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Prevalence of bacteriological and parasitological causes of diarrheic calves in middle Egypt

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ABSTRACT

Neonatal calf diarrhea (NCD), is one of the prime fundamental health issues facing the cattle industry, and has resulted in significant economic losses. This study aimed at detection of the enteric pathogens in diarrheic calves. Another objective was to correlate the existence of isolated enteric pathogens to the age and seasonal distribution of diarrheic parasitic and bacterial infections among calves. The bacterial isolates were identified biochemically and serologically. Moreover the samples were examined macro and microscopically to investigate the internal parasitic causes of diarrhea. Also, for *Cryptosporidium* spp. Oocysts were diagnosed using modified Ziehl-Neelsen stained smears. Out of 364 collected fecal samples, a total bacteria isolated was 356 and identified as 131 *Escherichia coli* (35.99%), 90 *Salmonella enterica* (24.72%); 50 *S.* Typhimurium (13.73%) and 40 *S.* Dublin (10.99%), 35 *Citrobacter diversus* (9.61%), 24 *Klebsiella pneumoniae* (6.59%), 20 *P. vulgaris* (5.49%), 19 *P. penneri* (5.22%) and 37 *Staphylococcus aureus* (10.16%). Regarding the parasitological findings; *Toxocara vitulorum* was 19.8% (72 out of 364), Oocysts of *Eimeria* spp. were found in 26.9% (98/364), *Cryptosporidium* spp. was 29.4% (107 out of 364) and *Giardia* spp. infection was 14% (51 out of 364). In conclusion, NCD problem is a multifactorial in which bacterial and parasitological causes were the prime causes. Thus, regular monitoring and efficient treatment of bacteriological and parasitological causes of NCD are highly recommended.

Introduction

Diarrhea is prevalent in newborn calves, lambs, and kids. Cattle production plays an essential role in the livelihood and economy of farmers worldwide. Calves are thought to be a great value around the world as sources of good quality meat (Karimzadeh *et al*., 2022).

Calf diarrhea (CD) is one of the greatest problems occurred in young animals, causing decreasing in productivity and enormous economic losses in bovine industry around the world (El-azzouny *et al*., 2020). Calf diarrheal illnesses are frequently noticed in calves up to four months old and could a result of multifactorial intricate pathogen-environmental interactions (Maier *et al*., 2022).

The cause of CD may be non-infectious, due to the (environment-managemental etiology) or factors related to the animal (nutritional status and immunological) (Izzo *et al*., 2011). The infectious CD is due to several enteropathogens including bacteriological; *E. coli* and *Salmonella*, parasitological; *Cryptosporidium* spp., *Eimeria* spp. and *Giardia* spp., rather than the viral causes; bovine corona and rotaviruses (Lee *et al*., 2019).

The bacteriological etiology of calf diarrhea is greatly common, *Escherichia coli* (*E. coli*) is one of the most prevalent reason of diarrhea and septicemia in newly born calves, impacting dairy, and beef production. Moreover, calves constitute a very substantial reservoir of pathogenic *E. coli*, and can transfer this pathogen to humans.

Salmonella enterica serovar Dublin (*S.* Dublin) and serovar Typhimurium (*S.* Typhimurium) are the most prevalent etiologic agents that colonize the gastrointestinal tract of cattle particularly calves less than 3 weeks of age (Casaux *et al*., 2023).

Despite *Salmonella* can induce diarrhea in both calves as well as adult cattle, infection is much more observed and often leads to grave symptoms in ten days to three month old calves (Cho and Yoon, 2014). Calves can excrete the organism intermittently and for variable periods of time, relying on the infection degree thereby infected animals can render as a source of zoonosis via direct contact or food-borne routes (Li *et al*., 2022).

Bovine coccidiosis and colibacillosis are important diseases of apicomplexan pathogens of both *Eimeria* and *E. coli* genera and considered the prime vital and common illnesses of cattle worldwide (Ibrahim *et al*., 2015; Bashahun and Amina, 2017). All calves reared in ordinary systems are disclosed to coccidia and can be infected early in life (Alemayehu *et al*., 2013). *Cryptosporidium* spp. is an opportunistic pathogen that can infect humans and a broad scope of animals (Cai *et al*., 2019). It is ubiquitous and is responsible for significant neonatal morbidity in cattle. Cattle as well as other hosts gain cryptosporidiosis primarily via the fecal-oral route, and manifest symptoms of delayed growth and weight loss, resulting in massive economic loss in the breeding industry (Inpankaew *et al*., 2017). *Giardia* spp. is a zoonotic protozoan infects domestic and wild animals all over the world with high levels of genetic divergence (Ramadan *et al*., 2020). *Toxocara vitulorum* (*T. vitulorum*) is a pathogenic gastrointestinal nematode parasitized cattle and buffaloes worldwide, particularly in tropical and subtropical regions with humid climates (Celik *et al*., 2022). Digestive disturbances such as decreased appetite, dehydration, weight loss, abdominal pain, diarrhea, or constipation are noticed in infected calves (Celik *et al*., 2022). In addition to the infection, *T. vitulorum* can cause visceral larval migrans contributing a zoonotic feature (Biswas *et al*., 2021).

This study was therefore performed to estimate the frequency and detection of enteric pathogens, in diarrhoeic calves. Another objective was to correlate the existence of isolated enteric pathogens to the age and seasonal distribution of diarrheic parasitic and bacterial infections

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among calves.

Materials and methods

Ethical Approval

The applied protocol was approved by the Ethics Committee belonged to Faculty of Medicine, Cairo University, Giza, Egypt under the number of (IACUC 03162023697). The Institutional Animal Care and Use Ethical Committee of Cairo University, Egypt, reviewed and accepted the protocols used for handling the animals and collecting samples.

Sample collection and fecal examination

Animals and samples collection

Three hundred and sixty-four cattle calves (*Bos taurus*) that suffered from diarrhea in different localities in Egypt were subjected to study. Fecal swabs aseptically taken from diarrheic calves in some of the Egyptian governorates (Giza, Beni Suef, Fayoum and Ismailia) as shown in Table 1, from January to December 2022. Also, fecal samples were directly gathered from the rectum of each calf, in a sterile plastic sac. The date and age of each sample were recorded. All samples were put in ice box and transmitted to the laboratory; those set for parasitological findings, were freshly examined. The clinical pictures of involved diseased animals are characterized by diarrhea, pyrexia, depression, paleness to redness in mucous membranes, complete anorexia, emaciation, dehydration and loss of weight.

Table 1. The number of samples that was recorded according to diarrheic calves old.

Ages	No. of Samples
1-4 weeks	87
<1-4 Months	202
< 4-6 Months	75
Total	364

Bacteriological examination

After collection, the samples set for bacteriological examination were transported on primary culture as Brain Heart Infusion broth (BHIB) and/or half-strength tryptone-soya broth (TSB). They were cultured in the same day or stored at 4ºC and cultured within 3 days. Subcultures were incubated on sheep blood agar (5%) and MacConkey agar plates for 24 to 72 hours at 37°C, followed by culture into differential and selective media such as Eosin Methylene blue (EMB) agar, *Salmonella* Shigella (SS) agar, Xylose Lysine Deoxycholate agar (XLD), and Brilliant Green agar (BG) plates as well as Mannitol agar (Cowan, 1985).

The bacterial isolates were identified through the morphological appearance of colonies (size, shape, hemolysis and pigment production). Not only bacterial colonies morphology but also Gram staining, biochemical and fermentative identification tests were used to study the cultural characteristics (Cruickshank, 1975). Moreover, pure colonies were biochemically analyzed by API kit (Analytical Profile Index) systems (BioMerieux, Marcy l'Etoile, France), such as API 20 E and API 20 NE Systems for members of *Enterobacter*iaceae and non- *Enterobacter*iaceae, rather than API STAPH IDENT 32 Staph, following manufacturer instructions.

Vitek identification

The obtained bacterial isolates were more identified via Vitek2 compact system Version 9.02 MIC Interpretation Guidelines (according to the manufacturer guidelines).

Serotyping of pathogenic bacterial isolates

The obtained *E. coli* isolates were serotyped using diagnostic antisera based on somatic antigen differentiation (O-antigen) according to Ørskov and Ørskov (1984). The identification of the obtained *Salmonella* isolates was based on White-Kauffman-Le Minor scheme according to Grimont and Weill (2007), the steps were carried out according to manufacturer guidelines.

Antimicrobial sensitivity assay

Antibiotic sensitivity standard discs of 28 antibacterial agents were used for the detection of antibiogram of the obtained bacterial isolates as following in Table 2 (Oxoid, Basingstoke, Hampshire, England, UK). The assay was performed for each isolate via diffusion method on Mueller-Hinton agar, followed the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI, 2019), was used.

Parasitological examination

Samples were examined grossly and microscopically on the same day of collection, to investigate the internal parasitic causes of diarrhea. Each sample was examined using both concentration -flotation with a saturated salt solution and sedimentation with distilled water techniques (Kabir *et al*., 2019; Ramadan *et al*., 2022). On the other hand, iodine solution was used to identify protozoans and cysts. For the diagnosis of enteric protozoa, thin fecal smears were fixed in Schaudinn's solution and stained by the Wright stain (Kalifa *et al*., 2023). Moreover, for detecting *Cryptosporidium* spp. oocysts in feces, the smears were stained with a modified Ziehl-Neelsen technique (Kalifa *et al*., 2016).

Results

Bacteriological Results

E. coli and *Salmonella enterica* were the most common etiologic agents that recovered in diarrheic cattle. Out of 364 collected fecal samples, a total bacteria isolated was 356 and identified as 131 *Escherichia coli* (35.99%), 90 *Salmonella enterica* (24.72%); 50 *S.* Typhimurium (13.73%) and 40 *S.* Dublin (10.99%). Moreover, 35 *Citrobacter diversus* (9.61%), 24 *Klebsiella pneumoniae* (6.59%), 20 *P. vulgaris* (5.49%) and 19 *P. penneri* (5.22%) and finally, 37 *Staphylococcus aureus* (10.16%).

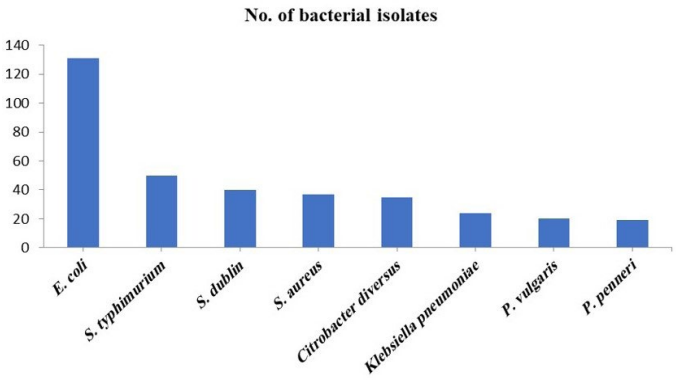

Fig. 1. Prevalence and typing of the isolated Bacteria.

Serological characterization

E. coli were serotyped into six serotypes; O26, O78, O86, O101, O111 and O119. No O157 was isolated in culture or identified in serological typing. Serological identification of *Salmonella* isolates displayed that, 90 *Salmonella enterica* (24.72%); 50 *S.* Typhimurium and 40 *S.* Dublin) iso-

lates were belonged to *S. enterica*, out of *Salmonella* spp. isolates.

Susceptibility of the bacterial isolates to the antibacterial agents

Full antibiograms were run for the most predominant common isolates, which demonstrated that the Gram negative bacteria were 100% sensitive to Imipenem, Amikacin, Cefoxitin, Ceftazidime, Cefotaxime, Ceftriaxone and Trimethoprim/sulfamethoxazole, while Staphylococcus isolates were 100% sensitive to Imipenem, Amikacin, Ciprofloxacin, Cefuroxime, Cefotaxime, Ceftriaxone, Rifampicin, Levofloxacin and Vancomycin.

Parasitological Results

Macroscopic examination

Twenty-three examined calves were positive for *T. vitulorum* adult by macroscopic examination with a 6.3 % prevalence rate. *T. vitulorum* was appeared as translucent, cylindrical, soft whitish in color, and large-sized worm; 30 cm in length. Additionally, the anterior end is characterized by small-sized lips (3 lips each divided into three).

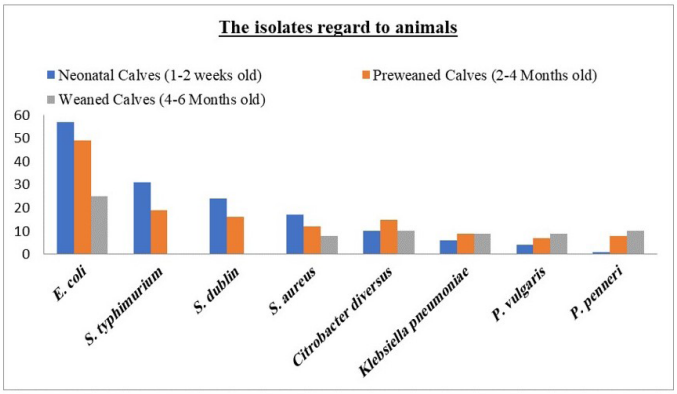

Fig. 2. The isolates in regard to animals.

Microscopic examination

The detected parasites were *Cryptosporidium* spp., *Eimeria* spp., *Giardia* spp. and *T. vitulorum*. By using light microscopic examination; *T. vitulorum* eggs were dark and subglobular thick-pitted albuminous shell with immature one-cell embryo of dimensions 73 - 91 × 58 - 73 µm. *Giardia* cyst was oval in shape, has 4-8 nuclei, a tuft of flagella and a parabasal body and the dimensional measuring was (6-12 X 7-9 µm). *Cryptosporidium* spp. oocysts were spherical to ovoid in shape, by the Modified Ziehl-Neelsen stain technique they appeared as acid-fast (red-pink) on a green to blue background. The oocyst pass sporulated and contains 4 sporozoites and oocysts residual body and measured 4.5 x 5.4 µm. *Eimeria* spp. oocyst has a typical ovoidal shape, varied in size and has a very distinct dark brown color, some species are very thick, others have microtubules and a smooth and homogeneous oocyst wall. as shown in Fig. 4.

Table 2. Antibacterial discs, abbreviations and concentration.

Antibacterial disc	Abbreviations	Concentration	Antibacterial disc	Abbreviations	Concentration
Amikacin	AK	30 µg	Doxycycline	DO	30 µg
Ampicillin	AM	10 µg	Erythromycin	E	15 µg
Amoxicillin + Clavulanic acid	AMC	20+10 µg	Gentamicin	GM	10 µg
Ampicillin + Sulbactam	SAM	10+10 µg	Imipenem	IPM	10 µg
Aztreonam	ATM	30 µg	Neomycin	N	30 µg
Cefalexin	CN	30 µg	Novobiocin	NV	30 µg
Cefepime	FEP	30 µg	Norfloxacin	NOR	10 µg
Cefotaxime	CTX	30 µg	Penicillin G	P	10 $_{IU}$
Cefoxitin	FOX	30 µg	Rifampicin	RA	5 µg
Ceftazidime	CAZ	30 µg	Streptomycin	S	10 µg
Ceftriaxone	CRO	30 µg	Trimethoprim/sulfamethoxazole	SXT	1.25+23.75=25 µg
Ciprofloxacin	CIP	5 µg	Tetracycline	TE	30 µg
Clindamycin	CD	2 µg	Tobramycin	TOB	30 µg
Colistin	CL	10 µg	Vancomycin	VA	30 µg

Table 3. The number of *E. coli* regard to serotyping.

E. coli serotype	Number	%
E. coli O26	32	24.43%
E. coli O78	32	24.43%
E. coli O86	22	16.79%
E. coli O101	15	11.46%
E. coli O111	14	10.68%
E. coli O119	16	12.21%
Total sum	131	100%

Prevalence of internal parasites in diarrheic calves

The overall prevalence of *T. vitulorum* was 19.8% (72 out of 364). Regarding prevalence by age and season, *T. vitulorum* was high in calves aged 1-4 months (24.2 %) (Table 4). The seasonal variation was 29.2% in winter, while the other seasonal variation were 21.1%, 14.1% and 12.04% in autumn, spring and summer respectively (Table 5).

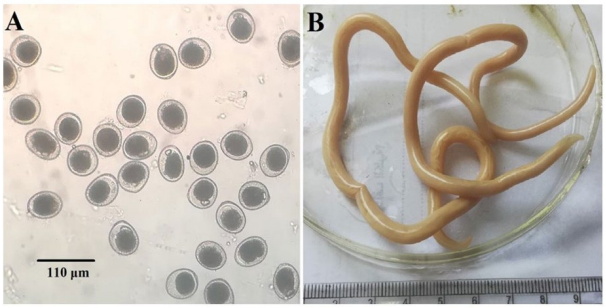

Fig. 3. A) *T. vitulorum* eggs in diarrheic calf feces (Heavy infection) (X10); B) adult worm of *T. vitulorum* with thin cuticle.

Oocysts of *Eimeria* spp. were found in 26.9% (98/364) of the examined fecal samples from diarrheic calves using a light microscope. Prev-

alence of *Eimeria* spp. based on age was high in group of four to six months of age (48%) followed by a prevalence rate of 25.2% and 12.6% in calves of <1-4months, 1-4 weeks respectively (Table 4). Concerning the season, the results revealed that high prevalence rate of *Eimeria* spp. in summer (42.2%) then winter (36.8%), autumn (16.7%) and finally spring (10.6%).

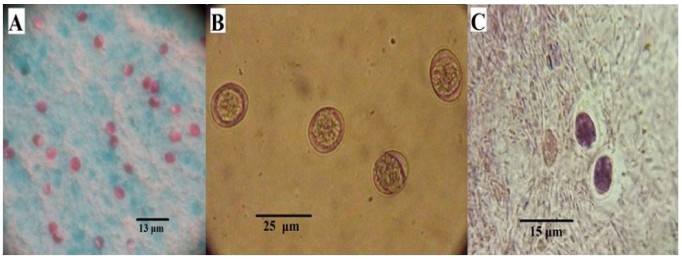

Fig. 4. Direct micrographs of: A) *Cryptosporidium* spp. oocysts (X100); B) *Eimeria* spp. oocysts (X40); C) *Giardia* spp. cyst (X100).

The overall prevalence of *Cryptosporidium* spp. was 29.4% (107 out of 364). Regarding age, the present study clarified that the highest prevalence rate of *Cryptosporidium* infection recorded young age from 1-4 weeks (62%) as shown in Table 4.

Prevalence rate of *Giardia* spp. infection was 14% (51 out of 364). Infection was top in the age group of 1–4 months. The prevalence was higher in samples collected during winter than those collected during summer as shown in Table 5.

Discussion

In young calves, diarrhea is looked one of the most fundamental illnesses, because the economic loss following decreasing in growth rate, treatment costs, and rather than deaths (Saleh *et al*., 2022). The clinical signs noticed in ill calves in this study corresponded well with those reported in former studies. We reported watery fecal matter that may be of color variance; yellow, green grey, or brown. At times, blood and mucus may be obvious in the feces. Calves are frequently depressed, weak to stand, and developed a sunken-eyed picture as a consequence of dehydration. If calves left untreated, death occurs within 24 hours. Relying on the etiology and the graveness of the infection, a case of scours in a calf can last one to two days or as long as two weeks (Gould, 2014).

Concerning the bacteriological examination of the current study, out of the collected 364 fecal samples, a total bacteria isolates was 356. *E. coli* was detected in 131(35.99%) belonged to six serotypes; O26 (24.43%),O78 (24.43%),O86(16.79%), O101 (11.46 %), O111 (10.68 %) and O119 (12.21 %).

Our isolation result was more than that mentioned by some Egyptian studies; 28.8% (Algammal *et al*., 2020), 26.8% (Meshref *et al*., 2021) and 22.7% (Eldesoukey *et al*., 2022). Contrarily, greatly less than others; 80% (Aref *et al*., 2018), 65.5% (Abed and Menshawy, 2019), 100% (Awad *et al*., 2020) and 76% (Shehta *et al*., 2022). Also these isolation variances were reported worldwide; (58.9%) in Ethiopia (Belete *et al*., 2022), (12.12%) in Brazil(Coura *et al*., 2015), (40%) in Belgium(Habets *et al*., (2022), (100%) in Bangladesh(Haque *et al*., 2022), and (37.77%) in India(Singh *et al*., 2022).

On the other hand, the obtained serotypes were coincided with those usually isolated from diarrheic calves in the previous mentioned Egyptian studies; Galal *et al*., (2013) reported (O78, O86 and O119), El-Seedy *et al*. (2016) mentioned (O26, O78,O86,O111andO119), Abed and Menshawy, (2019) recovered very near data; O26(21.1%), O86(13.2%), O111(10.5%) rather than O119 (5.3%). and Algammal *et al*. (2020) determined (O26, O111, and O119).

In the present study, the incidence rate of *Salmonella enterica* was (24.72%) as 90 *Salmonella enterica* isolates; 50 *S.* Typhimurium (13.73%) and 40 *S.* Dublin (10.99%). Our results harmonized with that recorded by Shehta *et al*. (2022) who reported an incidence of 24%. In contrast of our results, in Sharkia Governate an isolation rate of 16.25% was determined among diarrheic calves in two intensive dairy farms and distributed as four serotypes *S. Kiel, S. Agama* (31.25%), *S. Nitra* (12.5%) and *S. Koessen* (Gharieb *et al*., 2019). Also, El-Seedy *et al*., (2016) reported an incidence of 18.1% of three serotypes; *S.* Typhimurium, *S.* Dublin in addition to *S.* Enteritidis. More low isolation rate (8%) was determined by Tawfik *et al*., (2022) with three serotypes; *S.* Kentucky, *S.* Typhimurium, and *S.* Enteritidis in Kafr El-Sheikh farms. Moreover, *Salmonella* spp. was obtained from nine out of 220 diarrheic calves (4.09%) at farms belonged to four governorates located in central and northern Egypt (Younis *et al*., 2009). The nine *Salmonella* isolates were serotyped as *S.* Typhimurium and S. Enteritidis (Ahmed *et al*., 2009).

There were other incidence's variations of *Salmonella* isolation in calf diarrhea; A total of 12 *Salmonella* spp. (33%) were isolated from 36 fecal samples of diarrheic calves in a dairy farm in Bangladesh (Haque *et al*., 2022). Contrarily, in Uruguay, Casaux *et al*. (2019) reported fourty one *Salmonella* isolates as 15.65 % and serotyped as *S.* Typhimurium, S. Anatum and *S.* Dublin.

In addition to isolation of *Escherichia coli* and *Salmonella enterica*, our study determined other recovered bacterial species; 35 *Citrobacter diversus* (9.61%), 24 *Klebsiella pneumoniae* (6.59%), 20 *Proteus vulgaris* (5.49%), 19 *Proteus penneri* (5.22%) and 37 *Staphylococcus aureus* (10.16%).

The obtained data were coincided with that mentioned by Meshref *et al*. (2021) who reported *Citrobacter diversus* (18%), *Klebsiella pneumoniae* (6%), *Proteus* spp. (4%), *Klebsiella oxytoca* (2.7%), beside other bacteria; *Enterobacter* spp. (6%), *Serratia* spp. (12%), *Providencia* spp. (6%) and *Morganella morganii* (2%). Aly *et al*., (2016) reported *Proteus vulgaris* in an incidence of 10 %. Lee *et al*. (2020) identified two *K. pneumoniae*; one type 14 as first time and type 65 in two diarrheic calves in Korea. In Brazil, Ambrosim and co- authors isolated *Enterobacter* spp. (8.64%) strains, *Klebsiella pneumoniae* (6.76%), *Citrobacter* spp. (5.63%) (Ambrosim *et al*., 2002). Parallel to our results, Mohamed *et al*. (2022) mentioned isolation rates of *Staphylococcus aureus* (12 %), *Klebsiella pneumoniae* (6%) and *Proteus vulgaris* (4%) from 50 diarrheic buffalo calves in Giza Governorate.

In the current study, the vitro susceptibility of the recovered bacterial isolates revealed 100% sensitive to amikacin, cefoxitin, ceftazidime, cefotaxime, ceftriaxone, imipenem, levofloxacin, trimethoprim/sulfamethoxazole and vancomycin.

Meshref *et al*. (2021) mentioned that the obtained isolates were sensitive sulfamethoxazole –trimethoprim, cefotaxime, ciprofloxacin and norfloxacin. Also, it was demonstrated that *Salmonella* and *E. coli* serotypes obtained from diarrheic calves in Sharkia Governorate were sensi-

Table 4. Existence of internal parasites in examined diarrheic calves regarding age.

Age	No. of examined calves	*T. vitulorum*		*Giardia* spp.		*Cryptosporidium* spp.		*Eimeria* spp.	
		+ve	%	*+ve*	%	*+ve*	%	*+ve*	%
1-4 w	87	20	23.00%	3	3.40%	54	62.00%	11	12.60%
<1-4 M	202	49	24.20%	39	19.30%	47	23.30%	51	25.20%
<4-6 M	75	3	4.00%	9	12.00%	6	8.00%	36	48.00%
Total	364	72	19.80%	51	14.00%	107	29.40%	98	26.90%

Table 5. Existence of internal parasites in examined diarrheic calves regarding the season.

Age	No. of examined calves	*T. vitulorum*		*Giardia* spp.		*Cryptosporidium* spp.		*Eimeria* spp.	
		+ve	%	*+ve*	%	*+ve*	%	*+ve*	%
Autumn	90	19	21.10%	10	11.11%	25	27.80%	15	16.70%
Winter	106	31	29.20%	26	24.50%	48	45.30%	39	36.80%
Spring	85	12	14.10%	9	10.60%	27	31.80%	9	10.60%
Summer	83	10	12.04%	6	7.22%	7	8.40%	35	42.20%

tive to amikacin and imipenem but resistant to cephalosporins (Gharieb *et al*., 2019).

In other studies; *E. coli* strains isolated from diarrheic calves were 100 % susceptible to amikacin, cefoxitin, norfloxacin, and gentamicin while *Salmonella* spp. strains were sensitive to amikacin, ampicillin, amoxicillin, norfloxacin, tetracycline, gentamicin, cefoxitin, and trimethoprim-sulfamethoxazole (Souto *et al*., 2017). While, Ambrosim *et al*. (2002) observed high sensitivity to cephalothin, erythromycin and trimethoprim-sulfadiazine

Opposite to our results, *E. coli* and *S.* Typhimurium isolates demonstrated resistances versus cefotaxime, erythromycin, and trimethoprim sulfamethoxazole (Mohamed *et al*., 2022). Remarkable resistance to amikacin and trimethoprim/sulfamethoxazole was reported (Algammal *et al*., 2020). Atwa *et al*. (2012) revealed that the recovered *E. coli* isolates were resistant to erythromycin, gentamicin and lincomycin.

The present study revealed the detection of *T. vitulorum* in 72 (19.8%) out of 346 fecal samples examined. While in Egypt the overall prevalence rate was 28.4%, 12% and 63.4% which reported by Abdel-Rahman and El-Ashmawy (2013); Ramadan *et al*. (2015) and Osmana *et al*. (2016) respectively. In studies performed in various places in the world, the prevalence ratios were reported as follows: 3.01% in Turkey (Celik *et al*., 2022), 7.3% in Iran (Tavassoli *et al*., 2018), rather than 18.54 and 63.83% in Pakistan (Raza *et al*., 2013; Deeba *et al*., 2019). In Bangladesh; 2.4% and 22.9% (Mamun *et al*., 2011; Biswas *et al*., 2021), while in India, the ratios were 8.47%, 26.16% and 22.5% (Singh and Juyal, 2014; Das and Phukan 2018; Parihar *et al*., 2022). Rast *et al*. (2013) reported 25.5% in Lao and as well as 20.1% in Cambodia (Dorny *et al*., 2015) but 9% in North Central Florida (Davila *et al*., 2010). In our study, age groups were categorized into three groups with the highest prevalence (24.2 %) was found in the one to four months group, these findings were similar to those of other researchers. The prevalence is high in buffalo calves aged one to three months as mentioned; Davila *et al*. (2010); Abdel-Rahman and ElAshmawy (2013); Raza *et al*. (2013); as well as Osmana *et al*. (2016); Deeba *et al*. (2019); Biswas *et al*. (2021) and Parihar *et al*. (2022).

The variation in the incidence rate of the *T. vitulorum* infection may be attributed to different agents like poor hygienic statuses of the shed, health care management sample divergence, also varied geo-climatic conditions played a great role. In this study, the seasonal variation of *T. vitulorum* was achieved as 29.2% in winter, higher than other seasons; 21.1%, 14.1% and 12.04% in autumn, spring and summer respectively. These results agreed with Ramadan *et al*. (2015) who recorded a higher prevalence rate in winter (8.3%) followed by autumn (6%), spring (4.3%) and summer.

In the present study, a prevalence rate of *Giardia* duodenalis (Syn. G. intestinalis, G. lamblia) infection was 14% (51 out of 364). Infection was in the top among the age group 1–4 months. Also, the incidence was higher in samples collected during winter than in samples gathered during summer. In contrary, McAllister *et al*. (2005) recorded that the overall prevalence of *Giardia* in Columbia among calves was 36% and Hamnes *et al*. (2006) found *Giardia* in 49% (679 out of 1386) of the calves. The *Giardia* infection was high in the age group two to three months. While parallel to our study, the prevalence was also higher in samples gathered during winter than in samples collected during summer.

Coccidiosis induces massive economic losses in cattle as a consequence of a lowering in feed efficiency followed by slow weight gain and increased susceptibility to other diseases (Malek and Kuraa, 2018). In the current study, the overall incidence rate of *Eimeria* spp. infection was 26.9% (98/364). These results disagreed with El-Seify *et al*. (2012); Ramadan *et al*. (2015) and Malek and Kuraa (2018) whose recorded prevalence rate was 28.94%, 32% and 46.7% respectively. Concerning age, the incidence of *Eimeria* spp. was high in calves four to six months of age (48%) followed by a prevalence rate of 25.2%, 12.6% in calves of < 1-4 months, 1-4 weeks respectively. While El-Seify *et al*. (2012) recorded that the cattle group of 3-6-month-old demonstrated the highest rate of infection; 37.1%. Also, Malek and Kuraa (2018) reported a similar findings; higher incidence was found in calves of 3 - 6 months age (73.3%) than in calves of age (28.9%). Concerning the season, the results revealed that high prevalence rate of *Eimeria* spp. in summer (42.2%) followed by winter (36.8%), autumn (16.7%) and spring (10.6%). These results agreed with Malek and Kuraa (2018) who found a high prevalence rate of *Eimeria* spp. in diarrheic calves in summer (69.2%) followed by winter (36.4%), autumn (25%) and spring (7.7%). While El-Seify *et al*. (2012) displayed the highest rate of infection (33.3%) in winter, persuaded by spring (29.1%), summer (27.1%) and autumn (26.6%). On the other hand, Ramadan *et al*. (2015) recorded that the highest rate of infection was in spring (41.3%) followed by autumn (40%), summer (26.9%) and winter (13.3%).

In this study, it was observed that the overall prevalence rate of *Cryptosporidium* spp. infection was 29.4%. while In Egypt, Kafr El-Sheikh province, the overall prevalence of *Cryptosporidium* spp. recorded 34.1 % by El-Seify *et al*. (2012) and 7.07% by Mahfouz *et al*. (2014) in calves. Ghoneim *et al*. (2017) from different Egyptian governorates (Cairo, Giza, and Al-Bahira) reported that 30.4% prevalence rate. In Assiut Province, Elmahallawy *et al*. (2022) found a prevalence rate of 38.27%. In Columbia, McAllister *et al*. (2005), the overall prevalence of *Cryptosporidium* spp. in calves was 13%. Concerning age, the present study clarified that the highest prevalence rate of *Cryptosporidium* infection was recorded young age from 1-4 weeks (62%). This finding is similar to the earlier record of El-Seify *et al*. (2012); Essa *et al*. (2014) and Ghoneim *et al*. (2017). While Elmahallawy *et al*. (2022) recorded that the infection rates of *Cryptosporidium* spp. in cattle calves with ages of under one month, 1–3, and above than 3 months were 39.13, 34.04, and 54.54% respectively. Regarding the seasonal dynamics of *Cryptosporidium* spp. infection, the data exposed that the examined cattle manifested the highest rate of infection in winter (45.3%), followed by spring (31.8%), autumn (27.8%) and finally summer (8.4%). Our findings agreed with El-Seify *et al*. (2012) who reported that the highest incidence of infection was in winter (39.9%), followed by spring (36.6%), autumn (28.7%) and summer (24.7%). Elmahallawy *et al*. (2022) the infection rates of *Cryptosporidium* spp. in cattle calves were the highest in winter (52.63%) followed by spring (42.11%), summer (30.43%) and autumn (30%). This result disagreed with Essa *et al*. (2014) who reported that the highest prevalence of *Cryptosporidium* spp. infection was observed in summer (46.39%) then spring (45.65%), autumn (24.18%) and winter (16.33%). The reason of this prevalence divergence of infection in varied age groups in cattle may be attributed to the poor developed immunity of the young. This divergence might also be attributed to the variation in the grazing area and management diversity of cattle.

Conclusion

Calf diarrhea is one of the prime substantial issues facing cattle livestock resulting in great economic losses although the prevention and control measures. The problem is multifactorial in which bacterial and parasitological causes were implemented and may induce a public health hazard. Thus, the regular monitoring of bacteriological and parasitological causes to identify their actual role in calf diarrhea as well as the transmission routes to humans. The most effective antibacterial agents should be used, and there should be an efficient control programs for the prevention of calf diarrhea specially unweaned calves.

Conflict of interest

The authors declare that they have no conflict of interest.

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