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PREVALENCE OF INTESTINAL PROTOZOA IN CATTLE IN SOME AREAS OF WASIT PROVINCE, IRAQ

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Abstract

The current study was aimed to determine the infection rate with intestinal protozoa in cattle at Wasit province, during period from the beginning of December 2018 to the end of August 2019. Two hundred and seventy fecal samples were collected from cattle and examined by traditional methods (direct moist smears, flotation with saturated NaCl solution and stained with Modified acid fast, Giemsa and lugol's iodine) The total infection rate was 81,85% with intestinal protozoal oocysts and cysts. The infection rate with *Cryptosporidium* sp. was (28.51%), *Giardia duodenalis* was (22.96%), *Entamoeba* sp was (29.62%), *Eimeria* sp. was (42.59%) and *Buxtonella sulcata* was (11.48%), and touching on the risk factors (age, gender, season) on the infection rate. In conclusion, this study approved high incidence of intestinal protozoa infection rate in cattle.

Key words : Cattle, *Cryptosporidium, prevalence, Eimeria* oocysts, intestinal protozoa.

Introduction

Intestinal protozoa are invisible single cell organisms, more than 65,000 of which are species have been described. Many happen in such harmless commensals as either free-living form, but their figures include some of man and animal's most important parasitic diseases in the world. Ruminants carry a high number of protozoa in their intestinal tract, the vast majority of which are completely harmless. However, some protozoa species are important as causes of disease in domesticate cattle and sheep or because of their potential for zoonotic transmission (Taylor, 2000).

Intestinal protozoa are those able to cause intestinal infections; they are viewed endoparasites; they are accountable in creating counties for some of the most prevalent infectious illnesses such as amoebiasis and giardiasis (Geo *et al.,* 2001). In temperate regions areas in particular (Akanda *et al.,* 2014).

Intestinal protozoa infection requires special attention because it can be an barrier to increasing livestock growth in the more region can cause financial losses owing to lower animal efficiency, lower weight, meat quality, skin and inner organs, growth retardation in young animals, and the risk of zoonoses. Delay weight gain with protozoan infection in livestock can reach > 40% compared with healthy cows (Thompson and Smith, 2011; Maharana *et al.,* 2016).

Giardia intestinalis, Entamoeba histolytica and *Cryptosporidium* spp. are the most prevalent enteric protozoan parasites. The illnesses produced by these enteric protozoan parasites are severely referred to as giardiasis, amoebiasis, and cryptosporidiosis and are associated with diarrhea (Davis *et al.,* 2002). The objective of this research was to investigated prevalence of intestinal protozoa in cattle in Waist province regarding the age, sex, and month.

Materials and Methods

Fecal samples were collected two hundred and seventy from local breed of cattle from three distinct areas (Al-Hafriyah, Al-Swearah and Al-Azeziyah) at Wasit province. During the period from December 2018 to the end August 2019 include were used in this study . Fecal samples about (10-15 grams) were collected directly from the rectum of animal then put in a clean plastic container. Three age group was less than one year into >5 year. (<1-3, 3-5 and >5) year. All information's about age, sex and date of sampling collecting were recorded

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and transported in cold bag to the Parasitology Laboratory.

Laboratory examination

A. Macroscopic examination: involved color and consistency of fecal samples collected.

B. Microscopic examination:

Direct smear a small amount of feces as far as the head of the pin s stick mixed with a drop of distilled water on the glass slide and cover with glass slide and examine under the strength of (40 and 100x) magnification (Griffiths, 1978).

Anthers smears were prepared by mix with one drop of Lugol's iodine stain with a small amount of feces as far as the head of the pin s stick to a drop of distilled water on the glass slide (Levine, 1961) .

Concentration methods: flotation method by using saturated NaCl solution and Staining methods by used Giemsa stain for 20-25 minutes for detected expected protozoa as *Giardia* cyst, *Eimeria* oocysts, *Buxtonella sulcata* and *Entamoeba* sp. (Beaver and Jung, 1985). And used Modified Ziehl-Nelsen stains for detect *Cryptosporidium* oocysts (Garcia,2016).

Results and Discussion

Infection rate of intestinal protozoa

The overall infection rate with intestinal protozoa recorded in three regions (Al-Hafriyah, Al-Swearah and Al-Azeziyah) of Wasit province was 221 sample positive out of 270 sample were microscopically. Infection with *G. intestnalis* was 22.96%, *Cryptosporidum* spp. was 28.51%, *Entamobea* spp was 30%,, *Eimeria* spp. was 42.59% and *B. sulcata* was 11.48% (Fig. 1, 2, 3, 4 and 5). Fecal samples recorded high infection rate with *Eimeria* sp in Al-Hafriya (35.55%), Al-Suwaira (41.11%) and Al- Aziziyah (50%). (Table1 and 2). with high significant difference (P <0.01). Intestinal protozoa are the primary cause of cattle farms losses, including conversion-failure feeding and even death of highly infected livestock. (Penny *et al.*, 2016). The result of this research agreement with Poedji *et al.*, (2019) results reported the prevalence variety of gastrointestinal protozoa in cattle in Madura (Bangkalan Regency, East Java, Indonesia) which recorded six species of protozoa that infected gastrointestinal tract, those are *Eimeria* spp., *Balantidium* spp., *Isospora* spp., *Blastocystis* spp., *Entamoeba* spp. and *Cryptosporidium* spp., eimeria was recorded (53.42 %) the highest infection rate in studies, compared to other diagnosed protozoa. Ibukun and Oludunsin (2015), who recorded the incidence of gastrointestinal parasites in cattle *Eimeria* spp, reported a high infection rate of 42.1% in 2012, This could likely be due to the criteria used to collect samples as one of the leading signs of clinical coccidiosis is diarrhea (Hansen and Perry, 1990; Adejinmi and Osayomi, 2010) . Al-Saffar *et al.*, (2010) who recorded infection with *Buxtonella sulcata* was 24.16% in cattle in Mosul city. increase infection with protozoa due to overcrowding and poor hygiene practices that can significantly the dispersal of these parasites, As these animals become carriers of intestinal protozoa and

Table 1: Result of prevalence infection rate of intestinal protozoain cattle

Parasite	No. of cattle examined	No. of cattle infected	Percentage (%)	P-Value	X^2
Giardia sp	270	62	22.96		
Cryptosporidium sp	270	77	28.51	<0.00001	63.71
Entamoeba sp	270	81	30		(HS)
Eimeria sp	270	114	42.22		
Buxtonella sulcata	270	31	11.48		

according to species HS : high significant difference (P <0.01).

Table 2: Infection rate with intestinal protozoa species in cattle in relation to area

parasite	Al-Hafrea			Al-swearea			Al-Azezea		
	No. of cattle examined	No. of cattle infected	(%)	No. of cattle examined	No. of cattle infected	(%)	No. of cattle examined	No. of cattle infected	(%)
Giardia sp	90	20	22.22	90	18	20	90	24	26.66
Cryptosporidium sp	90	23	25.55	90	22	24.44	90	32	35.55
Entamoeba sp	90	20	22.22	90	31	34.44	90	30	33.33
Eimeria sp	90	32	35.55	90	37	41.11	90	45	50
Buxtonella sulcata	90	12	13.33	90	6	6.66	90	13	14.44
Total	270								
P-Value	0.013			<0.00001			<0.00001		
X^2	**12.70(HS)**			**33.76(HS)**			**27.92(HS)**		

HS : high significant difference (P <0.01).

Table 3: Infection rate with intestinal protozoa species in cattle in relation to sex

Parasite	Sex	No. of cattle examined	No. of cattle infected	(%)	P-Value	X^2
Giardia sp	Males	61	13	21.31	0.727	0.121
	Females	209	49	23.44		(NS)
Cryptosporidium sp	Males	61	16	26.22	0.653	0.203
	Females	209	61	29.18		(NS)
Entamoeba sp	Males	61	21	34.42	0.351	0.870
	Females	209	59	28.22		(NS)
Eimeria sp	Males	61	33	54.09	0.039	4.267
	Females	209	82	39.23		(S)
Buxtonella sulcata	Males	61	8	13.11	0.649	0.207
	Females	209	23	11		(NS)

S : Significant difference (P<0.05) NS : Non-significant difference (P>0.05)

Table 4: Infection rate with intestinal protozoa species in cattle in relation to Age group

Parasite	Age group (year)	No. of Samples examined	No. positive	(%)	P-Value	X^2
Giardia sp	<1-3	119	28	23.52	0.547	1.205
	>3-5	72	19	26.38		(NS)
	>5	79	15	18.98		
Cryptosporidium sp	<1-3	119	31	26.05	0.266	2.651
	>3-5	72	18	25		(NS)
	>5	79	28	35.44		
Entamoeba sp	<1-3	119	40	33.61	0.452	1.589
	>3-5	72	21	29.16		(NS)
	>5	79	20	25.31		
Eimeria sp	<1-3	119	50	42.01	0.334	2.191
	>3-5	72	35	48.61		(NS)
	>5	79	29	36.70		
Buxtonella sulcata	<1-3	119	10	8.40	0.113	4.355
	>3-5	72	7	9.72		(NS)
	>5	79	14	17.72		

NS : Non-significant difference (P>0.05)

continuously contaminate the environment with parasite eggs and oocysts.

Infection rate with intestinal protozoa species in relation with sex

This study recorded highest infection rate with *Giardia* sp *and Cryptosporidium* sp in females (23.44% and 29.18) without Significant difference (P<0.05), while infection with *Entamoeba* sp, *Eimeria* sp and *Buxtonella sulcata* recorded highest infection rate in males was (34.42,%, 54.09 and 13.11 %) respectively (Table3).

Current study with Al-Difaie, (2016) who observed that the prevalence of giardiasis between sex was 60% in females and 40% in males by lougal's iodine stain . Females are more exposed to giardiasis because of low immunity during pregnancy (Khudier, 2011). Al- zubaidi, (2012) reported overall prevalence of *cryptosporidium* sp ratio in female was 36.11% (65 positive samples out of 180 samples) compared with in male 34.09% (30 positive samples out of 88 samples) .And agreement with Ferid *et al.,* (2012) who observed infection with Eimeria sp (29.4%) greater incidence in male calves than female (20.7%) . Bit higher prevalence of male calves may be due to less care given to male calves than to female calves, which are considered to be future cattle. Nonetheless, previous research on adult cattle showed a higher prevalence of Eimeria in female animals than in males (Priti *et al.,* 2008; Tauseef *et al.,* 2011). This could, however, be due to the physiological stress that is loaded on female animals in relation to pregnancies and giving birth as compared to males (Radostits *et al.,* 2007).

This study recorded non-significant difference and agreement with AL-Saffar *et al., (*2013) observed non-significant difference between sex on the percentage of infection with *Buxtonella sulcata*. Our findings are consistent with the line of (Tomczuk *et al.,* 2005), which revealed that this protozoan parasite infection may result in GIT damage associated with parasite virulence, host susceptibility, infection sensitivity, parasite microenvironment, position in the GIT, and the secondary bacterial infection.

Infection rate of intestinal protozoa according to the age groups

This study recorded highest infection rate with *Giardia* sp *and Cryptosporidium* sp in females (23.44% and 29.18) without Significant difference (P<0.05), while infection with *Entamoeba* sp, *Eimeria* sp and *Buxtonella sulcata* recorded highest infection rate in males was(34.42,%, 54.09 and 13.11 %) respectively (Table3).

Current study with Al-Difaie, (2016) who observed that the prevalence of

Table 5: Infection rate of intestinal protozoa according to months

Months	No.of Samples Examined	No. positive	(%)
December/2018	30	29	96.66
January/2019	30	22	73.33
February/2019	30	21	70
March/2019	30	28	93.33
April/2019	30	27	90
May/2019	30	21	70
June/2019	30	24	80
July/2019	30	22	73.33
August/2019	30	27	90
Total	**270**	**221**	**81.85**

S: Significant difference (P<0.05)

Fig. 1: Cyst of *G.duodenalis* sp oocyst

giardiasis between sex was 60% in females and 40% in males by lougal's iodine stain . Females are more exposed to giardiasis because of low immunity during pregnancy (Khudier, 2011). Al- zubaidi, (2012) reported overall prevalence of *cryptosporidium* sp ratio in female was 36.11% (65 positive samples out of 180 samples) compared with in male 34.09% (30 positive samples out of 88 samples). And agreement with Ferid *et al.*, (2012) who observed infection with Eimeria sp (29.4%) greater incidence in male calves than female (20.7%). Bit higher prevalence of male calves may be due to less care given to male calves than to female calves, which are considered to be future cattle. Nonetheless, previous research on adult cattle showed a higher prevalence of Eimeria in female animals than in males (Priti *et al.*, 2008; Tauseef *et al.*, 2011). This could, however, be due to the

Fig. 2: *Cryptosporidium* sp sporulated

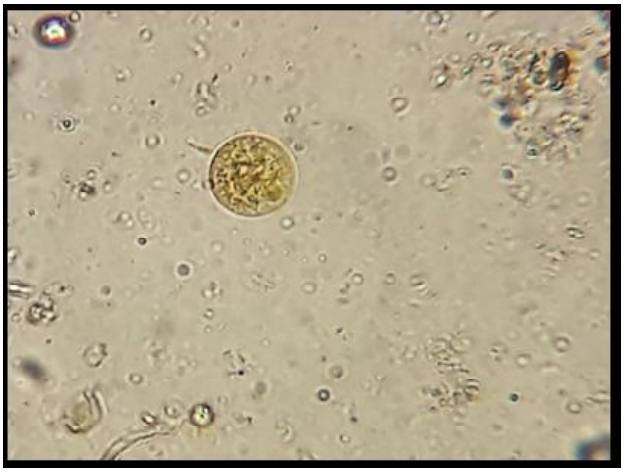

Fig. 3: Cyst of *Entamobea* sp

Fig. 4: Oocyst of *Eimeria* cattle fecal sample non sporulated in wet film (40x)

Fig. 5: Trophzoit of *B.Sulcata* in direct wet film (40x)

physiological stress that is loaded on female animals in relation to pregnancies and giving birth as compared to males (Radostits *et al.*,2007).

This study recorded non-significant difference and agreement with AL-Saffar *et al., (*2013) observed non-significant difference between sex on the percentage of infection with *Buxtonella sulcata*. Our findings are consistent with the line of (Tomczuk *et al.*, 2005), which revealed that this protozoan parasite infection may result in GIT damage associated with parasite virulence, host susceptibility, infection sensitivity, parasite microenvironment, position in the GIT, and the secondary bacterial infection.

Intestinal protozoa among age groups was recorded the highest infection rate with *Giardia* sp was (26.38%) at age group (3-5 year) and the lowest rate (18.98%) of age at (>5year), while infection rates with *Cryptosporidium* sp recorded hight rate(35.44%) among age of (>5 year) and in 3-5 year recorded lowest rate was (25%) . *Entamobea* sp recorded height infection among age (<1-3) which was (33.61%) and lowest infection (25.31%) among age (>5) *Eimeria* sp recorded height infection rate (48.61%) among age (3-5) and lowest infection rate (36.70%) among age (>5). *Buxtonella sulcata* recorded high (17.72%) among age (>5) and lowest infection rate (8.40%) among age <1-3 year (Table 4). with Non-significant difference (P>0.05). In Age, Poedji *et al.,(*2019) In this study, the incidence of protozoan infections in Madura cattle from 6 months to 2 years of age was also greater than those < 6 months of age due to their pasture habit in bigger regions contaminated with protozoa. Therefore, the incidence of calf diseases is always smaller as they have been less exposed to greater regions contaminated with protozoa and it is also because the calf is still suckling, agree with Chiu-Chen *et al.*, (2014) in this research . Heavy infection with *Buxtonella sulcata* (61.7%) was the most prevalent of all parasites, followed by *Cryptosporidium* spp. (32.6 %) and *Eimeria* spp. (11.8%). *B. sulcata* infection was found in 61.7% of the entire dairy cattle population, And the rising incidence associated with the growing area. This finding was similar to previous investigative outcomes (Wang, 1979). Fox and Jacobs (1986) noted that the quantity of carbohydrate in the diet would affect the development or decrease of *B. sulcata* in population Sulcata. Fooding of adult bovine animals with a significant amount of corn and wheat bran to recruit dietary losses lactation might reduce the prevalence of *B. sulcata* Infection, in adult cattle.

Infection rate of intestinal protozoa according to months of study

The study recorded infection rate with intestinal protozoa all over the months of the study, the highest infection rates was in December (96.66%), while the lowest infection rate (70%) was during May and February (Table5). According to months, the study showed high incidence in all study months with significant differences (P<0.05). The current study is close to the results of both Hasan and Khalaf, (2013) who recorded the lowest incidence in February was 23.68% with significant difference (P<0.05), and Hailu *et al.,* (2011), who recorded lowest infection in February month. This is due to the very low temperature in February and the fall of rain, which leads to the delay of hatching and vitality of oocyst and the life cycle of the parasite. (Beveridge *et al.,* 1989).

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