RESEARCH ARTICLE

AN AGE - CLASS STUDY OF SHEEP ENDOPARASITES IN BISKRA REGION (ALGERIA)

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ABSTRACT

The present study aims to evaluate the prevalence of gastrointestinal parasites in sheep of Biskra region according to age classes and to detect eventual zoonotic parasites. It included 372 sheep, females and males. 248 fecal sheep samples were coprologically examined and coprocultured, 119 being from adult sheep (≥ 12 months) and 129 from lambs (< 12 months). Also, 124 adult sheep were necropsied after the slaughter, focusing on the livers, lungs and intestines. The qualitative flotation technique was used by employing the McMaster microscope slides. All the statistics was done by using the SPSS 20. Prevalences were calculated according to age classes and parasites genera. Kendall test ($p < 0.05$) was applied to detect any correlation between total prevalence and parasite genera prevalences in adults versus lambs. The results showed that medium prevalence for five years in adults (63.02 %) was higher than in lambs (57.36 %). Coproscopy showed: coccidia, gastrointestinal strongyles, *Nematodirus* spp, *Moniezia* spp and *Marshallagia* spp. Coproculture showed *Protostrongylus* spp. and *Dictyocaulus* spp. Necropsy revealed *Fasciola hepatica,* Echinococcus polymorphus, *Thysaniezia ovilla, Moniezia expansa,* Cysticercus tenuicolis, Cysticercus ovis and *Paramphistomum daubenyi.* The diversity and simultaneousness of gastrointestinal parasites could have a negative impact on production parameters in infected sheep. The risks of contracting echinococcosis as a major zoonosis requires more respect of deworming programs in dogs and sheep and better vigilance in slaughterhouses.

Keywords: Gastrointestinal strongyles, lambs, flocks management, deworming programs, parasitic zoonosis.

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INTRODUCTION

Several studies show that nematode infection causes significant changes of haematological, biochemical and clinical parameters and reproductive performances in ruminants (Rouatbi et al., 2016). Also, it was reported that fasciolosis remained common in Algeria in ruminants, particularly in the north and causes direct losses due to the high rate of condemnation of infected livers (Ouchene-Khelifi et al*.,* 2018). Several investigations done in Algeria revealed that there was often a polyparasitism (Boulkaboul and Moulaye, 2006; Saidi et al*.,* 2009; Moussouni et al., 2018).

It is known that animal species influences, in general, the rate and the type of parasitic infection. Furthermore, the epidemiology of infections with gastrointestinal parasites (GIP) in livestock varies depending on the prevailing weather conditions and management practices (Moussouni et al. 2018). Variance analysis of annual parasite dynamics has shown a significant seasonal effect for some parasite species (Meradi, 2012; Titi et al. 2014). Also, some GIP species infect young animals, while others infect adults (Meradi, 2012).

Several studies were done in northern part of Algeria on GIP in ruminants. However, such studies are increasingly rare in the southeastern part of Algeria, although it contains a significant herd of domestic ruminants: sheep, cattle, goats and camels. In this context, the present study was carried out on a sample of sheep bred or slaughtered in Biskra governorate. It lasted from 2009 to 2013.

MATERIALS AND METHODS

Study area

The governorate of Biskra is located in southeast of Algeria, about 420 km from the capital Algiers and it occupies an area of 21 509.80 km^2. It is located between 4° 15' and 6° 45' East longitude and between 35°15 ' and 33° 30' North latitude and altitude between 29 and 1600 m. It is a rural area with an extensive small ruminants breeding system that supports a vocation of small ruminants breeder. The governorate of Biskra includes about 260 cattle farmers. There are 4995 bovines, 942 900 sheep, 293 000 goats and 5000 camels. Small ruminants' flocks are mainly reared in the form of nomadic and semi-nomadic systems (V.S.D, 2010; 2016; M.A.R.D, 2016; Mammeri, 2018). Municipalities included in the present study are: Biskra, Tolga, Sidi khaled, El'Hadjeb, Ouled

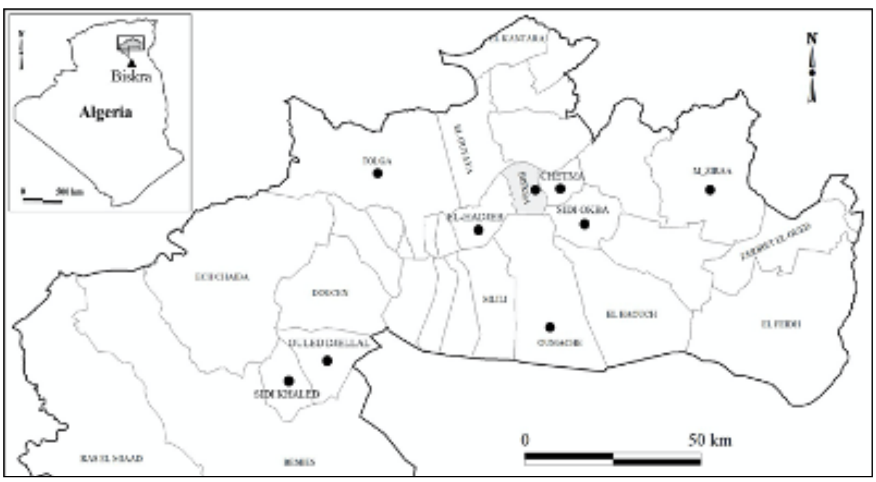

Figure 1
Administrative division of Biskra governorate (study area) (Chenchouni, 2010)

Djellal, M'zirâa, Sidi Okba, Chetma and Oumache (Figure 1). The selected municipalities are known by well-developed activities of small ruminants breeding, mainly sheep, and by vast pastoral areas.

Herds and animals sampling

Sampling was carried out in a reasonable manner according to the possibility of access. Breeders were called in advance after obtaining their telephone numbers from the Veterinary Services Directorate database, and if they agreed to participate in the survey, a suitable appointment was made with them to visit the flocks. Thus, fourteen sheep flocks distributed in the selected municipalities were visited. According to owners' records, sheep that have not received any antiparasitic drugs for at least two months, either orally or by injection or cutaneously , have been chosen. The sample 1 (E1) included 248 animals that underwent coproscopy and sometimes coproculture, out of which 119 adult sheep (AS) (≥12 months) and 129 lambs (L) (<12 months). Sample 2 (E2) included only 124 adult sheep that underwent post-mortem autopsy. E1 and E2 included both, males and females. The total sample (Et = E1 + E2) consisted of 372 sheep. It was not possible to obtain slaughtered L, given the Algerian legislation. All surveys were conducted in the spring, between March and May.

Fecal examination

Fecal samples (5-10 g) were collected using disposable latex gloves directly from the rectum of each animal. The samples were stored immediately in sterile plastic boxes, then transported on the same day to the laboratory (Department of Nature and Life Sciences, El'Hadjeb, University of Biskra). For the coprological analysis, the qualitative flotation technique was used with 25% saturated sodium chloride solution (250 g NaCl + 1l of distilled water). The faeces of each sample was crushed by a mortar while adding 70 mL of the saturated sodium chloride solution, and the resulting mixture was sieved to remove debris.

Then, the large test tube was completely filled and coverslip was placed on the surface of the tube. Floatation was allowed to proceed for 20 min.

The quantitative method was done also, from the mixture obtained after sieving. A sterile syringe was used to fill the two chambers of the McMaster microscope slide (Verrerie Dumas, Noisay, France) and left to rest for 20 min. Counting of parasitic forms was obtained according to recommendations of Urquhart (1996).

The flotation technique used for the detection of positive cases was able to find nematodes (eggs and larvae), cestodes (eggs) and coccidia (oocysts) (Kaufmann, 1996; Foreyt, 2001**)**, which were identified by their morphological characters. In the case of nematodes, the isolation of larvae 3 (L3) was carried out with the Baermann device after coproculture in Petri dish at 24 °C for 10 days. The identification of obtained L3 obtained by coproculture was done according to the keys of Niec (1968) and Audebert et al. (2004). Parasites were identified at 40x, 100x, and 400x microscope (Optika) magnification. It was necessary to add a few drops of 4% Lugol to inhibit the mobility of the larvae.

Postmortem necropsy

For sample E2, necropsies were carried out immediately after slaughter at two publicly owned slaughterhouses located in the municipalities of Biskra and Tolga. Livers, lungs and intestines were examined. Adult parasites were collected, preserved in 10% formalin and identified at El'Khroub Veterinary Institute.

Statistical analysis

Microsoft Excel XP. 2007 was used to determine descriptive histograms and tables. To evaluate the proportion of infection prevalence was calculated in two age classes according to the equation: (number of infected animals/number of examined animals) x 100. Then, the results were statistically analyzed by SPSS version 20 (IBM, 2011). A bivariate correlation using Kendall test ($p<0.05$) was applied to detect any correlation between the variables GIP general prevalence in AS and GIP general prevalence in L, which do not consider parasites genera but only age classes. Also, the Kendall test was applied between the variable GIP genera prevalence in AS in opposite to the variable GIP genera prevalence in L, which consider both of GIP genera and age classes.

RESULTS

Coproscopy

Using coproscopy method, total prevalence (Pt) of GIP total average count for five years, without considering age classes, is 60.08 %. Medium prevalence of GIP for five years in AS is higher than in L after coproscopy. More than a half of the sampled sheep are infected in both age classes (Table 1).

The highest prevalence results were recorded in 2010 (AS) then in 2011 (L). The lowest prevalence results were observed in 2013 (AS and L) then in 2009 (AS). Prevalence results in L were higher than in AS for three years (2009; 2011; 2013) (Table 1).

Table 1 Distribution of general prevalence of GIP according to year and sheep age classes using coproscopy method (2009-2013)

Year	2009			2010			2011			2012			2013			2009-2013		
Age	N[a]	I[b]	P[c]	N	I	P	N	I	P	N	I	P	N	I	P	N	I	MP[d]
AS	29	8	27.58	20	20	100	36	32	88.88	24	15	62.50	10	0	00	119	75	63.02
L	37	20	54.05	20	19	95	22	21	95.45	20	8	40	30	6	20	129	74	57.36

N[a] : Number of sampled animals – I[b] : Number of infected animals – P[c] : Prevalence (%)
MP[d] : Medium prevalence for five years (%)

For GIP count by year, the highest value is recorded for coccidia (CC) (AS; 2011), followed by gastrointestinal strongyles (GIS) (L; 2010) then by GIS (AS; 2010). Considering the sum of five years GIP count, the higher values are recorded for CC (AS) and GIS (L), while the lower values correspond to *Marshallagia* spp. (MA) (AS and L). Total count of all GIP genera detected by coproscopy in five years is higher in AS than in L. *Nematodirus* spp. (NE) and *Moniezia* spp. (MO) have higher prevalences in L than in AS. Consequently, regarding the prevalence of GIP for five years, the higher values are recorded for CC (AS) and DS (L), while the lower ones correspond to both, MA (AS and L) (Table 2 and Figure 2).

Table 2 Distribution of GIP count results and prevalences according to GIP genera, year and sheep age classes (2009- 2013)

Year / Period	Age classes/ Total prevalence and total count in five years	Coproscopy count results by GIP genera				
		GIS	NE	MA	CC	MO
2009	A	15	15	0	15	0
	L	15	0	0	105	0
2010	A	4275	2085	405	1020	1245
	L	5910	2325	255	1170	645
2011	A	1320	180	285	16290	615
	L	450	75	45	3735	915
2012	A	150	315	15	0	15
	L	15	30	0	0	150
2013	A	0	0	0	0	0
	L	15	60	0	0	15
2009-2013	TCAS[a] =28260	5760	2595	705	17325	1875
	TCL[b] =15930	6405	2490	300	5010	1725
	Genera PAS[c] (%)	20.38	9.18	2.49	61.30	6.63
	Genera PL[d] (%)	40.21	15.63	1.88	31.45	10.83

TCAS[a] : GIP total count in AS-TCL[b] : GIP total count in L
Genera PAS[c]: GIP genera prevalence in AS - Genera PL[d]: GIP genera prevalence in L

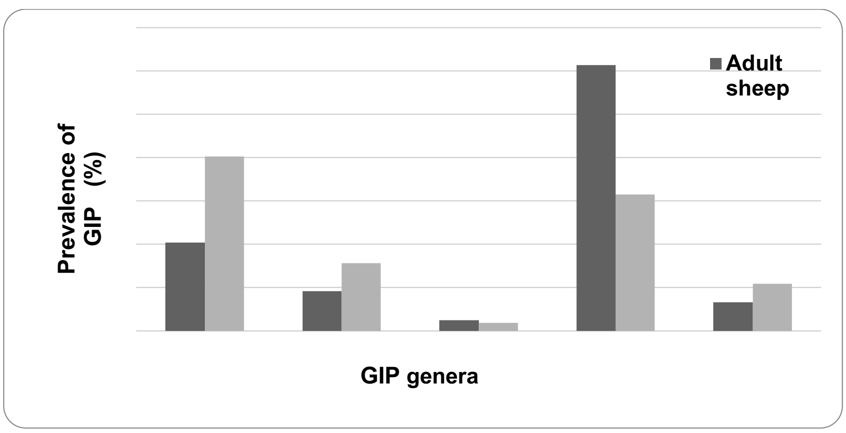

Figure 2 Prevalence of GIP (%) according to GIP genera and sheep age classes (2009-2013)

Coproculture and necropsy

After coproculture, the results showed two principal genera: *Protostrongylus* spp. and *Dictyocaulus* spp. The whole parasites species found after postmortem autopsy of 124 AS were: *Fasciola hepatica*, Echinococcus polymorphus, *Thysaniezia ovilla*, *Moniezia expansa*, Cysticercus tenuicolis, Cysticercus ovis and *Paramphistomum daubeneyi*. The latter is very difficult to identify as it has a lot of similarities with *Fasciola hepatica.*

Kendall test ($p<0.05$) was significant for the variable GIP genera prevalence in AS in opposite to the variable GIP genera prevalence in L. However, Kendall test ($p>0.05$) did not show any correlation between the variables GIP general prevalence in AS and GIP general prevalence in L (Table 3).

Table 3 Results of Kendall's correlation test between GIP general prevalences and genera prevalences in AS and L

Variable 1	Variable 2	Tau-B de Kendall Correlation	Signification
General PAS[b]	General PL[c]	0.600	0.071
Genera PAS	Genera PL	0.800	0.025[a]

[a]Test is significant at p<0.05-PAS[b]: Prevalence in AS –PL[c]: Prevalence in L

DISCUSSION AND CONCLUSION

In the present study, total GIP prevalence (Pt) without considering age classes, is 60.08 %. This prevalence rate is comparable to the one reported by Saidi et al. (2009), which reached 54%. His study about internal parasites, carried out in naturally infected *Rembi* breed sheep, was done in the region of Ain D'hab, an arid region similar to our study area (Biskra), and via coproscopy carried out between April and June. In the above mentioned studies, the overall rate of infection by sheep GIP exceeds 50% and reflects the importance of parasitism in sheep flocks. Comparable results were reported by Pinilla Léon et al. (2019), where the global prevalence of GIP in Colombian sheep was 56.3% and by Pedreira et al. (2006) in Galicia (NW Spain), where the prevalence of GIP was 100%.

Comparable to necropsy results, in a study conducted by Hazzaz Bin Kabir et al. (2010) at different abattoirs of Bangladesh, it was revealed that the parasitic infections due to one or more causal agents were found in (41.08 %) of 460 sheep.

The present study showed higher coccidia prevalence among other GIP. Comparable to our study in Algeria, Saidi et al. (2009) revealed the presence of the following parasites: *Nematodirus* spp., *Marshallagia marshalli*, various gastrointestinal strongyles as *Charbetia ovina*, *Skrjabinema ovis*, *Trichuris ovis*, *Moniezia* spp., *Dictyocaulus filaria* and coccidia (*Eimeria* spp.). The same study revealed the presence of two main parasites *Nematodirus* spp. and *Marshallagia* spp. in lambs and ewes. While abroad, Pinilla Léon et al. (2019) reported that the most prevalent parasites were *Eimeria* spp., *Fasciola hepatica*, and parasites of Strongylida order. In the same context, Pedreira et al. (2006) reported that the genera identified in sheep were *Chabertia*, *Cooperia*, *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Teladorsagia*, *Trichostrongylus* and *Trichuris* spp.

Furthermore, our results revealed that the prevalence of *Nematodirus* spp. is higher in L than in AS. on the contrary, the prevalence of *Marshallagia* spp. is higher in AS than in L. Thus, our results corroborate with those reported by Saidi et al. (2009) where an overall prevalence of 20.2% and 55.5% (ewes and lambs, respectively) was registered for *Nematodirus* spp., whereas the prevalences of 18.8% and 15% (ewes and lambs, respectively) were observed for *Marshallagia* spp.

In the present study, *Fasciola hepatica* was found only after postmortem autopsy. Comparable to this study, in a survey done in El Tarf region by Ouchene-Khelifi et al. (2018), the authors reported that for sheep the prevalence of fasciolosis varied from 1.1± 0.2% (in August) to 21.2±1.7% (in December), and that the highest prevalence rate was observed during winter season (9.2 %) (P <0.001). It is clear that fasciolosis is more common in humid regions than in arid areas as it is the case of Biskra region.

Fasciola hepatica eggs were not found after coproscopy because we didn't do the sedimentation technique and floatation with flotation solution of higher speciffic gravity.

Also, a study conducted by Boulkaboul and Moulaye (2006) revealed the chronicity and the multiplicity of gastrointestinal parasites in *Ouled Djellal* sheep in the semi-arid region of Algeria. It had a marked seasonal variation and was dominated by GIS, among which were often present those of the genera *Marshallagia* and *Nematodirus*. There was the dominance of coccidia then adult cestodes. According to Boulkaboul and Moulaye (2006), in addition to the weather conditions, some steppe plants used in sheep feeding and genetic traits of *Ouled Djellal* breed could play a key role in the relative minimisation of strongylian parasitism.

On the other side, the financial and agriculture losses caused by parasites have a substantial impact on farm profitability. For example, the annual cost associated with parasitic diseases in sheep and cattle are proposed to be tens of billions of dollars worldwide, according to the sales of anti-parasitic compounds by pharmaceutical companies, excluding production losses. In addition, the excessive and frequent use of anthelmintics has resulted in substantial and widespread problems with anthelminthic resistance in nematode populations (Roeber et al., 2013). In a study conducted in eastern Algeria, Bentounsi et al. (2007) confirmed that *Nematodirus* spp. was putatively resistant to albendazole and ivermectin on two and three farms, respectively, and *Marshallagia* spp. on four and three farms, respectively. According to a meta-analysis research, Mavrot et al. (2015) reported that the consequences of gastrointestinal nematode infections seemed to be similar for different species of parasites, but seemed to influence also a milk yield and weight gain more than wool production. Indirect losses would be due to antiparasitic drugs resistance such as loss of veterinary treatment costs and efforts.

Regarding the zoonotic impact of GIP, according to Barnes et al. (2017), nomadic and pastoral communities are at risk of infection with a variety of zoonotic GIS due to their living environment, cultural and dietary traditions and close relationship with animals. For example, in a study conducted in southwestern Iran by Beiromvand et al. (2019), the authors confirmed that people in the rural regions were more infected with intestinal parasite than those living in the urban areas. Furthermore, in a study conducted in Iran by Pezeshki et al. (2018) on slaughtered sheep and cattle, the hydatidosis in sheep was 2.48%. In regards to liver flukes, recorded were 0.62% of *Fasciola* spp. infection and 2.86% of *Dicrocoelium dendriticum* infection in sheep. In the study conducted by Hazzaz Bin Kabir et al. (2010), the prevalence of zoonotic parasitic diseases in slaughtered sheep were for hydatidosis (16.95%), fasciolosis (14.13%) and paramphistomosis (10%).

This study confirmed the multiplicity of gastrointestinal parasites genera in sheep of Biskra region. In addition, the total prevalence of gastrointestinal parasites seems very high in both adult sheep and lambs. This would have a negative impact on the lambs' growth, sheep production and health in the study area, especially with the high infection rates recorded for coccidia and gastrointestinal strongyles. Presence of zoonotic parasites as *Fasciola hepatica* and Echinococcus polymorphus is alarming in regards to the infection risks incurred by the concerned socioprofessionals and consumers. Breeding practices, feeding methods, transhumance, parasitic diseases prevention and treatment protocols as well as weather conditions, could influence the growth of one parasitic genera at the expense of another and from one year to another. More attention should be paid to deworming programs in sheep and dogs and better vigilance from veterinarians active in slaughterhouses should be required.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with this work and the preparation of the paper.

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STUDIJA ENDOPARAZITOZE OVACA PREMA STAROSNIM GRUPAMA U REGIJI BISKRA U ALŽIRU

SAŽETAK

Cilj našeg istraživanja jeste odrediti prevalencu gastrointestinalne parazitoze kod ovaca u regiji Biskra prema starosnim grupama i identificirati eventualne zoonotske parazite. U istraživanje su uključene 372 životinje oba spola: od 248 fekalnih uzoraka ovaca urađena je koprokultura, od čega za 119 odraslih (≥ 12 mjeseci) i 129 janjadi (< 12 mjeseci). Na 124 odrasle životinje je izvršena nekropsija nakon klanja pri čemu je posebna pažnja posvećena jetri, plućima i crijevima. Korištena je kvalitativna metoda flotacije upotrebom McMaster mikroskopskih stakalaca. U statističkoj obradi je korišten SPSS 20. Prevalenca je izračunata prema starosnim grupama i rodu parazita, a Kendallov test ($p<0.05$) je korišten za otkrivanje korelacije između ukupne prevalence i prevalenci rodova parazita kod odraslih naspram janjadi. Rezultati su pokazali da je srednja petogodišnja prevalenca kod odraslih (63.02%) veća nego kod janjadi (57.36%). Koproskopija je pokazala prisustvo kokcidije, gastrointestinalnog strongiloidesa, *Nematodirus* spp, *Moniezia* spp. i *Marshallagia* spp. Koprokulture su pokazale *Protostrongylus* spp. i *Dictyocaulus* spp. Nekropsijom je dokazana *Fasciola hepatica*, Echinococcus polymorphus, *Thysaniezia ovilla, Moniezia expansa,* Cysticercus tenuicolis, Cysticercus ovis i *Paramphistomum daubenyi.* Raznolikost i istovremeno prisustvo više gastrointestinalnih parazita može negativno djelovati na proizvodne parametre kod inficiranih ovaca. Rizici širenja ehinokokoze kao glavne zoonoze zahtijevaju posvećivanje više pažnje programima deinfestacije pasa i ovaca, kao i bolju kontrolu u klaonicama.

Ključne riječi: Gastrointestinalna strongiloidoza, janjad, upravljanje stadom, programi deinfestacije, parazitarna zoonoza