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Risk factors affect prevalence of diarrheal entero- pathogens in children, calves and broiler chickens in Assiut, Egypt

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Abstract

Diarrhea is a complex syndrome causing colossal economic losses. The objective of the current study was to diagnose Enter-pathogens in children, calves, and broiler chickens by a serological and molecular diagnosis of *Rotavirus*, evaluate *Escherichia coli* (*E. coli*) and *Salmonella spp.* with bacteriological examination beside conventional diagnosis of *Cryptosporidium* in addition to studying risk factors affecting these pathogens in Assiut Governorate. From February 2021 to April 2021, a total of 60 samples were collected from the stool of children (n=20), feces of calves (n=20) in addition to liver and pool intestine of broiler chickens (n=20) were examined by Immuno Chromatographic Assay (ICA), Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), microbiological examination and modified Ziehl-Neelsen stain smears. The prevalence of *Rotavirus* infection by ICA was 55% (11/20), 10% (2/20), and 5% (1/20) of examined children, calves, and broiler chickens, respectively. Two (18.18%) of serologically *Rotavirus* positive stools of children were positive molecularly by RT-PCR, while serologically positive fecal and pool intestines of calves and broiler chickens, respectively, were negative molecularly. There was no significant variation between *Rotavirus*, *E. coli*, and *Cryptosporidium* infections with gender and age of investigated children and between these enteropathogens with sex, age, and species of examined calves. In addition, there were no significant differences between *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* with the age of investigated broiler chickens. It is concluded that these enteropathogens in livestock and chicken living close to the human population necessitate better surveillance and control measures to protect vulnerable animal and human populations.

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Introduction

Livestock plays an outstanding role in the national economy and the socio-economic development of millions of rural households (1). Enteritis is a significant problem in livestock production in Egypt and worldwide (2). Fecal animals are a source of enteritis infection in young children in low- and middle-income countries by contamination of their food (3,4). Diarrhea is a complex multi-factorial etiology, usually influenced by environmental, management, nutritional, and physiological variations and different pathogens, including *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* that are described as essential agents causing enteritis (either separately or in combination) (2). The *Rotavirus* belongs to the genus *Rotavirus*, family *Reoviridae*, has 11 segments of double-stranded ribonucleic acid (dsRNA) in its genome, contains triple layered capsid protein, and encodes six viral structural proteins (VP) (VP1-VP4, VP6, and VP7) and six non-structural proteins (NSP) (NSP1-NSP6) (5). Depending on VP6, Rotaviruses are

classified into ten serogroups (A-J) (5). *Rotavirus* is the major etiological agent causing acute gastroenteritis in different species of mammals (6). Avian *Rotavirus* is morphologically identical and antigenically related to mammalian *Rotavirus*, and it is like mammalian *Rotavirus* in its genome (6). An avian-like group A *Rotavirus* has been isolated from enteric calf feces, and avian *Rotavirus* has been found to induce diarrhea in mammals (6). Laboratory diagnosis of *Rotavirus* enteritis is based on detecting viral antigens and nucleic acids in stool, fecal, and pool of intestine samples. Immune chromatographic assay ICA is a rapid and sensitive test requiring a shorter time than viral isolation for *Rotavirus* antigen detection (5,7). Virus confirmation can be performed at the genomic level using the molecular method of RT-PCR (7). *E. coli* and *Salmonella* belong to the family *Enterobacteriaceae* and are the most common and economically critical bacterial agents (2,8). Zoonotic enteropathogens include a wide range of microorganisms transmitted to humans by consuming dairy or meat products, food or water contaminated with animal feces, or direct contact with animal feces in the environment (3). Animals carry pathogenic *E. coli* strains that can cause gastrointestinal illness in humans, and they play an essential role in fecal contamination of sources of drinking water and crops that enable the direct transfer of zoonotic organisms to humans (9). *E. coli* infection is an important bacterial avian pathogen responsible for various disease syndromes in farmed birds, causing 5-50% mortality in the poultry industry (10). Several strategies have been adapted to isolates of *E. coli* by culturing that aiding the identification of pathogenic strains in humans, animals, and poultry (3,9,10). Salmonellosis is a food-born infection with worldwide importance and is a zoonotic bacterial disease that causes economic problems in both developed and developing countries (11). *Salmonella* infection can be diagnosed by identification of this bacteria in animals, humans, and poultry (11). The gold standard for *Salmonella* detection is bacteriological culture (11). *Cryptosporidium* is common enteric protozoa that belong to the phylum *Apicomplexa* (12). It is a coccidian protozoan that infects various vertebrate animals, humans, and birds (12). *Cryptosporidium* is most common in pre-weaned calves, resulting in clinical disease with the potential of zoonotic transmission to human contacts like children (12,13). On the other hand, birds such as chickens are infected with several *Cryptosporidium* species. Chicken acts as a reservoir for transmission to humans (12). *Cryptosporidium* infection in farm animals and humans is diagnosed under microscopy by detecting oocysts in smears by conventional techniques such as the modified Ziehl Neelsen staining method (12,13).

The present study aimed to investigate the presence of enteropathogens (*Rotavirus*, *E. coli*, *Salmonella* and *Cryptosporidium*) in children, calves, and broiler chickens by a serological and molecular diagnosis of *Rotavirus*. Also, evaluation of *E. coli* and *Salmonella* with the bacteriological examination. Besides, perform the conventional diagnosis of *Cryptosporidium* in them. Correlation between different factors, such as gender/sex, age, and species of children, calves, and broiler chickens with infection rates of these enteropathogens, was also assessed in Assiut Governorate/Egypt.

Materials and methods

Ethical approval

All study conditions were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt (aun/vet/3/0007).

Human, calves, and broiler chickens sample collection

A total of 60 samples were collected from the stool of children, feces of calves, and liver and pool intestine of broiler chickens during the period of investigation between February 2021 and April 2021. For the humans, stool samples were obtained from 20 children (11 male and 9 female) of variant ages that were in contact with 20 calves (7 male and 13 female) of different ages and species. For broiler chickens, liver and pools of intestine were collected from 20 broiler chickens aged from 20 - 36 days from areas that overlapped with calves and human beings in the North, Center, and South of Assiut governorate/Egypt. Clinical findings of investigated children were gastrointestinal tract disturbances like vomiting and diarrhea with fever, and examined calves suffered from diarrhea. In addition to investigated broiler, chickens showed diarrhea, depression, pasty vent, and ruffled feathers with swollen eyes.

Serological detection of *Rotavirus* antigen by ICA (*Rotavirus* rapid test device)

Sixty samples (20 feces of calves, 20 stools of children, and 20 pools of intestine of broiler chickens) were subjected to ICA for detection of *Rotavirus* antigen by a commercial kit (Atlas Medical, United Kingdom).

RNA extraction

The positive *Rotavirus* samples were prepared by 50% stool of children, fecal of calves, and pool of intestine of broiler chickens' suspension (w/v) in phosphate buffer saline was clarified by centrifugation at 12000 rpm for 30 min and supernatant was used for RNA extraction (14). Viral RNA extraction was performed using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

RT-PCR

The extracted RNA was denatured at 97°C for 3 minutes and immediately placed on ice (7). Then, OneStep RT-PCR was performed using Qiagen OneStep RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The primers pair was used to amplify the Viral

structural protein 7 (VP7) gene of *Rotavirus*. The primers sequences were as follows: an upstream primer: 5′- GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3′, downstream primers: 5′- GGT CAC ATC ATA CAA TTC TAA TCT AAG -3′ (7,15). The amplified products of *Rotavirus* of 1062 bp were visualized on 1.5% agarose gel stained with Ethidium bromide and photographed by a gel documentation system (BDA digital Biometra, Germany).

Isolation of *E. coli*

E. coli isolation was done according to Lee and Nolan (16). Collected stool of children, feces of calves, and liver samples of broiler chickens were cultured on tryptic soy broth (Oxoid CM129) and incubated at 37ºC for 18-24 hours. A loop full of incubated broth was streaked Eosin Methylene Blue (EMB) agar (Oxoid CM69) that was used to observe bacterial growth and incubated at 37ºC for 18-24 hours.

Isolation of *Salmonella*

Salmonella isolation was done according to ISO (17). Rappaport-Vassiliadis Enrichment Broth (RVS) (Himedia) was used to isolate *Salmonella* from the stool of children, feces of calves, and pool intestine of broiler chickens incubated at 37 °C for 18 hours. A loop full of culture was streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid CM0469) and incubated at 37 °C for 18-24 hours.

Identification of *E. coli* and *Salmonella*

Suspected colonies for *E. coli* and Salmonella were subjected to morphological and biochemical identification according to MacFaddin (18).

Lactose fermentation

Lactose fermentation was examined by culturing a single suspected pure colony on MacConkey agar (Himedia M081) and incubated at 37 °C for 24 hours; a rosy/pink colony was identicative of lactose fermentative bacteria, while the pale colony was identicative of non-lactose fermentative bacteria.

Triple Sugar Iron (TSI) agar

The heavy inoculum was stabbed into the bottom and streaked over the surface of the slope of TSI agar (Oxoid CM0277), incubated aerobically at 37 °C for 24 hours. Interpretation of the results was recovered by changing colors at the surface and the bottom, with or without H_2S and gas production.

Identification of *Cryptosporidium* oocysts

Direct smears were prepared from stool, fecal, and pool intestine specimens of children, calves, and broiler chickens, respectively, and processed for microscopical examination after staining of smears with modified Ziehl-Neelsen stain according to Bessat *et al.* (12), and oocysts were identified by examining the stained smears under the 100X-oil immersion objectives of the optical light microscope (Optika, Italy).

Statistical analysis

The obtained results were analyzed by Chi-square of independence to the association between prevalence and different parameters (gender/sex, age, and species) by using the Statistical package for the social sciences (SPSS) version 16 software program.

Results

Prevalence of enteropathogen infection among children, claves, and broiler chickens

In the present study, a total of 60 examined samples for *Rotavirus* antigen, 14 (23.33%) were serologically positive by ICA as indicated by a different red band (Result line) appearing in the white central zone of the test (Result region) beside the green band (Control line) (Figure 1). The prevalence of *Rotavirus* infection by ICA was 55% (11/20), 10% (2/20), and 5% (1/20) of investigated children, calves, and broiler chickens, respectively (Table 1). Two (18.18%) of the serologically positive stool of children were molecularly positive for *Rotavirus* by RT-PCR (Figure 2), while serologically positive fecal and pool intestines of calves and broiler chickens, respectively, were molecularly negative (Figure 2). The specific band showed 1062 bp after PCR amplification of the VP7 gene of *Rotavirus* group A. Culturing of suspected *E. coli* samples on EMB agar showed flat dark colonies with a green metallic sheen. The suspected colonies on MacConkey's agar showed medium-sized, rounded, and pink colonies that act as identification for lactose fermentation (Figure 3). In addition, the biochemical test of suspected colonies showed a yellow butt and slant with gases on TSI (Figure 4). The prevalence of *E. coli* infection was 10% (2/20), 10% (2/20), and 55% (11/20) of examined children, calves, and broiler chickens, respectively (Table 1). Colonies of suspected Salmonella culture appeared pink with a black center on XLD agar. On MacConkey's agar showed pale colonies (non-lactose fermenter) (Figure 3). Biochemical identification of cultures showed a yellow butt and red slant with H_2S (blacking) on TSI (Figure 4). Prevalence of *Salmonella* infection was detected only in broilers by 35% (7/20), while it did not find in children and calves (Table 1). Microscopic examination of stool, fecal, and pool intestine smears stained with modified Ziehl-Neelsen stain revealed the characteristic diagnostic stages of *Cryptosporidium* as acid-fast-stained oocysts on a blue background (Figure 5). The prevalence of *Cryptosporidium* in investigated children, calves, and broiler chickens was 10% (2/20), 40% (8/20), and 20% (4/20), respectively (Table 1). According to single infection in (Table 2), *Rotavirus* infection was most common in children, while *Cryptosporidium* infection was in calves. Single infection with *E. coli* and *Salmonella* was prevalent in broiler

chickens. Regarding double mixed infection, *Rotavirus* with *E. coli* was observed in calves, while *Rotavirus* with *Cryptosporidium* and *E. coli* with *Cryptosporidium* were recorded in children. In addition, *E. coli* with *Salmonella* and *Salmonella* with *Cryptosporidium* were reported in broiler chickens (Table 2). According to triple mixed infection, *Rotavirus*, *E. coli*, and *Cryptosporidium* were found in calves, while *E. coli*, *Salmonella*, and *Cryptosporidium* were shown in broiler chickens (Table 2).

Table 1: Number and percentage of enter pathogens in investigated children, calves, and broiler chickens

Variable	Total number	*Rotavirus*		*E. coli*		*Salmonella*		*Cryptosporidium*	
		+ve	%	+ve	%	+ve	%	+ve	%
Children	20	11	55	2	10	0	0	2	10
Calves	20	2	10	2	10	0	0	8	40
Broiler Chickens	20	1	5	11	55	7	35	4	20

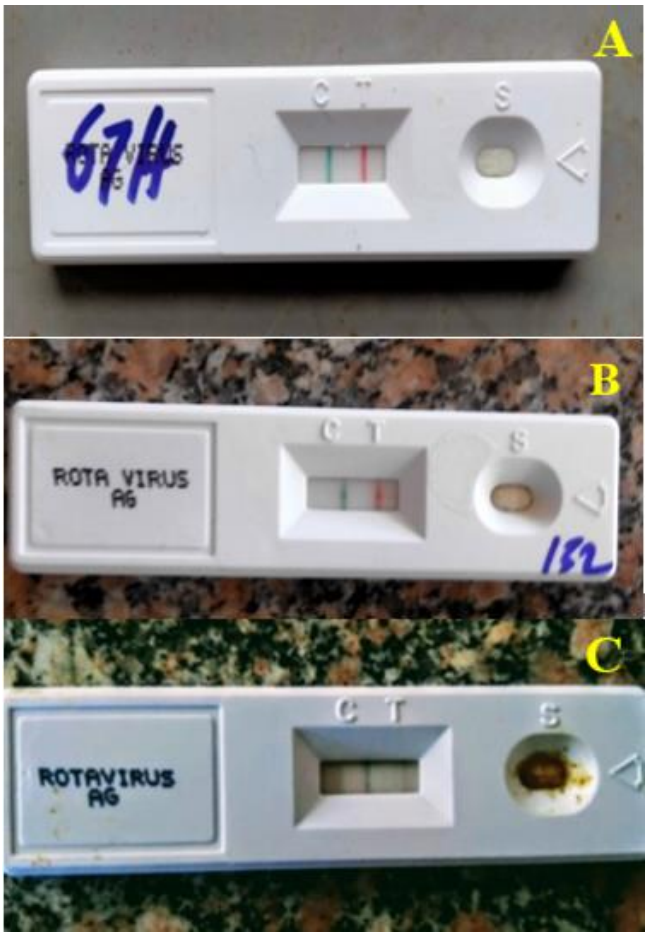

Figure 1: Rapid Test device for detection of *Rotavirus* antigen: Positive result (In addition to the green band (Control line), a different red band (Result line) appears in the white central zone of the test (Result region). (A): Children's stool sample, (B): Calf fecal sample, and (C): broiler chicken pool intestine sample.

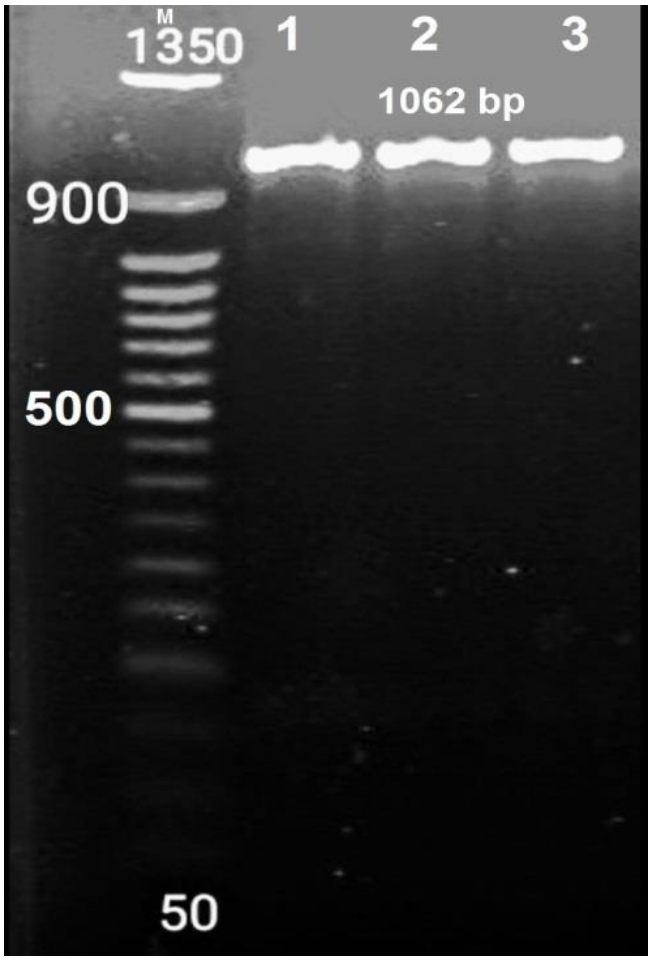

Figure 2: Agarose gel electrophoresis of RT-PCR after amplification of VP7 gene of *Rotavirus*. Children samples, Lane M: DNA Marker of 100 bp, Lane 1: Control positive sample, Lanes 2 and 3: Positive samples of Children with an amplified product at 1062 bp.

Table 2: Number and percentage of enter pathogens in investigated children, calves, and broiler chickens

Variable	Enteropathogen infection type	Children		Calves		Broiler Chickens	
		No.	%	No.	%	No.	%
Single infection	*Rotavirus* only	10	50	0	0	1	5
	E. coli only	1	5	0	0	5	25
	Salmonella only	0	0	0	0	2	10
	Cryptosporidium only	0	0	7	35	0	0
Double infection	*Rotavirus* + *E. coli*	0	0	1	5	0	0
	Rotavirus + *Cryptosporidium*	1	5	0	0	0	0
	E. coli + *Cryptosporidium*	1	5	0	0	2	10
	E. coli + *Salmonella*	0	0	0	0	3	15
	Salmonella + *Cryptosporidium*	0	0	0	0	1	5
Triple infection	*Rotavirus* + *E. coli* +*Cryptosporidium*	0	0	1	5	0	0
	E. coli + *Salmonella* + *Cryptosporidium*	0	0	0	0	1	5

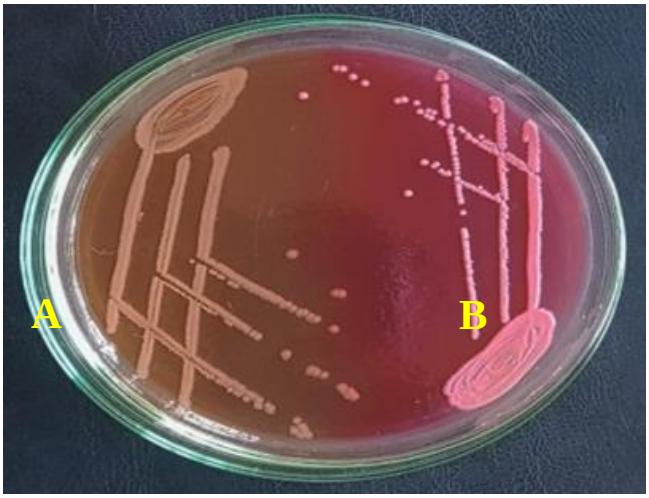

Figure 3: Colonies of isolated bacteria on MacConkey's agar. (A): Non-lactose fermenter (yellow colonies): *Salmonella* and (B): Lactose fermenter (pink colonies): *E. coli*.

Prevalence of different enteropathogens in relation to sex and age of investigated children

Concerning sex susceptibility, the results revealed no significant difference between male and female children to infection by *Rotavirus*, *E. coli*, and *Cryptosporidium*. However, mathematically male children 63.64% had a higher infection rate with *Rotavirus* than females 44.44%, but female children 11.11% had a higher infection rate with *E. coli* than males 9.09% (Table 3). According to the aging susceptibility of children to infection with *Rotavirus*, *E. coli*, and *Cryptosporidium*, there was no significant variation between different age groups and these enteropathogens. However, mathematically age group 6 months - 1 year of children 60% was more infected with *Rotavirus* than other age groups, while the age group> 1 year was more infected with *E. coli* 25% and *Cryptosporidium* 50% (Table 3).

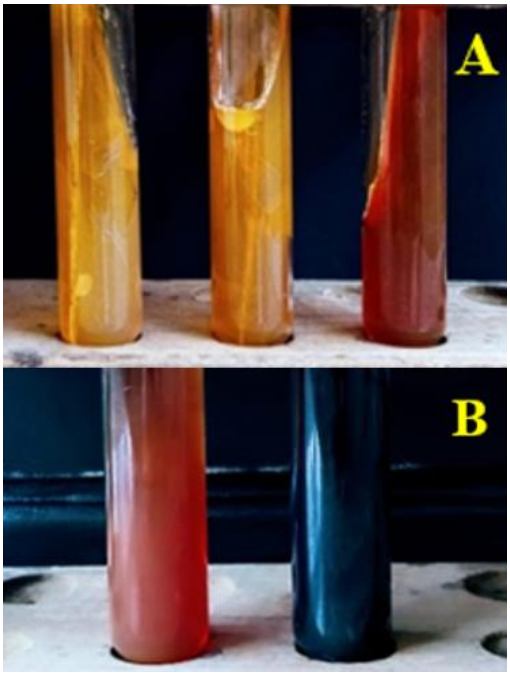

Figure 4: Inoculated isolated colonies on TSI agar. (A): *E. coli* and (B): *Salmonella*.

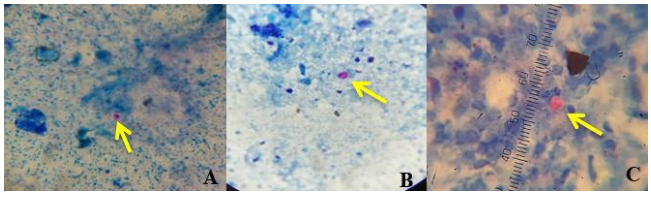

Figure 5: Stool, fecal, and pool intestine smears stained with modified Ziehl-Neelsen stain showing oocysts of *Cryptosporidium* spp. with 100X. (A): Children's stool sample, (B): Calf fecal sample, and (C): Broiler chicken pool intestine sample.

Table 3: Prevalence of enteropathogens according to gender/sex, age, and species susceptibility

Variable				*Rotavirus*		*E. coli*		*Salmonella*		*Cryptosporidium*	
			n	+ve(%)	p	+ve (%)	p	+ve (%)	p	+ve (%)	p
Children	Gender	Male	11	7(63.64)	0.39	1 (9.09)	0.88	0	-	2 (18.18)	-
		Female	9	4(44.44)		1 (11.11)		0		0	
	Age	<6m	6	3(50)	0.90	1 (16.67)	0.30	0	-	0	-
		6m-12m	10	6(60)		0		0		0	
		> 12m	4	2(50)		1 (25)		0		2 (50)	
Calves	Sex	Male	7	1(14.29)	0.63	1 (14.29)	0.63	0	-	4 (57.14)	0.25
		Female	13	1(7.69)		1 (7.69)		0		4 (30.77)	
	Age	4-45d	14	1(7.14)	0.51	1 (7.14)	0.51	0	-	5 (35.71)	0.55
		>45-3m	6	1(16.67)		1 (16.67)		0		3 (50)	
	Species	Cattle	14	2(14.28)	-	2 (14.28)	-	0	-	6 (42.86)	0.15
		Buffalo	6	0		0		0		2 (33.33)	
Broiler	Age	20-30d	9	1(11.11)	-	5 (55.56)	0.96	3 (33.33)	0.88	2 (22.22)	0.82
		>30-40d	11	0		6 (54.55)		4 (36.36)		2 (18.18)	

Prevalence of different enteropathogens in relation to the age of investigated broiler chickens

Regarding table 3, there was no significant variation between age groups of examined broiler chickens and different enteropathogens, although mathematically age group 20 - 30 days was more susceptible to infection with *Rotavirus* 11.11%, *E. coli* 55.56%, *Salmonella* 33.33% and *Cryptosporidium* 22.22% than age group > 30 - 40 days.

Prevalence of different enteropathogens in relation to sex, age, and species of examined calves

Regarding the sex susceptibility of investigated calves to different enteropathogens, our findings reported no significant difference between sex and *Rotavirus*, *E. coli*, and *Cryptosporidium*. However, mathematically, male calves were more infected with *Rotavirus* 14.29%, *E. coli* 14.29%, and *Cryptosporidium* 57.14% than female calves (Table 3). According to the aging susceptibility of examined calves to variant enteropathogens, statistically, there was no significant variation between different age groups and these enteropathogens. However, mathematically age group > 45 - 3 months had a higher infection rate with *Rotavirus* 16.67%, *E. coli* 16.67%, and *Cryptosporidium* 50% than the age group 4 - 45 days (Table 3). Concerning to species susceptibility of investigated calves, there was no significant difference between cattle and buffalo calves to these enteropathogens, although mathematically, cattle calves had higher infection rates with *Rotavirus* 14.28%, *E. coli* 14.28% and *Cryptosporidium* 42.86% than buffalo calves (Table 3).

Discussion

Diarrhea is a common syndrome caused by management, environmental, nutritional, and physiological differences and variants of enteropathogens such as *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* are described as critical infectious agents causing enteritis (either single or mixed) (18). Defining the map of children, calves, and broiler enteritis-causing enteropathogens allows for the application of effective preventative measures. In our study, the prevalence of *Rotavirus* infection by ICA was 55% (11/20) of examined children, and this finding was higher than recorded by Biswas *et al.* Simo-Fouda *et al.* (19,20), who elucidated that the prevalence of *Rotavirus* infection in investigated children was 51% and 7.7%, respectively. On the contrary, the highest rate of *Rotavirus* infection in investigated children was offered by Shafik *et al.* (21), that mentioned that *Rotavirus* infection was detected in 82% of examined children. In the current study prevalence of *Rotavirus* infection by ICA was 10% (2/20) of investigated calves, and this finding was higher than that obtained by Abdel-Rady *et al.* (22), who found that *Rotavirus* infection was observed in 8.54% of examined calves.

In contrast, the highest rate of *Rotavirus* infection in investigated calves was concluded by Abdulazeez and Abed, Youssef and Zaitoun (23,24), which revealed that the prevalence of *Rotavirus* infection in examined calves was 53.6 and14.92%, respectively. In the present study, the prevalence of *Rotavirus* infection by ICA was 5% (1/20) of investigated broiler chickens. This result was higher than that reported by Wani *et al.* (6), who mentioned that the prevalence of *Rotavirus* infection was 4% (3/75) of examined broiler chickens, but this finding was lower than the results observed by Karim *et al.* (25), Kattoor *et al.* (26), Pauly *et al.* (27) that found that the prevalence of *Rotavirus* infection was 13.81%, 15% and 36.1% in investigated chicken. In the present work, the prevalence of *E. coli* infection was 10% (2/20) in examined children, and this finding was lower than recorded by Vasco *et al.* (3), which reported that the prevalence of *E. coli* infection was 17.20% in investigated children. In the present result, the prevalence of *E. coli* infection was 10% (2/20) in examined calves,

which was lower than that obtained by Merera *et al.* (1), and Vasco*et al.* (3), whom recorded that the prevalence of *E. coli* infection was 28.60% and 50.30% in examined calves. In the current result, the prevalence of *E. coli* infection was 55% (11/20) of investigated broiler chickens, and this result was higher than shown by Vasco *et al.* (3) Zahid *et al.* (10) that obtained that prevalence of *E. coli* infection was 7.10% and 45% in examined chickens but lower than reported by Ezz El-Deen *et al.* (28) who isolated *E. coli* from chickens with the prevalence of 75%. In our data, the prevalence of *Salmonella* infection was detected only in broiler chickens by 35% (7/20), and this result was higher than that obtained by Khan *et al.* (29), who isolated *Salmonella* in 20.50% of examined chickens but lower than reported by Byomi *et al.* (11) that mentioned that prevalence of *Salmonella* in investigated chickens was 71.15%. In the current study, the prevalence of *Cryptosporidium* oocysts in investigated children and calves was 10% (2/20) and 40% (8/20), respectively. These findings were higher than that previously obtained by Abu Samra *et al.* (30), that concluded that the prevalence of *Cryptosporidium* oocysts in examined children and calves was 5.60% and 0.6%, respectively, but lower than previously recorded by Bessat *et al.* (12) showed that the prevalence of *Cryptosporidium* in children and calves was 16.60% and 43.20%, respectively. In our data, the prevalence of *Cryptosporidium* in examined broiler chickens was 20% (4/20), and this result was higher than reported by Bessat *et al.* (12), and Cao *et al.* (31), they noted that the prevalence of *Cryptosporidium* in examined chickens was 6% and 2.43%, respectively but lower than concluded by Jarad (32) who recorded that prevalence of *Cryptosporidium* in investigated chickens was 51.40%. Such variations in the rate of infection of *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* in examined children, calves, and broiler chickens may be attributed to the clinical phase of the disease is a suitable time for sampling collection to obtain the better result from a test, hygienic measures and environmental conditions.

In our study, two serologically *Rotavirus*-positive stools of children were molecularly positive by RT-PCR after amplification of the VP7 gene of *Rotavirus* group A while the serologically positive fecal samples of calves and pool intestine of broiler chickens were molecularly negative. This finding may be ascribed to three plausible reasons; a) non-specific inhibition of PCR reaction by the components of fecal and intestinal content samples, b) mismatches in primer binding sites or c) the sequence variation in *Rotavirus* genome due to the segmented nature of RNA genome, *Rotavirus* is continuously changing and leading to the emergence of new genotypes (7) or Rotaviruses that possess an antigenic A group is considered typical of mammals, while typical avian Rotaviruses are those designated as groups D, F, and G (33).

According to Single infection, *Rotavirus* infection was the most common in children, and this result was in agreement with Shrivastava *et al.* (34), and Msolo *et al.* (35), whom observed that *Rotavirus* infection was more prevalent enteropathogens in children. This finding may be attributed to *Rotavirus* remains one of the peak causes of childhood diarrhea which causes hospitalizations and mortalities in children every year, especially in impoverished countries, despite improvements in the development of vaccines for *Rotavirus* over the years (35). Regarding double mixed infection, *Rotavirus* with *Cryptosporidium* and *E. coli* with *Cryptosporidium* were recorded in children, and these findings were similar to those previously reported Shrivastava *et al.* (34), and Msolo *et al.* (35). These results may be explained by co-infection that causes an exacerbated diarrheal condition, and there was a synergistic effect between *Rotavirus* and *Cryptosporidium* beside *E. coli* and *Cryptosporidium* on diarrheal disease in addition to supports the above fact where virus- parasite and bacterial parasite co-infections are the aggravating factors of severe diarrhea in children (34,35). Concerning Single infection, *Cryptosporidium* infection was in calves, and this result was matched with Abou EL-Ella *et al.* (36), and Wei *et al.* (37). This finding may be attributed to several factors, such as the tremendous proliferative capacity of protozoa and resistance of oocysts, besides poor hygiene and sanitary conditions found on much ruminant exploitation, which lead to the presence of the parasite in regions that raise sheep and goats, which serve as asymptomatic carriers of the disease (36). In referring to double mixed infection, *Rotavirus* with *E. coli* was observed in calves, and this finding was in agreement with Uhde *et al.* (38), Içen *et al.* (39) and Smith (40). According to triple mixed infection, *Rotavirus*, *E. coli*, and *Cryptosporidium* were found in calves, which were similar Abou EL-Ella *et al.* (36), Wei *et al.* (37), Uhde *et al.* (38), and Içen *et al.* (39). These results may be due to *Rotavirus* damage small intestine that facilitates systemic invasion by normal intestinal flora, including *E. coli*, and colonization of the small intestine by *E. coli* has been associated with impaired glucose and lactose absorption, decreased serum glucose concentration and possibly increased susceptibility to *Cryptosporidium* infection (41). Regarding single infection, single infection with *E. coli* and *Salmonella* were prevalent in broiler chickens, and these findings were parallel with Shaltout *et al.* (42). According to double mixed infection, *E. coli* with *Salmonella* and *Salmonella* with *Cryptosporidium* were reported in broiler chickens. Concerning triple mixed infection, *E. coli*, *Salmonella* and *Cryptosporidium* were shown in broiler chickens, and these results were matched with Hauck (43). These findings may suggest a synergism between *Cryptosporidium* and the bacteria such as *E. coli* and *Salmonella* (43).

Focusing on the effect of gender, the results revealed no significant difference between male and female children to infection by *Rotavirus*, *E. coli*, and *Cryptosporidium*. However, mathematically male children had a higher infection rate with *Rotavirus* than females, and these findings

were similar to that of Biswas *et al.* (19), Shafik *et al.* (21), Ibrahim *et al.* (44), and Kalantari *et al.* (45). This finding may be attributed to the high possibility of males being infected with *Rotavirus* than females to the tendency of parents to take care of males than females concerning the treatment in hospitals, and the hypothesis that females are more resistant to infection than males due to hereditary factors represented in XX chromosomes in females (21). In our result, female children had a higher infection rate with *E. coli* than males, and this was in agreement with Gould *et al.* (46). This result matched a previous study by Chang and Tserenpuntsag (47), who recorded that females were more likely than male children to develop enteritis by *E. coli.* According to the aging susceptibility of children to infection with *Rotavirus*, *E. coli*, and *Cryptosporidium*, there was no significant variation between different age groups and these enteropathogens. However, mathematically, the age group 6 months - 1 year of children 60% was more infected with *Rotavirus* than other age groups, and this finding agreed with Ibrahim *et al.* (44), Ojobor (48), and Shrestha *et al.* (49). This result may be due to the high exposure of children in this age group to contaminated materials, and children in this group were known to consistently put their hands into their mouth beside passive immunity acquired by infants from their mothers protected from *Rotavirus* gastroenteritis during 0-6 months of age, these immunities disappear post 6 months of infant's age (21,44). In our study, the age group> 1 year of children was more infected with *E. coli* 25% and *Cryptosporidium* 50%, and these findings were parallel with Al-Alousi and Mahmood (13), and Ochoa *et al.* (50). These results may be attributed to the children at the weaning period being exposed to a contaminated environment, food, and water and, after six months of age, increased exposure to enteropathogens as potentially contaminated foods are introduced into the diet (13,50).

According to the sex susceptibility of investigated calves to different enteropathogens, the present study reported no significant difference between sex and *Rotavirus*, *E. coli*, and *Cryptosporidium*. However, mathematically male calves were more infected with *Rotavirus* 14.29%, *E. coli* 14.29%, and *Cryptosporidium* 57.14% than female calves. These results were similar to Merera *et al.* (1), Youssef and Zaitoun (24), and Mahmoud *et al.* (51). These findings may be attributed to male calves not being as needed in the production system, and producers mainly weaned the female calves for replacements (1). Regarding the aging susceptibility of examined calves to *Rotavirus*, *E. coli*, and *Cryptosporidium*, statistically, there was no significant variation between different age groups and these enteropathogens. However, mathematically age group > 45 - 3 months had a higher infection rate with *Rotavirus* 16.67%, *E. coli* 16.67%, and *Cryptosporidium* 50% than the age group 4 - 45 days. These findings agreed with Aminu *et al.* (52) finding that older calves had a higher prevalence rate with Rotavirus than younger ones, and also Awad *et al.* (53) recorded the presence of *E. coli* in enteric older calves till 12 weeks. In addition, Bessat *et al.* (12), and Wegayehu *et al.* (54), recorded those older calves had a higher infection rate with *Cryptosporidium* than younger calves. These results may be attributed to the fact that young calves can be protected from *Rotavirus* more than older calves through maternally derived antibodies. The possible explanation for *E. coli* in older diarrheic calves may be attributed to concurrent infection with other enteropathogens (52,53).

Concerning to species susceptibility of investigated calves, there was no significant difference between cattle and buffalo calves to different enteropathogens, although mathematically, cattle calves had higher infection with *Rotavirus* 14.28%, *E. coli* 14.28% and *Cryptosporidium* 42.86% than buffalo calves. These findings were previously recorded by Youssef and Zaitoun (24), Mahmoud *et al.* (51), and Awad *et al.* (53), in which the susceptibility of cattle calves to be infected with enteropathogens was higher than buffalo calves. This may relate to the difference in natural immunity of the two species, and buffalo calves had strong body immunity (24,54).

Regarding the aging susceptibility of broiler chickens, there was no significant variation between the age groups of investigated broiler chickens and different enteropathogens. However, mathematically age group 20 - 30 days was more susceptible to infection with *Rotavirus* 11.11%, *E. coli* 55.56%, *Salmonella* 33.33%, and *Cryptosporidium* 22.22% than the age group > 30 - 40 days. These findings were previously reported by Abd El-Mohsen *et al.* (8), Karim *et al.* (25), Matin *et al.* (55), and Guechtouli *et al.* (56), they concluded that younger chickens could be more infected with *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* than older chickens. The probable reasons for *Rotavirus* may be due to a meager amount of virus in samples or the absence of *Rotavirus* particles in samples of age group > 30 - 40 days (25). Our finding of *E. coli* may be attributed to colibacillosis, usually seen in young chicks up to three weeks of age, besides immunosuppression and viral infection acting as predisposing factors for this disease (57). Our result of *Salmonella* may be due to intestinal colonization of *Salmonella*, invasion of internal organs and persistence in colonized tissues are all higher in newly hatched chicks than in older birds due to their acquisition of protective microflora that either compete with *Salmonella* for intestinal receptors or produces negative factors (8). The explanation of our finding of *Cryptosporidium* may be attributed to the poor hygienic conditions at the hatchery as a large percentage of chicks were affected by *Cryptosporidium* in age groups 20 - 30 days. Furthermore, these may have favored the infestation through their debilitating action on the immune system in addition to lower infestation with *Cryptosporidium* at age group > 30 - 40 days due to the resistance of broiler chicken, which likely has antibody levels against *Cryptosporidium* sufficient to counteract the infection at this age (56).

Conclusion

According to the results of this study, *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium* play a role in the etiology agents of enteritis in children, calves, and broiler chickens. Identification of the possible causative agent of diarrhea is crucial because it allows knowing possible risk factors or sources of infection. It is worth applying to field diagnosis for *Rotavirus*, *E. coli*, *Salmonella*, and *Cryptosporidium*.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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عوامل الخطورة المؤثرة على مدى انتشار ممرضات الإسهال المعوية لدى الأطفال والعجول ودجاج التسمين بمحافظة أسيوط، مصر

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الخلاصة

يعد الإسهال متلازمة معقدة تسبب خسائر اقتصادية فادحة. الهدف من الدراسة الحالية هو تشخيص الممرضات المعوية لدى الأطفال والعجول ودجاج التسمين بالطرائق المصلية والجزيئية لفيروس الروتا، العصيات القولونية والسالمونيلا بالفحص الجرثومي بجانب التشخيص التقليدي للأبواغ الخبيئة ودراسة عوامل الخطورة لهم في محافظة أسيوط/مصر. تم جمع ٦٠ عينة من براز الأطفال (عدد = ٢٠)، براز العجول (عدد = ٢٠) بالإضافة إلى الكبد والأمعاء من دجاج التسمين (عدد = ٢٠) من شباط ٢٠٢١ ولغاية نيسان ٢٠٢١ تم فحصهم باختبار المقايسة المناعية، تفاعل البلمرة المتسلسل العكسي واختبارات الأحياء

المجهرية ومسحات صبغة زيل-نيلسن المحورة وجد أن معدل انتشار الإصابة بفيروس الروتا وباستخدام المقايسة المناعية ٥٥% (٢٠/١١)، ١٠% (٢٠/٢) و٥% (٢٠/١) من الأطفال المختبرة، العجول ودجاج التسمين، على التوالي. اظهرت عينتان فقط ١٨,١٨% من عينات براز الأطفال إيجابية لفيروس الروتا بوساطة تفاعل البلمرة المتسلسل العكسي بينما كانت عينات البراز والأمعاء الإيجابية لفيروس الروتا الخاصة بالعجول والدجاج على التوالي سلبية بوساطة تفاعل البلمرة المتسلسل العكسي. لم يظهر اختلاف معنوي بين عدوى فيروس الروتا العصيات القولونية والأبواغ الخبيئة مع الجنس والعمر للأطفال وأيضًا بين البكتريا المعوية مع الجنس والعمر وفصيلة العجول المختبرة بالإضافة إلى عدم وجود فرق معنوي بين فيروس الروتا، العصيات القولونية والسالمونيلا والأبواغ الخبيئة مع عمر دجاج التسمين المختبرة. ولخص أن البكتريا المعوية في الماشية والدجاج التي تعيش على مقربة من البشر تتطلب إجراءات مراقبة أفضل لحماية الحيوانات والبشر المعرضين للخطر.