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DETECTION OF PARASITES CAUSING DIARRHOEA IN LAMBS OF BABYLON GOVERNORATE, IRAQ

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Abstract

Ruminants infects with large numbers of parasites in their gastro-intestinal tract. *Giardia duodenalis*, *Eimeria* spp. and *Cryptosporidium* spp. are the main gastro-intestinal protozoa that affect a wide range of mammals, including sheep, causing economic losses. This study was carried out to record the prevalence of intestinal protozoa in lambs. A total of 120 fecal samples was collected from diarrheic lambs aging from (less than one month to one year old) of both sexes in different areas of Babylon governorate during winter season. Routine diagnostic tools, such as the direct smear and floatation technique and sporulation were used to detect and identify these parasites. The results showed detection of three species of parasite infecting sheep, including: *Eimeria* spp. 47 cases (39.16%), *Cryptosporidium parvum* 29 cases (24.16%) and *Giardia duodenalis* 13 cases (10.83%). Thus, this study revealed that *Cryptosporidium parvum* was the common intestinal protozoa infecting lambs in this area.

Key words: Parasite, Diarrhea, *Cryptosporidium, Eimeria*, *Giardia.*

Introduction

Diarrhea in lambs are caused by infectious and non-infectious agents. Strongylida nematodes, protozoa, bacteria and viruses are the major pathogens which are responsible for production losses include growth reducing rates and the occurrence of diarrhea in lambs (Belloy *et al.*, 2009, Jacobson *et al.*, 2009). It is important to understanding and quantifying the economic losses associated with these infectious pathogens (Hamzah and Hasso, 2019).

Digestive tract protozoa are common parasites in the gut of mans and mammals such as enteric bacteria and viruses can be detected in water following direct or indirect contamination by the feces of mans or other animals (Karanis *et al.*, 2007). For example, *Cryptosporidium* parasite of medical and veterinary importance that may cause gastroenteritis in a variety of vertebrate hosts, like sheep (Bouzid *et al.,* 2013). *Eimeria* species also cause a disease called coccidiosis which typically results in diarrhoea, weight loss and dehydration, leading to poor growth and death of the animal, particularly amongst young (Foreyt, 1990). In addition, *G. duodenalis* causes symptomatic acute or chronic giardiasis, the symptoms of which include, diarrhea, abdominal pain, bloat, dehydration, weight loss and mal-absorption (Buret and Cotton, 2011). However, asymptomatic infections are also reported in different hosts, including in farm animals (Geurden and Olson, 2011).

The ability of these microorganisms to produce cysts or oocysts that are extremely resistant to environmental conditions and conventional drinking water sterilization has facilitated their ability to spread and cause infection (Health Canada, 2012). Enteric protozoa are wide-spread occurrence in domestic animals (Taylor, 2000). Many types of micro-organisms have intracellular life-cycle phases in the intestinal epithelial layer and have the ability to induce disease, due to this widespread occurrence of infection, economic importance associated with restricted options of treatment and sometimes zoonotic importance have need most investigations of protozoan parasite infections in farm animals have come from outbreaks on farms or from experiments including production animals (Craig *et al.,* 2007). This study focused on identification and prevalence of common intestinal protozoa that infect lambs during winter.

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Materials and Methods

Sample collection

Fecal samples (10-15) grams were collected from 120 pre, post-weaned lambs, of different ages ranged from less than 1 month to 1 year old, for both sexes (55 male- 45 female) during the period from the beginning of November 2017, to end of April 2018, from different regions of Babylon province, samples were collected directly from the rectum, in a clean plastic container and were tightly closed, given sequential numbers, all information included age, sex and date of sampling, the samples were transported in refrigerated bag to the lab.

Laboratory tests: Microscopic examinations for the fecal samples were carried out, where each sample was divided into four parts:

1. Direct Smear: The smears were prepared and examined by using Lugol's Iodine stain (Levine, 1961).
2. Staining methods: The smears prepared and stained by Giemsa and Modified Ziehl-Neelsen stains to investigate *Cryptosporidium* oocysts and *Giardia* cysts according to (Mor, 1981, Beaver and Jung, 1985).
3. Flotation technique: This technique was conducted using Sheather's and Zinc Sulphate solutions to investigate *Cryptosporidium*, *Eimeria* oocysts and *Giardia* cysts according to (Chermette and Boufassa, 1988, Ruest *et al.*, 1997).
4. Sporulation of fecal samples that contain oocysts (Bowman and Lynn, 1995).

Statistical analysis: The Chi-square test was used for comparison between the results. Differences were considered statistically significant at $P<0.05$ (Al-Mohammed *et al.,* 1986).

Results and Discussion

Infection rates of intestinal protozoa

Out of the total number of 120 fecal samples, 89 (74.16%) are infected with intestinal protozoa in lambs at Babylon province, the microscopic examination showed the presence of (*Eimeria* spp., *Cryptosporidium parvum*, *Giardia duodenalis*). This finding highlighted the importance of these parasites on lambs health which might be leading to economic losses.

Several studies demonstrated that the prevalence of intestinal protozoa recorded various ratios in Iraq. A study conducted by Abd Al-Wahab (2003) found the prevalence rate 85% in lambs of Baghdad province and Dawood *et al.,* (2007) recorded the rate 50.2% in lambs of Al-Diwaniya province. Other studies in 2011 also reported infected rates 34.36% and 75.1%, in Al-Sulaymaniyah and Baghdad provinces, respectively (Nassrullah, 2011, Fadl *et al.,* 2011). The slight variation in these infection ratios compared to the current study might be ascribed to different areas and dates of samples collection.

As mentioned above, three genera were detected in the present survey, the first genus was *Eimeria*, out of the total number of 120 fecal samples, 47 (39.16%) were infected with different species of *Eimeria*. Table 1, showed the *Eimeria* spp. oocysts that were detected and distinguished in this study according to (Deniz, 2008 and Dittmar *et al.*, 2010). In Iraq, some local studies were carried out to record the prevalence of *Eimeria* spp in in lambs. For example, Abd Al-Wahab (2003) showed that the prevalence of *Eimeria* spp. was 79.19%, while Fadl *et al*., (2011) recorded 49.0% in Baghdad province. In addition, Mohammed (2013) found that the prevalence of *Eimeria* spp. in AL-Muthana province was 67.5%. These different prevalence might be ascribed to age of animals, shorter period of study and the number of animals used in the study. In the meantime, the prevalence rate in

Table 1: Oocysts of *Eimeria* species.

No.	Species	Characteristics		
		Shape	Wall	Color
1	*E.ahsata*	Ellipsoidal	Smooth	Yellowish brown
2	*E. bakuensis* (*ovina*)	Elongate to ellipsoidal	Smooth	Yellowish
3	*E.crandalis*	Broad ellipsoidal to spherical	Smooth	Pale- yellowish
4	*E.granulosa*	Urn-shape	Smooth	Yellowish- brown
5	*E.intricata*	Ellipsoidal	Thick	Brown
6	*E.marsica*	Ellipsoidal	Smooth	Colorless
7	*E.punctata*	Ovoid	Smooth	Pale- yellowish
8	*E. weybridgensis*	Broad ellipsoidal to spherical	Smooth	Colorless
9	*E.faurei*	Ovoidal	Smooth	Pale- yellowish brown
10	*E.ovinoidalis*	Ellipsoidal	Smooth	Colorless to pale-yellowish
11	*E.pallida*	Ellipsoidal	Very- smooth	Colorless
12	*E.parva*	Spherical to sub spherical	Smooth	Colorless

Table 2: Total rate of infection according to sex.

Sex	No.	Positive	Negative
Male	50	33	17
Female	70	56	14
Total	120	89	31

Table 3: Percentage of infection according to species.

Species	No. of samples	No. Positive	Percentage %
Eimeria spp.	120	47	39.16
Cryptosporidium parvum		29	24.16
Giardia duodenalis		13	10.83
Total		89	74.16

current study was lower than that established by Arslan *et al.,* (1999) in Turkey (97.9%) (Table 1, 2, 3, 4 and 5).

The second genus was *Cryptosporidium parvum*, the results revealed that 29 (24.16%) out of 120 fecal samples were infected with *cryptosporidium parvum*. The morphological characteristics of *C. parvum* oocysts in Modified Ziehl Neelsen stain appeared as spherical densely stained pink to red bodies with a clear halo around the oocyst, against a dark blue background of the methylene blue stain. Furthermore, in Sheather's sugar floatation, the oocysts appeared as round or oval retractile bodies with a thin greenish membrane, the four sporozoites looked like black bodies inside the oocysts.

In comparison with previous studies in Iraq, the current findings were in agreement with Abdullah (2005), who reported 26.66% in Nineveh Province. Moreover, Dawood and Abdullah (2007) showed that the prevalence of *C. parvum* in Diwaniya province was 27.5% and Yakoob and Kathim (2009) detected the infection rate 15.8% in Baghdad province. In addition, the present results were approximately in accordance with other findings conducted in some countries. For instance, EL-Wahed *et al.,* (1999) in Egypt, Sultan *et al.,* (2007) in Tunisia, Panousis *et al.,* (2008) in Greece, Vahedi *et al.,* (2009) in Iran and Gokce *et al.,* (2010), whom recorded the prevalence rates of *C. parvum* were 30%, 16.7%, 29%, 28.6% and 21.05%, respectively.

Table 4: Percentage of infection according to species and sex.

Species	Total number of samples	Total number of infection	Sex	No. Positive	Percentage %
Eimeria spp.	120	47	M	23	19.16
			F	24	20
Cryptosporidium parvum		29	M	11	9.16
			F	18	15
Giardia duodenalis		13	M	5	4.16
			F	8	6.66
Total		89			74.16

However, a high percentage of infection (81.46%) was found in lambs of Baghdad province (Abd Al-Wahab, 2003). furthermore, the prevalence rate of *C. parvum* in current study was lower than those established by Misic *et al.,* (2006) in Serbia (42.1%), Sari *et al.,* (2009) in Turkey (38.8%), Silva-Fiuza *et al.,* (2011) in Brazil (47%) and Connelly *et al.,* (2013) in Scotland (37%). These differences were due to most of these studies were carried on rearing farms with high number of density which facilitate the contamination of soil and drinking water and considered the main sources of infection transmission, other differences might also be due to dates and areas of samples collection, shorter period of study, in addition to other factors affecting the prevalence such as number of samplings, methods of rearing and techniques of diagnosis used which have highly effective on determination such ratios particularly the density of the flocks.

Nevertheless, other studies recorded low ratios of infection, such as, Wang *et al.,* (2010b) in china reported 4.8%. In Iran, Khezri *et al.,* (2013) and Gharekhani *et al.,* (2014) recorded (10.24%, 11.3%, respectively). The most important reason of those lower rates of infection was age of animals that used in these studies (more than one year old) which were more resistant to infection (Sari *et al.*, 2009) (Table 2, 3, 4 and 5).

The third genus was *Giardia duodenalis*: 13 samples (10.83%) out of total number of 120 were infected with *Giardia duodenalis*. Morphologically, cyst of *G. duodenalis* was oval in shape with thick wall. It contains four nuclei which tend not to be obvious. Longitudinal fibrils consisting of the remains of axostyle and parabasal bodies may also be seen.

This result was in accordance with Rhaymah and Mohammed (2006) in Ninevah province, Khalil (2009) in Baghdad province and AL-Fetly *et al.,* (2010) in Al-Qadisiya province, whom recorded the rates of infection of *Giardia duodenalis* in lambs 15.5%, 13.71% and 13.5%, respectively.

While the infection rate in this study was lower than those recorded by Swadi (2008) in Baghdad province (52.17%), Geurden *et al.,* (2008) in Bulgaria (25.5%) and Wilson and Hankenson (2010) in United States (68%).

However, Abd Al-Wahab (2003) in Baghdad and Giangaspero *et al.,* (2005) in Italy showed that the infection rates of *G. duodenalis* in lambs were (6.92%) and (1.5%) respectively, which were lower than the result in the current study.

Table 5: Percentage of infection according to species and sex.

Species	Total number of samples	Total number of infection	Age months	No. Positive	Percentage %
Eimeria spp.	120	47	<1-4	11	9.16
			5-9	17	14.16
			>10	19	15.83
Cryptosporidium parvum		29	<1-4	7	5.83
			5-9	9	7.50
			>10	13	10.83
Giardia duodenalis		13	<1-4	2	1.66
			5-9	5	4.16
			>10	6	5.00
Total		89			74.16

The variation among these studies might be ascribed to some important factors, such as, season of samples collection, study area, number of samples collected, methods of rearing and methods of diagnosis used which have highly effective on determination such ratios particularly the density of the flocks (Craig *et al*., 2007) (Table 2, 3, 4 and 5).

In summary, the current study found that three important genera *Eimeria*, *Cryptosporidium* and *Giardia* were detected in lambs of Babylon province using classical tools. The infection ratios of these protozoa were approximately moderate compared with other studies. This study also revealed that season of samples collection, study area, number of examined animal, age and diagnosis method could be important factors to detect and determine the intestinal protozoa in sheep, particularly lambs. Thus, further studies using modern techniques would be useful to confirm these findings, as well as identifying other significant genera.

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