increasing egg count. The correlation between lymphocytosis and gastrointestinal parasitism was therefore consistently negative in both management systems except with oocysts of *Eimeria* spp. (Tables III and IV). Lymphocytosis is a rare physiological phenomenon and would likely occur only under chronic lymphatic disorders or severe inflammation (Walter, 1982).

Gastro-intestinal parasitism is ranked globally and in West Africa with the highest index as an animal health constraint to resource-poor farmers due to their wide geographic distribution, host species range, and high economic implications in all production systems particularly in sheep, goats, poultry and camels (Perry *et al.,* 2005).

CONCLUSION

Gastrointestinal parasites cause significant deviation in the normal leukocyte physiology of sheep in rural extensive management systems that can have implications on productivity of rural sheep. The high prevalence of *Strongyles* even in the semi-intensive management despite routine anthelmintic administration may also suggest drug resistance while the consistent increase in the egg count of *Strongyloides* and oocysts of *Eimeria* spp. in the extensively managed flock could suggest the need for good husbandry practices including routine administration of coccidiocides and nematode anthelmintics in extensively managed sheep. The principal physiological index for assessing the degree of gastrointestinal infestation in the Djallonké sheep could probably be the degree of eosinophilia.

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