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Gastrointestinal parasitic infection in cow and buffalo calves causing diarrhea.

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ABSTRACT

Diarrhea is a major cause of illness and death in calves, especially during their first three months of life, with significant financial implications for the cattle industry. In this study, fecal samples from 100 diarrheic calves were examined, revealing that 26 (26%) were infected with gastrointestinal parasites. The incidence rates were 15 (27.27%) in cattle calves and 11 (24.44%) in buffalo calves. The identified species included *Eimeria* species, *Cryptosporidium parvu*m, *Buxtonella sulcata*, *Moneizia* species, *Toxocara vitulorum*, *Trichostrongylus* species and *Cooperia* species in suckling and post weaning calves. Molecular characterization (PCR) using 18S rRNA gene of *Cryptosporidium* species. *Cryptosporidium parvum* was identified as the species affecting calves with amplicon 655 pb. Phylogenic analysis of *Cryptosporidium parvum* revealed three isolates deposited in the GenBank database under the accession numbers PV208384 – PV208385 – PV208386. Affected calves respond to the treatment with 100 µg/kg/day halofuginone lactate (Halocur ®) orally once daily for 7 consecutive days.

INTRODUCTION:

Diarrhea frequently affects newborn calves, lambs, and kids, posing a major challenge to their health and survival. Cattle production plays a vital role in farmers' livelihoods and the global agricultural economy. Calves, in particular, are considered an important source of high-quality meat worldwide **(Karimzadeh et al. 2022)**. Diarrhea is recognized as the leading cause of morbidity and mortality in calves **(Heath, 1992)**. Approximately 75% of mortality losses are due to acute diarrhea, which typically results in The greatest economic losses **(Heinrichs and Radostits, 2001)**. In Egypt, neonatal calf diarrhea (NCD) is the primary cause of calf mortality which ranges between 27.4-55% of the total mortality in young calves **(Ahmed, 1980)**.

There are non-infectious causes of calf diarrhea, such as environmental management etiology, or factors related to the animal as nutritional status and immune response **(Izzo et**

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al. 2011). The infectious calf diarrhea is due to several enteric pathogens including parasitological, bacteriological*; E. coli* and *Salmonella* rather than the viral causes; bovine *corona* and *rota viruses* **(Lee et al. 2019)**.

Eimeria spp., *Cryptosporidium* spp., *Giardia* spp., *Buxtonella sulcata* and *Toxocara vitulorum* are the parasite agents most frequently found to cause diarrhea **(Tomczuk et al. 2005)**.

Cryptosporidium is one of the most prevalent enteropathogens that calves encounter in the early days of life. Calves with a *Cryptosporidium* infection may exhibit nonspecific diarrhea, dehydration, anorexia, depression, and stomach pain. Diarrhea often begins 3–5 days after infection and lasts 4–17 days in calves **(De Graaf et al. 1999 and Nydam et al. 2001**).

The zoonotic nature of cryptosporidiosis and the possibility for animals to infect humans via contaminated water and food should also be taken into consideration, in addition to the disease's effect on animal health and production. Despite the mortality rate from the infections is low, serious economic losses can occur because of the costs involved in the treatment **(De Graaf et al. 1999 and Elmahallawy et al. 2020).**

Eimeria is considered to be among the five most economically significant diseases in the cattle industry **(Carlson et al. 2011).** *Eimeria* species are highly host-specific, with over 20 species identified in cattle. The most prevalent pathogenic species in calves worldwide are *Eimeria bovis* and *Eimeria zuernii* causing morbidity and mortality by interfering with intestinal absorption and frequently associated with diarrheal stools that contain intestinal tissues, blood, and fibrin ((**Daugschies and Najdrowski, 2005; Pandit, 2009 and Bangoura et al. 2012)**. The highest infection rate is usually observed in calves younger than one year of age **(Daugschies and Najdrowski, 2005).**

Toxocara vitulorum is a nematode which inhabits the small intestine of cattle and water buffalo calves, can induce diarrhea in addition to anemia, weight loss and anorexia in calves aged 1–3 months **(Rehman et al. 2011).**

The present study was aimed to investigate the gastrointestinal parasite fauna in cow and buffalo calves at Alexandria governorate, Egypt, with special reference to their prevalence, seasonal dynamics, molecular identification of selected possible parasitic species and a trial to control cryptosporidiosis in calves.

MATERIALS and METHODS

Study area:

This study was conducted from July 2024 to June 2025 on selected cattle farms in Alexandria governorate of Egypt where calf diarrhea was frequently reported. These farms are located in different localities at Latitude, 31.205753 and Longitude coordinates, 29.924526. Alexandria are situated on the northern shores of Egypt and lies in the delta of Mediterranean Sea.

Animals and samples collection:

This study involved 100 calves (cattle and buffalo) that showing clinical signs such as anorexia, weight loss, pale mucous membrane, and diarrhea. The examined calves included 55 cattle calves (28 suckling and 27 post weaning calves) and 45 buffalo calves (24 suckling and 21 post weaning calves). Fresh fecal samples were collected directly from the rectum of each calf using sterile plastic bags. For every sample, the age of the animal and the date of collection were recorded. All collected samples were kept in an ice box and immediately transported to Parasitology Department, Animal Health Research Institute; Alexandria lab. During field collection, samples were preserved at 4 ◦C for no longer than of two days until further processing.

Examination techniques:

Each faecal sample was examined microscopically by direct smear and concentration (sedimentation and flotation) techniques according to **Solusby (1986)**. The detected *Eimeria* oocysts were identified after sporulation in 2.5% potassium dichromate at 27ºC according to **Norton (1986)**. The Modified Ziehl-Neelsen (MZN) staining method was applied for detection of *Cryptosporidium* species oocysts, as

described by **Henrikson and Pohlenz (1981)** and examined microscopically under oil immersion lens. The obtained parasites were identified according to **Yamagutti (1961)** and **Levine (1985)**.

Confirmation of *Cryptosporidium* species by PCR:

Positive specimens with *Cryptosporidial* oocysts were confirmed by polymerase chain reaction (PCR). Detection of 18S rRNA gene of Cryptosporidium was carried out using primers supplied from Metabion (Germany) are listed in table (1)

DNA extraction. A total of 220 mg of stool samples were processed using the QIAamp DNA stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

PCR amplification. The PCR reaction was carried out in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. A T3 Biometra thermal cycler was used to carry out the reaction.

Analysis of the PCR Products. Amplified products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For the gel analysis, each gel slot was filled with 15 µl of the products. The sizes of the fragments were measured using the Gene ruler 100 bp DNA Ladder (Fermentas, Germany). The gel was photographed and the results were analyzed using computer software.

Phylogenetic analysis:

PCR products were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit(Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul et al. 1990)** was initially performed to establish sequence identity to GenBank accessions. With Laser gene DNA Star version 12.1, the MegAlign module generated the phylogenetic tree **(Thompson et al.1994)** and MEGA6's maximum likelihood, neighbor joining, and maximum parsimony were used for phylogenetic analysis **(Tamura et al. 2013).**

Trial for Control of Parasitic Diarrhea:

Positive calves with *Cryptosporidial* oocysts were started treatment with 100 µg/kg/day halofuginone lactate (Halocur ®) orally for 7 consecutive days, Ringers lactate solution (IV) 1.5-2 litres / twice daily depending on dehydration degree and oral rehydration solution 500 ml / 2-3 times daily given 2 hours after feeding for suckling and post-weaning calves **(Constable et al. 2017).** Following therapy, fecal samples were taken on the 0th, 4th, and 7th. The absence of oocysts in smears stained using the mZN staining method was used to evaluate the calves' recovery.

RESULTS

Our results revealed that, out of 100 examined fecal samples of diarrheic calves 26 (26%) were infected with gastrointestinal parasites with prevalence 15 (27.27%) in cattle calves and 11 (24.44%) in buffalo calves. In cattle calves, the incidence was 25% in suckling calves and 29.62% in post-weaning calves, but in buffalo calves, it was 25% and 23.80% in suckling and post-weaning calves, respectively as shown in table (2).

Eimeria spp., *Cryptosporidium parvu*m, *Buxtonella sulcata*, *Moneizia* spp., *Toxocara Vitulor*um, *Trichostrongylus* spp. and *Cooperia* spp. were reported in examined diarrheic calves, there are mixed infections two species or more. In post weaning calves, *Eimeria* spp. showed highest infection rate (25.92% and 19.04%) in cattle and buffalo calves respectively, while *Cryptosporidium parvu*m and *Buxtonella sulcata* showed highest infection rate in suckling calves (10.71%, 8.33%) and (10.71%, 16.66%) in cattle and buffalo calves respectively, in addition, the helminths caused diarrhea, with an increased appearance of Moneizia spp., *Trichostrongylus* spp. and *Cooperia* spp.in post weaning calves but *Toxo-*

cara Vitulorum with highest infection rate in suckling calves (14.28% and 16.66%) in cattle and buffalo calves respectively as shown in table (3). Five eimerian species were discovered after sporulated *Eimeria* oocysts were identified under a microscope. (*E. cylindrical*, *E. Auburnesis*, *E. bovis*, *E. ellipsoidalis* and *E. zurnii*) as shown in fig. (1&2).

Eimeria spp. showed the highest infection rate in summer (30.76%), while most of parasites showed highest infection rates in winter as *Cryptosporidium parvum* (14.28%), *Buxtonella sulcata* (17.85%), *Moneizia* spp. (10.71%) and *Toxocara vitulorum* (17.85%) but *Trichostrongylus* spp. and *Cooperia* spp. showed highest infection rates in autumn (4.76% and 9.52%) resp. as shown in table (4).

Sequencing and genotyping of isolates: There are 7 positive faecal samples of *Cryptosporidium* spp. that examined by Modified Ziehl-Neelsen staining technique were analyzed by PCR. The PCR procedure that based on 18s rRNA gene sequences showed a positive band size of 655 bp. (Fig. 3) and revealed the presence of three isolates of *Cryptosporidium* spp. with accession number PV208384 – PV208385 – PV208386 in GenBank. As shown in sequence distance figure, the sequenced strains showed 100% identity to *Cryptosporidium parvum* strains confirming the clustering of the study strain with *Cryptosporidium parvum* (Fig. 4a). Phylogenetic tree cleared the clustering of the collected *Cryptosporidium parvum* with *Cryptosporidium parvum* strains. (Fig. 4b).

Treatment is effective for the diarrheic calves, and no further diarrheal symptoms are seen. Only two samples had a few oocysts visible after 4th day of treatment, following staining with the mZN staining procedure. No oocysts were found in the calves' stained fecal smears following a week of treatment as shown in table (5).

DISCUSSION

Parasitic diseases constitute one of the major problems affecting the general condition of farm animals causing significant losses in animal productivity or even mortality, which will have an impact on the economic condition of the country.

26 (26%) of the 100 diarrheic cattle and buffalo calves were infected with gastrointestinal parasites with prevalence 15 (27.27%) and 11 (24.44%) in cattle and buffalo calves respectively. The prevalence rate of parasitic infection among the diarrheic cattle calves was 25% and 29.62% in suckling and post-weaning calves respectively, while the prevalence rate in buffalo calves was 25% and 23.80% in suckling and post-weaning calves respectively. Similar results were reported by **El-Ashram et al. (2019)** (20.36%) but higher prevalence was recorded by **Ramadan et al. (2015)** (60%). The most common parasites in our study that found in calves were *Eimeria* spp., *Cryptosporidium parvum*, *Buxtonella sulcata*, *Moneizia* spp., *Toxocara vitulorum*, *Trichostrongylus* spp. and *Cooperia* spp. These findings agree with those reported by ***Ramadan et al.*** **(2015) and El-Ashram et al. (2019).**

Eimeria spp. showed highest infection rate (25.92% and 19.04%) in post weaning cattle and buffalo calves respectively (below 6 months of age), this result is agreed with the earlier recorded by **Fouad et al. (2024)** who recorded higher prevalence of *Eimeria* spp. in calves with age 3-6 months but, disagreed with **Ramadan et al. (2015) and El-Ashram et al. (2019)** who reported higher prevalence of *Eimeria* spp.in suckling calves. Additionally, different incidence rates of *Eimeria* species were identified, **Ramadan et al. (2015)** (32% and 30.4% in cow and buffalo calves), **El-Ashram et al. (2019)** (37.14% and 40.82% in cattle and buffalo calves) and **Fouad et al. (2024)** (48% in cattle calves). These variations could be attributed to many factors as environmental, mangemental and immunological factors of the examined animals.

Cryptosporidium parvum showed highest prevalence rate (10.71%, 8.33%) in suckling cattle and buffalo calves respectively. This result is agreed to that recorded by **Ramadan et al. (2015)**, **El-Ashram et al. (2019) and Fouad et al. (2024),** that reported higher prevalence rate in calves aged under 3 months but

dissimilar to **Elmahallawy et al. (2022),** which reported higher prevalence in calves aged more than 3 months. Different prevalence rates of *Cryptosporidium* spp. were recorded, **Ramadan et al. (2015)** (56% and 52.2% in cow and buffalo calves) and **El-Ashram et al. (2019)** (10% and 10.2% in cattle and buffalo calves). The variation may be due to the young's undeveloped immunity, as well as differences in the cattle's grazing area and management diversity.

The highest infection rate of *Buxtonella sulcata* was 10.71%, 16.66% in suckling cattle and buffalo calves respectively. **El-Ashram et al. (2019)** recorded highest infection rate of *Buxtonella sulcata* in suckling calves (32.86% and 36.73%) in cattle and buffalo calves respectively. The variation in the prevalence may be due to farm management practices, environmental, and stress factors.

Moneizia spp. showed highest infection rate in post-weaning cow and buffalo calves (14.81% and 14.28%) but not detected in suckling calves. This result is agreed with the earlier reported by **El-Ashram et al. (2019)** and **Sayed et al. (2024).** This might be as a result of the calves being exposed to more contaminated pasture and green fodder after weaning.

Some nematodes caused diarrhea as *Toxocara vitulorum* with highest infection rate (14.28% and 16.66%) in suckling cattle and buffalo calves respectively this result agreed with the earlier recorded by **Ramadan et al. (2015)** and **Fouad et al. (2024)** but disagreed with **El-Ashram et al. (2019),** but *Trichostrongylus* spp. and *Cooperia* spp. detected in post weaning calves.

Regarding the seasonal dynamics, our results showed that high infection rate of *Eimeria* spp. was in summer but, *Cryptosporidium parvum*, *Buxtonella sulcata, Moneizia* spp. and *Toxocara vitulorum* showed the highest rate of infection in winter. This result agreed with **Elmahallawy et al. (2022), Fouad et al. (2024)** and **Sayed et al. (2024).**

Molecular methods were further applied to identify the pathogenic genotype and assess its zoonotic potential **(Xiao, 2010)**. In the present study, 18S rRNA gene was used for the differentiation of *Cryptosporidium* species in calves. As shown in sequence distance figure, the sequenced strains showed 100% identity to *Cryptosporidium parvu*m strains in diarrheic calves. This is in agreement with **Mahfouz et al. (2014) and Abu El-Ezz et al. (2020)** who reported the predominance of *C. parvum* from cattle calves.

Few medications or compounds have demonstrated a partial protective effect against animal cryptosporidiosis when administered prophylactically in ruminants, despite the fact that many have been evaluated for the disease. They include halofuginone lactate, paromomycin, cyclodextrins, azithromycin **(Nasir et al. 2013)**, nitazoxanide, activated charcoal and wood vinegar liquid **(Villacorta et al. (1991), Watarai, Koiwa (2008).** Halofuginone lactate is a synthetic quinazolinone exhibits cryptosporidial activity in both the merozoite and sporozoite phases of *C. parvum* **(Jarvie et al. 2005)**. Halofuginone lactate has been shown to decrease the severity and mortality of cryptosporidiosis in calves. **(Joachim et al. 2003)**. In our investigation, the affected calves respond to the treatment with halofuginone lactate, this finding is agreed with that reported by **Keidela and Daugschies (2013) and Aydogdu et al. (2018**).

CONCLUSION

Eimeria spp., *Cryptosporidium parvu*m, *Buxtonella sulcata*, *Moneizia* spp., *Toxocara vitulor*um, *Trichostrongylus* spp. and *Cooperia* spp. were found to be responsible for parasite diarrhea in cattle and buffalo calves in this investigation. The detection of *Cryptosporidium parvum* genotyping indicated the possibility of public health hazards transmitted from diarrheic calves to animal farms and humans. Treatment with 100 µg/kg/day halofuginone lactate (Halocur ®) orally once daily for 7 consecutive days is effective to control cryptosporidiosis in Alexandria governorate

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target agent	Primers sequences	Target gene	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Cryptosporidium	CAA TTG GAG GGC AAG TCT GGT GCC AGC CCT TCC TAT GTC TGG ACC TGG TGA GT	18S rRNA	655	94˚C 5 min.	94˚C 30 sec.	68˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Yusof *et al.*, 2017

Table 2. Age-wise prevalence rate of parasitic infection in diarrheic calves (n = 100):

Species	Cattle calves		Buffalo calves		Total
	Ex. No.	Inf. No.	Ex. No.	Inf. No.	
Suckling calves (1d–60d)	28	7 (25%)	24	6 (25%)	13 (25%)
Post weaning calves (below 6 months of age)	27	8 (29.62%)	21	5 (23.80%)	13 (27.08%)
Total	55	15 (27.27%)	45	11 (24.44%)	26 (26%)

Table 3. Prevalence rate of gastrointestinal parasites causing diarrhea in cattle and buffalo calves (n = 100).

Age	Species	*Eimeria* spp.	*Cryptos-poridium parvum*	*Buxtonella sulcata*	*Moneizia* spp	*Toxocara vitulorum*	*Tricho-strongylus* spp.	*Cooperia* spp.
Suckling calves	Cattle 28	5(17.85%)	3(10.71%)	3(10.71%)	0	4(14.28%)	0	0
	Buffalo 24	3(12.50%)	2 (8.33%)	4(16.66%)	0	4(16.66%)	0	0
Post-weaning calves	Cattle 27	7(25.92%)	1 (3.70%)	2 (7.40%)	4(14.81%)	2 (7.40%)	3(11.11%)	3(11.11%)
	Buffalo 21	4(19.04%)	1 (4.76%)	2 (9.52%)	3(14.28%)	2 (9.52%)	2 (9.52%)	3(14.28%)
Total	100	19 (19%)	7 (7%)	11 (11%)	7 (7%)	12 (12%)	5 (5%)	6 (6%)

(There are mixed infections 2 species or more)

Table 4. Seasonal dynamics of gastrointestinal parasites in diarrheic calves (n = 100).

Season	Ex. No.	*Eimeria* spp.	*Cryptos-poridium parvum*	*Buxtonella s ulcata*	*Moneizia* Spp.	*Toxocara vitulorum*	*Tricho-strongylus* spp.	*Cooperia* spp.
Winter	28	5(17.85%)	4(14.28%)	5(17.85%)	3(10.71%)	5(17.85%)	1 (3.57%)	1 (3.57%)
Spring	25	3 (12%)	2 (8%)	3 (12%)	1 (4%)	2 (8%)	3 (12%)	3 (12%)
Summer	26	8(30.76%)	1 (3.84%)	1 (3.84%)	1 (3.84%)	2 (7.69%)	0	0
Autumn	21	3(14.28%)	0	2 (9.52%)	2 (9.52%)	3(14.28%)	1 (4.76%)	2 (9.52%)

Table 5. Treatment with halofuginone lactate (Halocur ®) in diarrheic calves.

DPI	Ex. No.	Parasitological response (Mzn staining method)
Zero day	7	All faecal samples were positive
4th DPI	7	Few oocysts were seen only in two samples
7th DPI	7	All samples were negative for oocysts

DPI days post infection

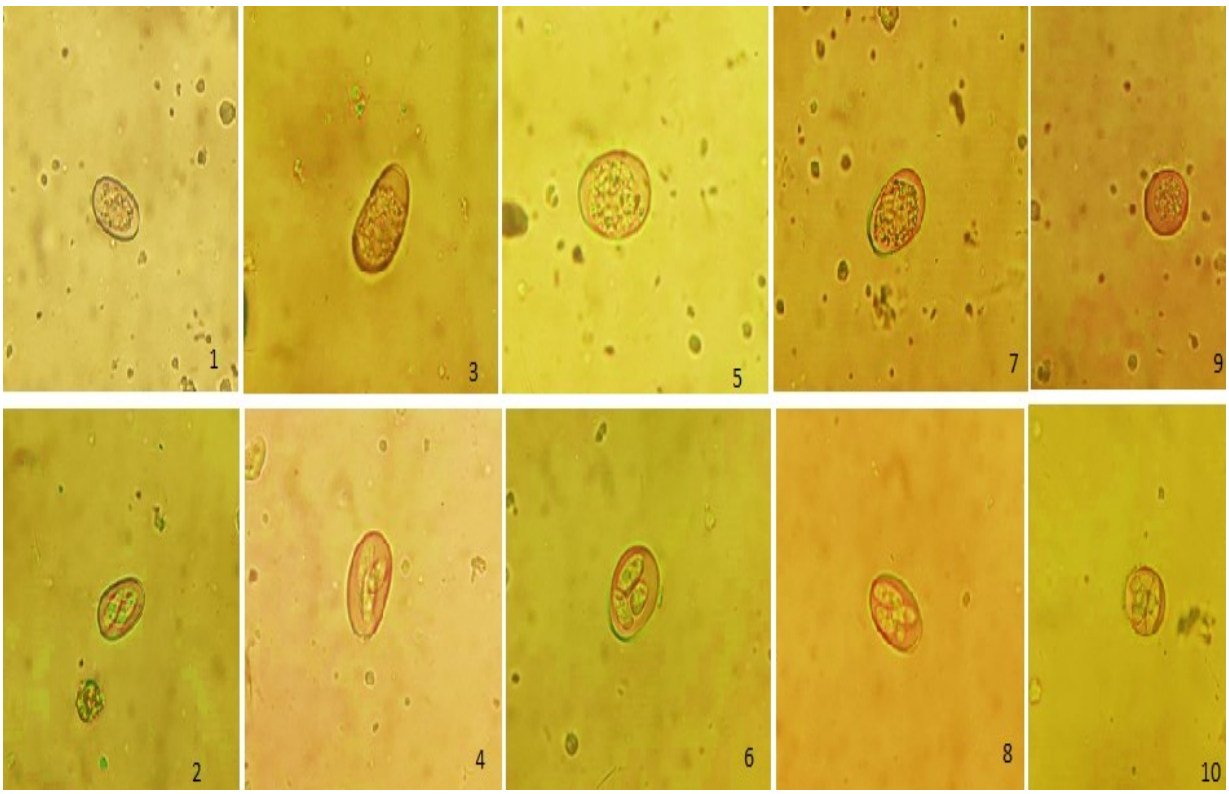

Fig. (1): 1. Unsporulated oocyst of *E. cylindrica* X 40 2. Sporulated oocyst of *E. cylindrica* X 40
3. Unsporulated oocyst of *E. Auburnesis* X 40 4. Sporulated oocyst of *E. Auburnesis* X 40
5. Unsporulated oocyst of *E. bovis* X 40 6. Sporulated oocyst of *E. bovis* X 40
7. Unsporulated oocyst of *E. ellipsoidalis* X 40 8. Sporulated oocyst of *E. ellipsoidalis* X 40
9. Unsporulated oocyst of *E. zurnii* X 40 10. Sporulated oocyst of *E. zurnii* X 40

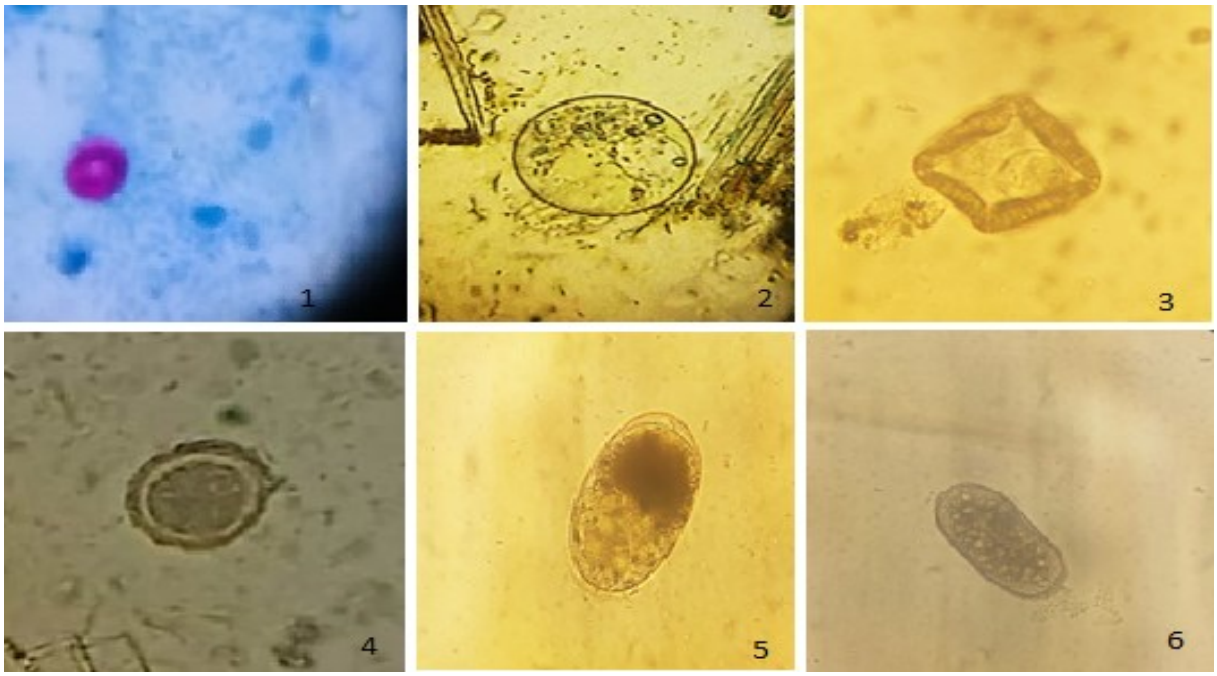

Fig. (2): 1. *Cryptosporidium parvu*m X 100 2. *Buxtonella sulcata* cyst X 40 3. *Moneizia* spp. X 40
4. *Toxocara Vitulorum* X 40 5. *Trichostrongylus* spp. X 40 6. *Cooperia* spp. X 40

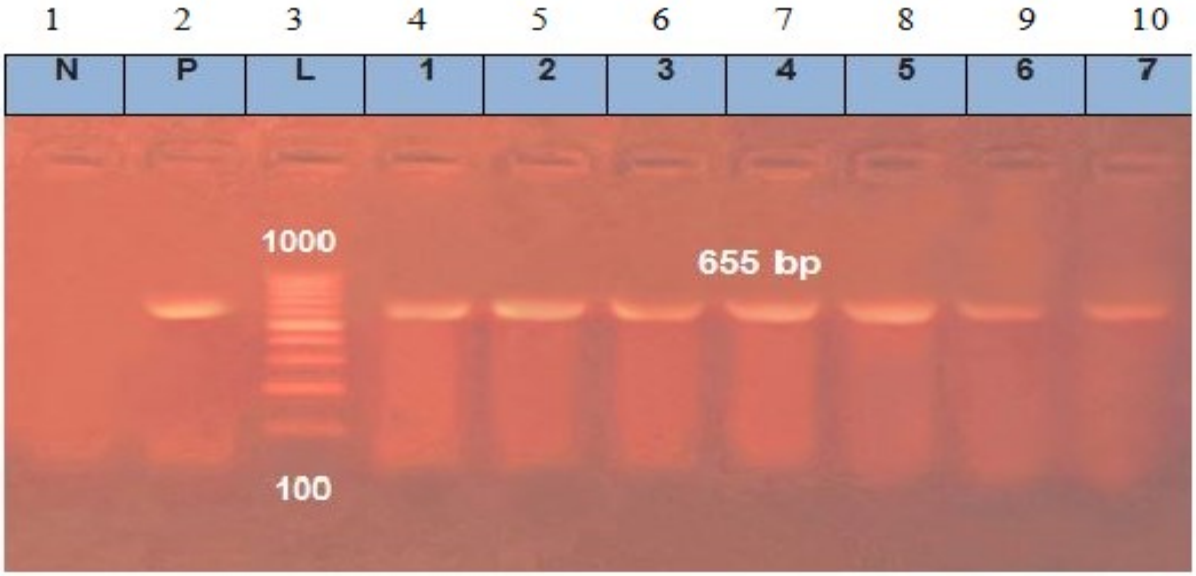

Fig. (3) PCR results for *Cryptosporidium* 18S rRNA gene showing positive amplification of 655 bp of 18S rRNA gene in tested sample. L [Gene ruler 100 bp ladder (Fermentas, thermo 100-1000 bp)].
Lane 1 Negative control Lane 2: Positive control Lane 3: 100-1000 bp. Ladder
Lane 4: Sample1 Lane 5: Sample2 Lane 6: Sample3
Lane 7: Sample4 Lane 8: Sample5 Lane 9: Sample6
Lane 10: Sample7

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
1		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	1	AB513859 C. parvum Sakha104
2	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	2	AF161856 C. parvum MT
3	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	3	AF040725 C. parvum
4	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	4	AY204237 C. parvum HMb
5	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	5	AF108864 C. parvum C1
6	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	6	AH006572 C. parvum KSU-1
7	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	7	AY204238 C. parvum HMa
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	8	PV208384 C. parvum Alex1
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	9	PV208385 C. parvum Alex2
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		93.6	90.0	99.0	97.1	97.1	91.8	91.0	98.8	97.3	89.3	95.9	95.3	96.9	86.7	86.3	89.5	96.1	92.8	10	PV208386 C. parvum Alex3
11	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1		90.0	93.9	94.1	93.9	89.5	90.4	93.2	94.3	89.3	93.4	93.9	93.9	86.5	85.0	89.5	92.6	94.3	11	KT151528 C. baileyi IQ.Cb-4
12	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.0		90.0	90.4	90.4	84.8	97.5	89.8	91.2	98.8	89.1	89.3	88.9	85.5	84.8	94.9	88.3	88.5	12	AB089285 C. andersoni
13	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	4.3	7.7		97.1	97.1	92.0	91.0	97.7	96.9	89.3	96.1	95.7	97.3	86.7	86.3	89.5	96.1	93.2	13	U11440 C. wrairi
14	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	4.8	8.4	1.5		98.4	92.2	91.0	97.1	97.5	89.8	95.1	94.9	95.9	86.9	86.7	89.8	95.5	92.6	14	MW043441 C. ubiquitum SUC69
15	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	4.3	8.1	1.3	0.7		92.4	91.2	97.7	98.2	89.8	94.9	94.3	95.5	86.3	85.9	90.0	95.3	92.4	15	OQ456427 C. suis HY3
16	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	5.5	11.3	4.2	3.8	3.8		86.3	92.4	92.0	84.2	91.0	90.6	91.8	83.8	82.8	85.0	92.0	88.3	16	AF108862 C. felis
17	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	7.8	2.4	6.2	7.4	6.9	9.3		91.0	92.0	96.7	89.5	89.8	89.3	85.5	85.0	96.1	89.1	90.0	17	AF093499 C. serpentis Snake
18	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	4.8	9.0	1.3	2.2	1.7	4.4	7.4		97.3	89.3	95.1	94.5	96.1	85.9	85.5	89.8	95.3	92.0	18	MK990042 C. hominis ET91
19	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	4.1	8.3	1.3	1.8	0.9	4.0	7.1	2.0		90.6	95.5	95.3	95.5	87.1	86.3	90.8	95.3	93.2	19	KT151537 C. meleagridis IQ.Cm-1
20	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	8.5	1.1	8.6	9.3	9.1	12.3	3.1	9.8	9.3		88.1	88.7	88.5	84.6	84.6	94.7	87.7	87.9	20	AF093497 C. muris Mouse
21	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	4.6	8.9	2.7	3.6	2.7	4.7	8.2	3.1	2.7	9.9		98.6	95.9	86.7	85.5	88.9	94.3	93.6	21	OR428363 C. ditrichi bb2
22	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.9	8.9	2.9	3.6	3.1	4.5	8.2	3.6	3.1	9.4	0.9		95.9	86.9	85.7	89.1	94.5	94.9	22	AB210854 C. canis
23	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	4.1	9.1	2.6	3.1	3.3	4.7	8.4	3.3	3.1	9.8	3.1	2.9		86.3	86.1	88.7	96.9	93.2	23	LC483882 C. fayeri
24	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	11.3	10.9	13.0	11.3	10.8	10.8	11.7	12.8	11.5	11.0	13.8	11.1	11.1	11.6		92.0	86.7	85.0	86.1	24	HM243548 C. molnari A1
25	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	12.5	13.5	11.5	10.7	11.0	12.7	12.9	11.7	11.7	13.5	12.3	12.4	11.5	7.9		86.9	85.0	85.0	25	MW075514 C. abrahamseni 443
26	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.0	4.3	7.7	8.6	8.1	10.6	2.7	8.6	8.3	4.3	8.7	8.7	8.9	11.9	11.3		87.7	88.5	26	KX345065 C. testudinis 15093
27	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	4.6	8.9	3.1	2.7	2.7	5.3	7.7	3.3	2.4	9.9	4.0	3.6	2.4	12.1	11.7	9.2		92.2	27	MK522270 C. viatorum YWWR2992
28	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	3.9	9.1	4.8	5.3	4.8	6.2	7.6	5.5	4.6	9.6	5.5	4.8	5.0	11.2	12.2	8.6	5.3		28	PP023917 C. bovis DL36
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		

Fig. (4a) Sequence distance of the 18S rRNA gene of the tested *Cryptosporidium* strain (generated by lasergene software) showing identity range of 100% with *Cryptosporidium parvum* strains.

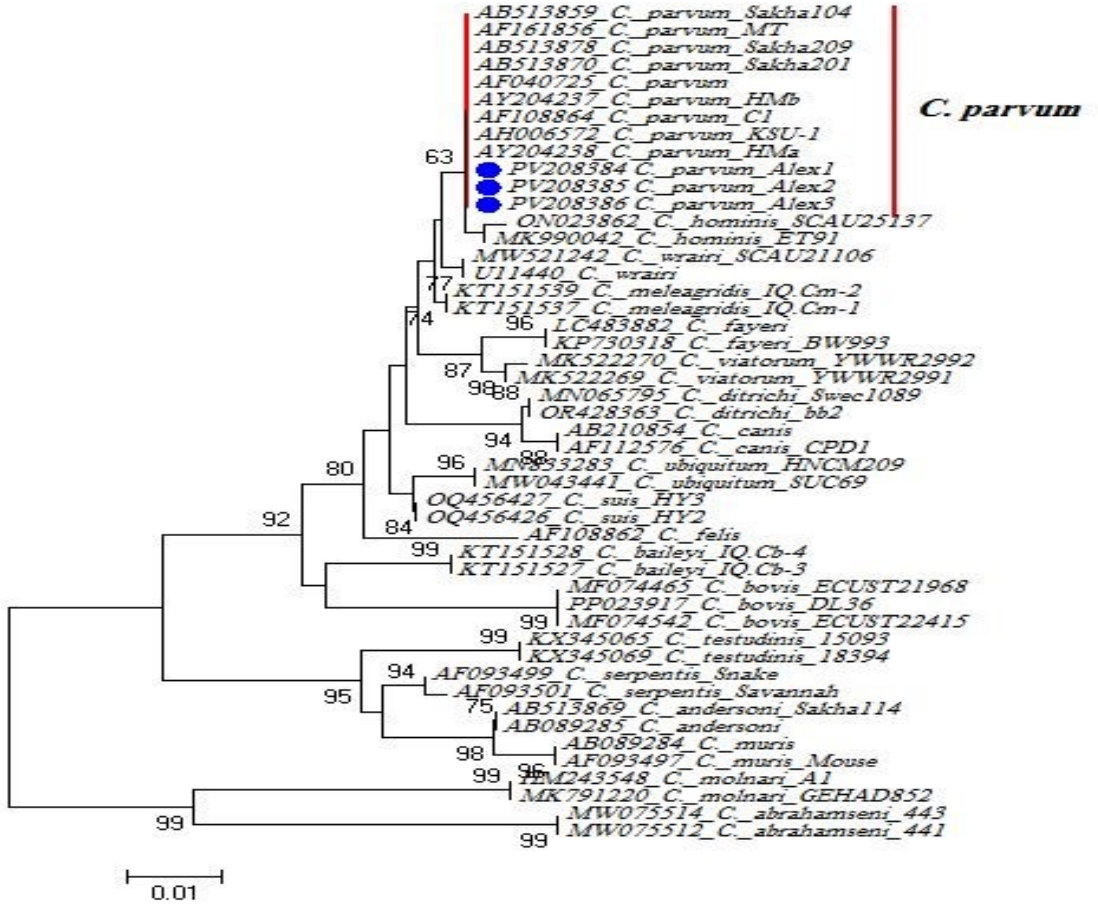

Fig. (4b): Phylogenetic relatedness of the 18S rRNA gene. Maximum-likelihood un rooted tree indicated clustering of the tested strain with *Cryptosporidium parvum* strains apart from other *Cryptosporidium*.

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