Veterinary Integrative Sciences 2023; 21(3): 735 - 749 DOI; 10.12982/VIS.2023.053

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

The role of *Cryptosporidium parvum* in diarrhea in calves and lambs

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Abstract

Cryptosporidiosis is an important parasitic disease that leads to morbidity and mortality among young ruminants. In our study, 200 neonatal calves and lambs were examined for *Cryptosporidium parvum (C. parvum)* to compare Enzyme-linked immunosorbent assays (ELISA) with microscopic examination. The positive rate of infection in cattle calves, buffalo calves and lambs was 38.7%, 22% and 40%, respectively, by microscope and was 82.7%, 84% and 62.7%, respectively using ELISA in Assiut Governorate. The total positive rate in young ruminants was 75.5%. According to the sex, the positive rate in cattle calves, buffalo calves and lambs was 82.1%, 90.5% and 54.2%, respectively in males and was 83.3%, 79.3% and 77.8%, respectively in females. Concerning the age, it was 90.6%, 96.2% and 96% in cattle calves, buffalo calves and lambs, respectively, with age less than 1 month and was 96.6%, 63.6% and 26.9%, respectively, with age 1-2 months. While, it was 35.7%, 76.9% and 66.7% in cattle calves, buffalo calves and lambs, respectively, in age more than 2 to 3 months. The positive rate of cattle calves, buffalo calves and lambs was 97.1%, 90% and 100% of farm rearing, respectively, and was 70%, 75% and 37.8% of household rearing, respectively. The positive rate according to the feces consistency of cattle calves, buffalo calves and lambs was 77.3%, 78.9% and 49% of normal feces, respectively, and was 90.3%, 87.1% and 91.7% of diarrheic feces, respectively. This finding showed a high infection rate with *C. parvum* in calves and lambs.

Keywords: Calves, Cryptosporidium parvum, ELISA, Lambs, Microscopic

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Article history; *received manuscript: 25 March 2023,*
revised manuscript: 15 April 2023,
accepted manuscript: 30 May 2023,
published online: 7 June 2023
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Cryptosporidiosis is a global distributed disease that results in major economic losses in livestock and animal production (Paoletti et al., 2009). *Cryptosporidium parvum* (*C. parvum*) is one of the most important parasitic zoonotic protozoa, common in young domesticated ruminants (calves, lambs and goat kids) and responsible for outbreaks of serious diarrhea in humans and newborn calves (Zhang et al., 2021). Severe diarrhea in calves is causing severe losses on dairy farms and the beef industry around the world due to calf mortality and morbidity within the early few weeks of life (Lombardelli et al., 2019) Furthermore, Wang et al. (2021) added that cryptosporidiosis leads to high morbidity and mortality in the developing countries.

Cryptosporidium infection is distinguished clinically by variable degrees of diarrhea. High production dairy cattle, overcrowding, poor hygiene and inappropriate management measures are risk factors for diarrheal diseases on farms. The clinical signs of *C. parvum* infection are obvious 3–5 days after infection, and its duration may range from 4 to 18 days (de Aquino et al., 2020). It causes fever, severe persistent watery diarrhea, anorexia, dehydration and laziness and often leading to death. Recovered or chronically infested calves are liable for tremendous economic losses through the reduction in growth rate, meat and milk yield, delayed maturity age and high production and drugs costs, which impedes the livestock industry development (Maurya et al., 2013; Elshahawy and AbouElenien 2019; Santín, 2020).

Infected ruminants with cryptosporidiosis, especially diarrheic calves, provide a direct source of infection for other farm animals (Olson et al., 2004). It is considered a major reservoir of zoonotic *Cryptosporidium*, because subtypes of *C. parvum* infecting peoples have been insulated from cattle and they excrete huge numbers of oocysts that cause contamination of the environment in numerous outbreaks (Xiao, 2010; Santín, 2020). Furthermore, few studies have proposed that cattle and buffaloes act as a significant reservoir of *Cryptosporidium* (de Aquino et al., 2020). In addition, Elmadawy et al. (2017) and Kabir et al. (2020) suggesting that sheep constitute a main *C. parvum* reservoir and a potential source of zoonosis.

Many outbreaks of cryptosporidiosis have been recorded in neonatal calves worldwide, with detrimental effects on animal welfare and its production (Thomson et al., 2017; Niine et al., 2018; Ouakli et al., 2018). As, the infected calves do not respond to treatment with antibiotic and in more severe cases occur dehydration and cardiovascular collapse which lead to deaths (Olson et al., 2003). Diarrhea and enteritis are the primary causes of calf mortality in the early weeks of life (Díaz-Lee et al., 2011).

Cryptosporidium infection is detected in farm animals by a variety of methods which can categorize into microscopy, serology, and molecular diagnostic methods (Khurana and Chaudhary, 2018). The microscopic examination is the most of these time consuming methods and requires the experience and competence of the microscopist (Shams et al., 2016). It may not be sufficient to detect the presence of *Cryptosporidium* (Weber et al., 1991). So, there is the necessity to perform a confirmatory test to rule out or indicate the existence of the zoonotic type in animal feces (Santín et al., 2004). *Cryptosporidium* specific coproantigens can detected by ELISA which

considered the simplest, quickest and least labor intensive one (Fereig et al., 2016; Elshahawy and AbouElenien 2019).

The purpose of this study was performed to determine the infection rate of *C. parvum* in calves and lambs and compare coproantigen ELISA assays with microscopic examination in Assiut governorate, Egypt.

MATERIALS AND METHODS

Sample collection and preparation

This study was conducted between March 2021 to September 2022 at Assiut Governorate, Egypt. Fecal samples collected from 200 young ruminants (75 cattle calves, 50 buffalo calves and 75 lambs). For each animal, the sex, age, housing system (farm, household) and consistency of the feces (diarrheic or normal) were registered to establish the presence of *C. parvum.* Based on animals' age, the examined animals (less than 3 months) were categorized into three groups: (< 1 months, 1-2 months and > 2-3 months).

Fresh fecal samples were collected in labelled plastic containers and transmitted within 2–3 hours to the laboratory and preserved at 4ºC until the examination. Each fecal sample was examined macroscopically. Then, thin fecal smears were made, air dried, fixed and stained by "modified Ziehl-Neelsen stain" for microscopic examination by the oil immersion lens (Swain et al., 2018). About 2 gm of each feces was preserved at -20 ºC for ELISA test. Each sample was subjected to *Cryptosporidium* coproantigens detection by ELISA.

Fecal samples preparation

The concentration of feces was done by using formalin-ethyl acetate sedimentation concentration technique (Anusz et al., 1990). After that, the supernatant removed and suspended the pellet in "0.5 mL of phosphate buffered saline (PBS) - 2% BSA".

ELISA plates preparation

Flat-bottom plates with 96 well were coated by "5 µg of *C. parvum* oocysts polyclonal antibody" (Invitrogen, REF: PA1-73183, LOT: UC2741154, USA) per mL in (0.05 M sodium carbonate, bicarbonate buffer pH 9.6), then added 50µL per well and incubated at 4 ºC overnight. Then, the plate were washed five times by 200µL of blocking buffer [(PBS-2% BSA) PBS containing 2% bovine serum albumin (Bioscience, LOT:078489)] and incubated for 2 hr at 4ºC with 200µL of blocking buffer (Anusz et al., 1990).

ELISA procedures

The plates were blocked a second time immediately before use, and the samples (50 µL of the concentrated fecal pellet) added and the plate incubated for (30 min at 37 ºC) and washed five times with (PBS-2% BSA). Then 50 µL of goat serum (1:1000) added. After incubating, the plates washed five times. Thereafter, Protein G conjugate (Molecular Probes by Life Technologies Corporation, Catalog No. P21041, Lot:1495870, USA) diluted by PBS (1:10.000 with pH 7.2) before use (Schaefer et al., 2011), and added 50 µL per well

and incubated for 30 min. The plates washed five times with (PBS containing 0.05% Tween 20). Then, 50µL TMB substrate (3, 3, 5, 5 tetramethyl Benzidine dihydrochloride) (Bioshop ® Canada Inc., Burlington, ON. L7L 6A4) added and incubated at room temperature for 15 min in the dark. Finally, 50 µL of stop solution (sulfuric acid) added to the plate. The reactions inspected visually and the optical density (OD) estimated at 450 nm within 15 minutes by ELISA reader (Titertek Multiskan MCC/340 MKII; Flow Laboratories, McLean, Va.).

Statistical analysis

A chi square Test (χ^2) (SPSS, 16.0) used for calculation of *C. parvum* positive rate in calves and lambs (Wang et al., 2021). It used to compare the effect of sex, age, housing system and fecal consistency on the positive rate between the examined animals. The results were considered statistically significant when ($P < 0.05$) (AL-Megrin, 2015).

RESULTS

In the present study, the positive rate of *C. parvum* infection in cattle calves, buffalo calves and lambs was 38.7% (29/75), 22% (11/50) and 40% (30/75), respectively by microscopic examination in Assiut Governorate. The oocysts of *C. parvum* showed round or oval structures (4–6 µm) with pink to deep purple in blue background using modified Zeihl- Neelsen stain (Figure 1, 2). In addition, the positive rate of *C. parvum* in cattle calves, buffalo calves and lambs was 82.7% (62/75), 84% (42/50) and 62.7% (47/75), respectively using ELISA. The total positive rate of *C. parvum* infection in examining ruminants was 75.5% (151/200). Very highly significant differences in positive rate between two diagnostic tests in cattle calves, buffalo calves and lambs (Table 1).

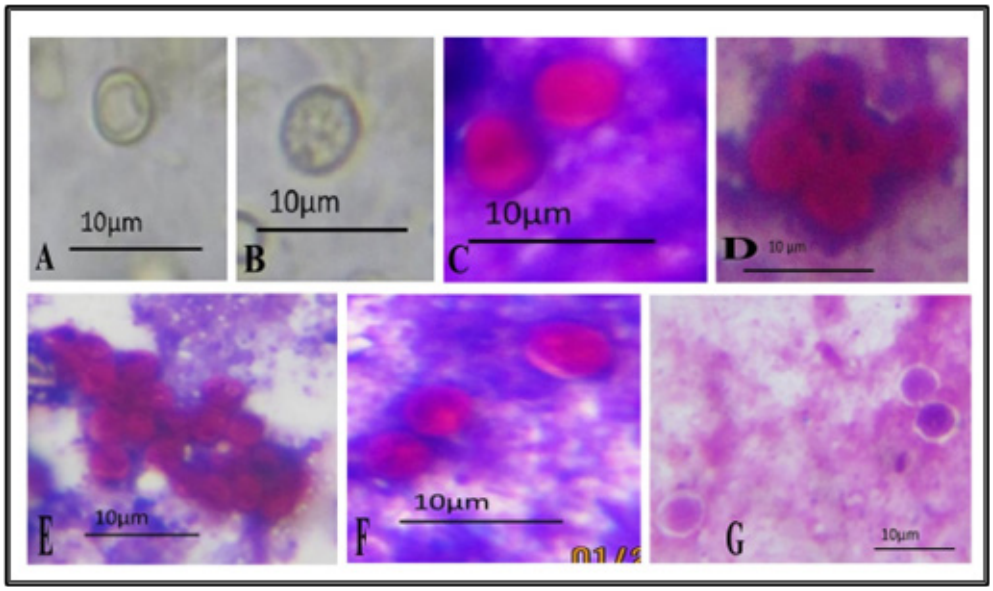

Figure 1 Showing Cryptosporidium parvum oocysts: A, B: oocysts in fresh lamb fecal sample (X1000). C, D, E multiple oocysts in the fecal smears stained by modified Zeihl- Neelsen stain of cattle calf. F: multiple oocysts in the stained fecal smears of buffalo calf and G: multiple oocysts in the stained fecal smears of lamb. (X1000) (scale bar = 10um).

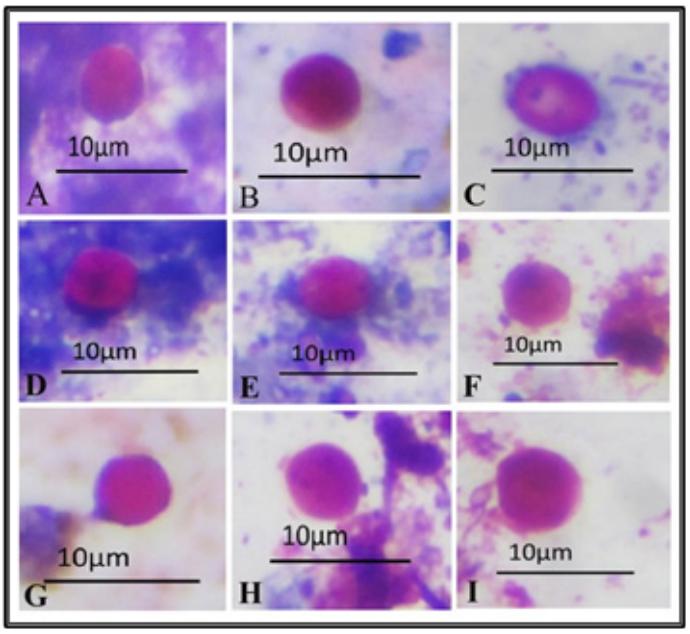

Figure 2 Showing Cryptosporidium parvum oocysts in the fecal smears stained by modified Zeihl- Neelsen stain: A, B, C, D, E: cattle calves, F: buffalo calf, G, H, I: lamb (X1000) (scale bar = 10um).

Table 1 Positive rate of *Cryptosporidium parvum* in cattle calves, buffaloes calves and lambs by using light microscope and ELISA.

Animal spp. / Test	Cattle calves		Buffaloes calves		Lambs		Total	
	Examined number	Positive (%)	Examined number	Positive (%)	Examined Number	Positive (%)	Examined number	Positive (%)
Light microscope	75	29(38.7)	50	11(22)	75	30(40)	200	70(35)
ELISA	75	62*** (82.7)	50	42(84) ***	75	47 ** (62.7)	200	151*** (75.5)
	χ^2 38.58 p 0.000		χ^2 30.425 p 0.000		χ^2 7.71 p 0.005		χ^2 66.34 p 0.000	
Total	75	62(82.7)	50	42(84)	75	47 (62.7)	200	151(75.5)

** High significant p values (p< 0.01)
*** Very high significant p values (p< 0.001).

According to the sex, the positive rate of *C. parvum* in cattle calves, buffalo calves and lambs was 82.1%, 90.5% and 54.2%, respectively in males. While it was 83.3%, 79.3% and 77.8% in female cattle calves, buffalo calves and lambs, respectively. The total positive rate of testing ruminants was 71.3% in males and was 80.4% in females. High significant differences were recorded among female lambs that were more susceptible to the infection than male. While, no significant difference regard to sex was recorded in cattle calves, buffalo calves and total young ruminants (Table 2).

Table 2 Positive rate of *Cryptosporidium parvum* among cattle calves, buffaloes calves and lambs according to the sex

Animal spp. / Sex	Cattle calves		Buffaloes calves		Lambs		Total	
	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)
Male	39	32 (82.1)	21	19 (90.5)	48	26 (54.2)	108	77(71.3)
Female	36	30 (83.3)	29	23 (79.3)	27	21* (77.8)	92	74(80.4)
	χ^2 0.021 p 0.88		χ^2 1.13 p 0.288		χ^2 4.12 p 0.042		χ^2 2.45 p 0.293	
Total	75	62 (82.7)	50	42(84)	75	47 (62.7)	200	151(75.5)

* Significant *p* values (p< 0.05)

The positive rate of *C. parvum* in cattle calves, buffalo calves and lambs were 90.6%, 96.2% and 96%, respectively, with age less than 1 month and was 96.6%, 63.6% and 26.9%, respectively with age 1-2 months. While, the positive rate in an age > 2-3 months was 35.7%, 76.9% and 66.7%, respectively in cattle calves, buffalo calves and lambs. The total positive rate of *C. parvum* in young ruminants was 93.9%, 63.6% and 60.8% with age < 1 month, 1-2 months and age more than 2 to 3 months, respectively. Very highly significant differences in positive rate were more frequently recorded in < 1 month old of lamb, and total young ruminants and significant of buffalo calves in < 1 month old. While, very highly significant difference in positive rate was more frequently recorded in 1-2 months old of cattle calves (Table 3).

Table 3 Positive rate of *Cryptosporidium parvum* among cattle calves, buffaloes calves and lambs according to age

Animal spp. / Age group	Cattle calves		Buffaloes calves		Lambs		Total	
	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)
<1 month	32	29 (90.6)	26	25 * (96.2)	25	24*** (96)	83	78*** (93.9)
1-2 months	29	28*** (96.6)	11	7 (63.6)	26	7 (26.9)	66	42 (63.6)
>2-3 months	14	5 (35.7)	13	10 (76.9)	24	16 (66.7)	51	31 (60.8)
	χ^2 26.86 p 0.000		χ^2 6.74 p 0.034		χ^2 26.24 p 0.000		χ^2 26.31 p 0.000	
Total	75	62 (82.7)	50	42(84)	75	47 (62.7)	200	151(75.5)

* Significant *p* values (p< 0.05)
*** Very high significant p values (p< 0.001).

The positive rate of *C. parvum* according to the housing system of cattle calves, buffalo calves and lambs was 97.1%, 90% and 100%, respectively, of farm rearing and was 70%, 75% and 37.8%, respectively of household rearing. The total positive rate of *C. parvum* of testing ruminants was 95.8% of farm rearing and was 57.1% of household rearing. There were high significant differences in positive rate between two housing systems of cattle calves and were very high significant differences of lambs and total young ruminants. There were no significant differences in positive rate between two housing systems of buffalo (Table 4).

Table 4 Positive rate of *Cryptosporidium parvum* among cattle calves, buffaloes calves and lambs according to the housing system

Animal spp. / Housing system	Cattle calves		Buffalo calves		Lambs		Total	
	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)
Farm	35	34** (97.1)	30	27(90)	30	30*** (100)	95	91*** (95.8)
Household	40	28 (70)	20	15(75)	45	17 (37.8)	105	60(57.1)
	χ^2 9.59 p 0.002		χ^2 2 p 0.156		χ^2 29.79 p 0.000		χ^2 42.27 p 0.000	
Total	75	62(82.7)	50	42(84)	75	47 (62.7)	200	151(75.5)

** High significant p values ($p< 0.01$)
*** Very high significant p values ($p< 0.001$).

The positive rate of *C. parvum* according to the feces consistency of cattle calves, buffalo calves and lambs was 77.3%, 78.9% and 49%, respectively, of normal feces and was 90.3%, 87.1% and 91.7%, respectively of diarrheic feces. The total positive rate of *C. parvum* of young ruminants was 64.9% of normal feces and was 89.5% of diarrheic feces. There were very high significant differences in positive rate between normal and diarrheic feces of lambs and total young ruminants but there were no significant differences between normal and diarrheic feces of cattle and buffalo calves (Table 5).

Table 5 Positive rate of *Cryptosporidium parvum* among cattle calves, buffaloes calves and lambs according to feces consistency

Animal spp. / Feces consistency	Cattle calves		Buffaloes calves		Lambs		Total	
	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)	Examined number	Positive (%)
Normal	44	34 (77.3)	19	15 (78.9)	51	25 (49)	114	74 (64.9)
Diarrhea	31	28 (90.3)	31	27 (87.1)	24	22*** (91.7)	86	77*** (89.5)
	χ^2 2.16 p 0.142		χ^2 0.582 p 0.445		χ^2 12.69 p 0.000		χ^2 16.07 p 0.000	
Total	75	62 (82.7)	50	42 (84)	75	47 (62.7)	200	151 (75.5)

*** Very high significant p values ($p < 0.001$).

DISCUSSION

Cattle and buffaloes are the major asset of the livestock industry in Egypt with public health apprehensions. *Cryptosporidium* is recognized as an opportunistic parasite which can increase in numbers rapidly if calves expose to stress due to environmental agents (Naag et al., 2015). Also, Kabir et al. (2020) elucidated that *C. parvum* was the predominant species between the calves, lambs.

Diarrheic and healthy calves less than one month of age can enable the disseminate of cryptosporidiosis in both animals and humans (Díaz et al., 2018). *Cryptosporidium spp.* are transmitted indirectly and directly through the fecal-oral route by ingesting contaminated food or water with oocysts (Ahmed and Karanis, 2020). *C. parvum* is a main parasitic disease agent of newly born calves with diarrhea in dairy farms (Thomson et al., 2017; Wang et al., 2021).

In the current study, the positive rate of *C. parvum* in cattle calves, buffalo calves and lambs were higher using ELISA than by microscopic examination. This may be attributed to the deficiency of microscopic ability to distinguish the oocysts. Also, Shams et al. (2016) stated that antibodies targeting antigens in fecal samples are up to 10 times more sensitive than acid-fast staining for diagnosis. Thus, ELISA tests are recommended for the *Cryptosporidium species* antigen detection in feces (AL-Megrin, 2015; Gündüz and Arslan, 2017).

This rising positive rate might be as a result of environmental pollution with the huge amounts of oocysts in the sheds of diarrheic animal, pasture, bedding, soil and contaminated drinking water, which agree with (Thomson et al., 2017; Kabir et al., 2020). Also, diseased calves excrete numerous million oocysts in the diarrheic feces (up to 4×10^7 per gram of feces) (Fayer et al., 1998).

Our result was higher in cattle calves than that reported "in Egypt (Amer et al., 2010) 25% in Kafr El Sheikh (Helmy et al., 2013) 65.7% in Ismailia and (Fereig et al., 2016) 35.9% in southern Egypt (46.1% in Sohag and 31.6% in Qena)". Also, it was higher than that described “in the world recovered by

(Díaz-Lee et al., 2011) 49.8% in Chile, (Kaupke and Rzeżutka, 2015) 22.5% in Poland, (Díaz et al., 2018) 31.3% of calves in Italy, (Ouakli et al., 2018) 72.4% in Algeria, (Lee et al., 2019) 4.4% in Korea, (Lombardelli et al., 2019) 25.5% in Argentina and (Mammeri et al., 2019) 70.4% in France". While, present results were similar to that reported "in the world by (Thomson et al., 2017) 80.0% in the United Kingdom". While, it was lower than detected by (Nguyen et al., 2007) 96.6% in the United States. This variation could be ascribed to differences in the breed, management practices, husbandry regimens, climate, geographic distribution in addition to applied diagnostic methods (Santín, 2020).

Cryptosporidiosis is currently known to be endemic to newborn buffalo calves on a global scale (Bharti et al., 2020). In the current study, the positive rate of *C. parvum* in buffalo calves was higher than that found "in Egypt by (Hamza et al., 2020) 50% in Cairo". This high positive rate may be due to different rearing conditions and the existence of *Cryptosporidium* oocysts in asymptomatic water buffaloes feces, which suggesting that they act as potential sources of infection (Ribeiro et al., 2000).

Diarrheic lambs are the most essential reservoirs for *Cryptosporidium* and *C. parvum* is the most common species among pre-weaned diarrheal lambs (Papanikolopoulou et al., 2018; Mammeri et al., 2019; Dessì et al., 2020). Likewise, Mammeri et al. (2019) mentioned that the feces of sheep are the main source of human infection, specially who in close contact with sheep, such as veterinarians and farmers (Dessì et al., 2020). Also, Bahrami et al. (2014); Ibrahim et al. (2016); Hingole et al. (2017) noticed a relationship between *Cryptosporidium* infection and diarrhea in buffalo calves, with a higher manifestation within the 1st months of age.

The positive rate of *C. parvum* in lambs was generally high 62.7% in comparison with that recorded "in Egypt by (Helmy et al., 2013) 32.2% in Ismailia, (Elmadawy et al., 2017) 16.1% in Qalyubia, (Elshahawy and AbouElenien, 2019) 42.9% in Qena". Likewise, it was higher than that reported "in the world by (Paoletti et al., 2009) 17.45% in central Italy, (Kaupke et al., 2017) 3.2 % in Poland, (Papanikolopoulou et al., 2018) 12.1% in northern Greece, (Dessì et al., 2020) 16.4% from Sardinia, (Kabir et al., 2020) 19.4% in Turkey". While it was lower than that recorded by (Díaz et al., 2015) 74.4% in Spain and (Romero-Salas et al., 2016) 67.5% in Mexico. Nevertheless, differences in positive rates globally can be attributed to different locations and rearing conditions (Elmadawy et al., 2017).

Our results coincide with the findings of Dessì et al. (2020) who illustrated the high positive rate of *C. parvum* in lambs as a result of that they are weaned from 30–40 days after birth, so a high hazard of maternal transmission may happen. The natural suckling and sharing the same night shelter may contribute to the transmission. Whereas, Kabir et al. (2020) added that *C. parvum* is not host-specific; accordingly, an environment contaminated with oocysts during an outbreak in calves can give rise to infection in lambs that subsequently use the same grazing area.

This study showed the presence of *C. parvum* oocysts in the form of round or oval structures (4-6 µm) by microscopic examination faecal smears stained by modified Ziehl-Neelsen. Similar to that observed by (Bessat et al., 2019; Wang et al., 2021). The positive rate of *C. Parvum* infection in small

ruminants was 35%, respectively, by microscopic examination and was 75.5%, respectively using ELISA. This agrees with Omoruyi et al. (2014) who stated that *Cryptosporidium* incidence were 37.1% and 74.3% by microscopic and ELISA, respectively, and AL-Megrin (2015) who found the prevalence of *Cryptosporidium* oocysts was 11.1 and 22.2% by microscopic and ELISA, respectively in sheep feces and confirmed that the microscopic method was less efficient at detecting *Cryptosporidium* oocysts than ELISA, which may be due to the small number of oocysts in fecal specimens (Elsafi et al., 2013). Our results revealed very high significant differences in positive rate between two diagnostic tests. The high positive rate of *C. parvum* in this study in young ruminants may be due to carriers (adult animals) produce a large feces volume and thus cause oocysts environmental contamination (Ghoshal et al., 2018). Moreover, the infected calves are shed a substantial large number of infective oocysts in their feces, which may comprise a potential infection risk if excreted in the drinking water (Thomson et al., 2017). *Cryptosporidium* oocysts remains infectious in the environments for months with favorable humidity and temperature, which enables the biological cycle maintenance and the spreading of parasite (O'Handley and Olson, 2006).

The positive rate of *C. parvum* in cattle calves, buffalo calves and lambs according to gender was 82.1%, 90.5% and 54.2%, respectively in males. While, it was 83.3%, 79.3% and 77.8% in female cattle, buffalo calves and lambs, respectively. These results could be ascribed to an unequal female: male ratio in flock and it could also be related to physiology, host genetics and immunology or environmental and management practices (Ayinmode and Fagbemi, 2010).

In the present study, there was a higher positive rate in female cattle calves than males. This was disagree with Ibrahim et al. (2016) was higher in males 12.4% than females 9.1%. While, in our study, the positive rate in buffalo calves was higher in males than females. This result is in accordance with Maurya et al. (2013) and Ibrahim et al. (2016) who demonstrated that the *Cryptosporidium* positive rate in buffalo was higher among males 14.5% than females 10.7% and Bharti et al. (2020) who documented that the presence of *Cryptosporidium spp*. was 58.33% among male and 23.07% among female. On the other hand, it contrasted with that described by Swain et al. (2018) who found that female calves had a higher prevalence (10.2%) than male (7.6%). These differences could be linked to the variety of the natural immunity of the two species (Abou El-Ella et al., 2013). Whereas, the positive rate in lambs was higher in females than males similar to Khan et al. (2019) who found higher prevalence was recorded in females sheep (18.8%) than males (17%) and which was disagree with Elmadawy et al. (2017) who recorded the *Cryptosporidium* infection rate higher among male sheep than female in Qalyubia, Egypt.

Age is a significant factor in the pathogenicity of *Cryptosporidium*, as young animals are more liable to infection than adults (Xiao et al., 2004; Santín, 2020). As well, young buffaloes are more liable than older buffaloes (Ibrahim et al., 2016; Bahrami et al., 2014). Meanwhile, Dessì et al. (2020); Chen et al. (2022) showed that the prevalence was higher in lambs compared to adults.

In our study, the highest positive rate of *C. parvum* was found in buffalo calves in an age < 1 month and > 2-3 months, while it was the highest in cattle calves with age 1-2 months. In accordance with Helmy et al. (2013) who

concluded that *C. parvum* was more common in cattle and buffaloes less than 3 months of age in Egypt. Also, our result agrees, with Santín et al. (2008) who found that *C. parvum* constitutes the most dominant species among pre-weaned dairy calves in different countries worldwide. The highest level of *C. parvum* infection was found with age < 1 month, which coincided with Santín et al. (2004) who detected highest prevalence of *C. parvum* within animals under than a month of age. Also, Abou El-Ella et al. (2013); Hingole et al. (2017); de Aquino et al. (2020) added that the infection rate for *C. parvum* has been decreased as cattle calves and buffalo calves became older, suggesting an inverse correlation between the prevalence rate and the age of the host. This can be ascribed to newborn calves whose immune system is immature, which makes them susceptible to *C. parvum* infection leading to the development of clinical signs (Santín et al., 2008; Swain et al., 2018; Zhang et al., 2021).

According to the housing system, the total positive rate of *C. parvum* of young ruminants was 95.8% and 57.1% of farm rearing and household rearing, respectively. This in agree with Swain et al. (2018) who recorded high prevalence of the *Cryptosporidium* in organized farm (26.7%) as in comparison with unorganized farm (6.44%) in India and El-Saharty et al. (2005) who mentioned that the semi intensive and intensive farming practices of the cattle livestock comprise a major infection source disseminates in Egypt. The highest positive rate was found in lambs of farm rearing in the present work, the Similar finding registered by Dessì et al. (2020) who stated that *Cryptosporidium* present in most Sardinia sheep farms. The high positive rate of *C. parvum* of a farm than household raising in the current work may be due to inadequate or poor hygiene, overcrowding and management measures in the farm comparing to household as described by (Díaz-Lee et al., 2011).

The positive rate of *C. parvum* according to the feces consistency of cattle calves, buffalo calves and lambs revealed very high positive rate of *C. parvum* in diarrheic feces than normal feces of all young ruminants. This matching with Maurya et al. (2013); Ibrahim et al.(2016); Swain et al.(2018); de Aquino et al.(2020); Chen et al.(2023) who registered a higher prevalence of *C. parvum* infection in diarrhea than normal feces of calves. Also, Zhang et al. (2021) discovered that *C. parvum* was the predominant species in newborn calves and its prevalence were 61.5% and 27.3% in diarrheal and non-diarrheal calves, respectively. On the contrary, with Bessat et al. (2019) who recorded that the infection rates of *Cryptosporidium* in calves was 47.9% and 42.9% with formed and watery feces, respectively. In the present study, there were very high significant differences in the positive rate between normal and diarrheic feces of lambs, which supports a similar observation by Dessì et al. (2020); Chen et al. (2022) who declared that the prevalence of *Cryptosporidium* was significantly higher in diarrhea than normal feces. Also, many studies presented that cryptosporidial infections are linked to outbreaks of diarrhea in newly born lambs (Papanikolopoulou et al., 2018; Santín, 2020).

In conclusion, we found that *C. Parvum* infection is prevalent among calves and lambs in Assiut Governorate, Egypt. ELISA is an effective tool for diagnosing *C. Parvum* than fecal examination because of its great sensitivity and usability. Elevated ELISA- positive rate necessitates more efficient strategies to combat these infections and efficiently diminish its transmission by improving farm hygiene practices and management, considering the *Cryptosporidium* oocysts strong resistance in the environment.

AUTHOR CONTRIBUTIONS

Kuraa, H.M. and Malek, S.S. designed the study and helped in the ELISA procedure and microscopic examination, data analysis, and interpretation. Malek, S.S. collected samples. Kuraa, H.M. wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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How to cite this article;

Huda Mohammed Kuraa and Safaa Sayed Malek. The role of the Cryptosporidium parvum in diarrhea in calves and lambs. Veterinary Integrative Sciences. 2023; 21(3): 735 - 749.