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Investigation of viral and bacterial enteropathogens of diarrheic sheep and goats in Medina, Saudi Arabia

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Abstract. Diarrhea is a serious problem in sheep and goat farming, causing great economic losses. Most viral and bacterial enteropathogens of diarrheic sheep and goats are considered foodborne pathogens with a potential to be zoonotic. The present study investigated the prevalence of some viral and bacterial enteropathogens in diarrheic sheep and goats between October 2015and February 2016 in Medina, Saudi Arabia. A total of 310 fecal samples were collected from diarrheic sheep (n=193) and goats (n=117). The samples were screened for the presence of rotavirus and *bovine coronavirus* (BCoV) using Sandwich (ELISA) technique. The bacterial enteropathogens were isolated and identified biochemically and the virulence factors of *Escherichia coli* were determined by using PCR. *Escherichia coli* was the most prevalent agent in both sheep and goats (34.7% and 30.7%, respectively). According to the expressed virulence genes, Enterotoxigenic *Escherichia coli* (ETEC) was detect in 34.3% of sheep isolates and 30.6% of goats isolates. *Salmonella* species was isolated from 3.6% of sheep and 2.6% of goats. *Klebsiella* species was isolated from 1.6% of sheep but not from goats. Regarding viral agents, rotavirus was found in 31.6% of sheep and 27.4% of goats, while *BCoV* was detected in 19.6% of the sheep and 16.2% of the goats. The prevalence of bacterial and viral enteropathogens was significantly higher in the 0-12 months age group compared to the older age groups. Double infection was the most common (53.0%) infection pattern compared to single (37.5%) and triple (9.5%) infections. Rotavirus infection was significantly associated with ETEC infection. In conclusion, we report high prevalence of rotavirus and ETEC in sheep and goats, which are of veterinary and public health importance. This study provides valuable data on the prevalence of viral and bacterial enteropathogens in sheep and goats in Medina, Saudi Arabia that will be useful to develop control measures for these pathogens.

INTRODUCTION

Sheep and goats are considered to be among the most important economic and food sources of the Kingdom of Saudi Arabia (KSA), as its meat is the most preferable among the people in the kingdom. Among the livestock population in KSA, sheep accounted for7.4 million and goats for 4.2 million, while camel accounted for half a million and cattle for a quarter of a million (House, 2012). Diarrhea is a major problem in livestock worldwide, causing great economic losses due to deaths, poor growth rates, and veterinary costs (Weiss & Navas-Martin, 2005). Its etiology in complicated by the co-detection of multiple pathogens. The causative agents and the epidemiology of diarrhea have been widely studied worldwide, however, few studies have been carried out on farm animals in KSA. Enteropathogenic bacteria and viruses are

important causes of diarrhea in livestock worldwide (Adesiyun *et al.*, 2001). The most important enteropathogens associated with diarrhea in livestock include: rotavirus, *bovine coronavirus* (BCoV), enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* species and cryptosporidium either singly or in combination (Steiner *et al.*, 1997). Other pathogens may also have a role in enteric diseases including: *Clostridium perfringens*, Giardia, *Eimeria* species, *Campylobacter*, *Klebsiella* and *Proteus* (Muñoz *et al.*, 1996).

Rotaviruses are double-stranded RNA viruses, family *Reoviridae*. They are the most common causative agents of viral enteritis with a worldwide distribution in both human and animal populations (Luchs & Timenetsky, 2016). The availability of complete genome sequences of human and animal rotaviruses rotavirus provided evidence for interspecies transmission and adaptation (Martella *et al.*, 2010).

Bovine coronavirus (BCoV) is a major causative agent of diarrhea in cattle and small ruminants (Muñoz *et al.*, 1996). Coronaviruses are more commonly identified in neonatal food animals and to a lower extent in adults, while infected adult animals do not always show symptoms, clinically and subclinically infected animals display virus shedding and are a potential sources of infection (Ozmen *et al.*, 2006).

Escherichia coli is a Gram-negative, motile, facultative anaerobes, non-spore forming Enterobacteriaceae. Most *E. coli* strains are part of gastrointestinal tract flora, but some strains possess virulence factors that enable them to cause diarrhea in neonatal farm animals and humans (Nguyen *et al.*, 2005). Enterotoxigenic *Escherichia coli* (ETEC) stick to the intestinal microvilli and producing enterotoxins acting on enterocytes. The important virulence factors of enterotoxigenic *Escherichia coli* are enterotoxins and adhesins. The main adhesins of animal-origin ETEC are the fimbriae (pili): F4 (K88), F5 (K99), F6 (987P), F42, F41, F165, F18, and F17. ETEC produce either heat-stable (ST) or heat-labile (LT) (Gaastra & Svennerholm, 1996). Our previous study provided a molecular evidence of human infections with the same *E. coli* strains of cattle, sheep, goats and camel (Shabana *et al.*, 2013).

Salmonella is a member of family Enterobacteriaceae, which are Gram-negative rods, non-capsulated, non-sporulated and most of the species are motile by peritrichous flagella (Adesiyun *et al.*, 2001). *Salmonella* infection is primarily an enteric infection which has two forms, clinical and subclinical. An animal may have a latent infection whereby the pathogen remains dormant in the lymph nodes and may be shed in the feces (Acha & Szyfres, 2001). Salmonellosis is a foodborne and zoonotic disease, and of a public health problem in developing countries (Carvajal-Restrepo *et al.*, 2017). *Klebsiella* is a Gram-negative, non-motile, rod-shaped, capsulated Enterobacteriacae that is also associated with diarrhea in farm animals (Ryan & Ray, 2004; Herrera-Luna *et al.*, 2009).

There are not studies that investigated the incidence of viral and bacterial enteropathogens in sheep and goats in Medina. Therefore, our study is the first to determine their prevalence to permit the comparison of data with other epidemiological studies worldwide and to provide information for the governmental agencies to implement the necessary control measures to reduce the economic losses and zoonotic infections.

MATERIALS AND METHODS

Study design and sample collection

A prospective study was conducted between October 2015 and February 2016 to identify bacterial and viral enteropathogens among diarrheic sheep and goats from different farms in Medina, KSA. Most farms reared more sheep than goats under semi-extensive husbandry. Sheep are raised for their milk and/or meat, while goat are generally raised for their meat. A total of 310 fecal samples were collected from diarrheic small ruminants (193 sheep and 117 goats) for the detection of the bacterial and viral enteropathogens. A rectal swabs was collected from each animals and transported

on ice within few hours to the laboratory of biology department, Faculty of Science, Taibah University. Demographic data of the animals such as sex, age, and source were recorded. The age distribution was as follows: 0 to 12 months; 1 to 2 years; 2 to 3 years. The animals were classified into local or imported.

Isolation and identification of *E. coli*

E. coli isolates were enriched, isolated, and identified according to the standard methods (Sayah *et al.*, 2005). Swabs were placed in tryptic soy broth (TSB) and incubated at 35°C for 24 h. About10 µl of the turbid broth were streaked onto Eosin methylene blue (EMB) (Hardy Diagnostics agar, USA) and incubated for 18 to 20 h at 35°C, and the plates were examined for metallic sheen green. Separate colonies were then streaked onto MacConkey agar (MAC) (Difco, Sparks, Md.) and incubated at 35°C for 18 to 20 h, and the plates were examined for pink colonies that had a dark red center. A single colony was selected and streaked onto tryptic soy agar (TSA) (Hardy Diagnostics agar, USA) and incubated for 18 h. The plates were then examined for separate colonies that were round, mucoid, whitish, and slightly convex. For biochemical confirmation, a single colony was placed in trypticase soy broth (TSB)(Hardy Diagnostics agar, USA) and incubated at 37°C for 24 h, then confirmed biochemically by using API 20E (Biomerieux Vitek, Inc., Hazelwood, Mo.).

***E. coli* serotyping**

E. coli isolates were overnight grown on nutrient agar at 37°C. The cells were suspended in 0.9% sterile normal saline, then autoclaved at 121°C for 15 min. The suspension centrifuged and resuspended in sterile normal saline. O-serogrouping was carried out using a commercially available O-serogrouping kit (Denka Seiken, Tokyo, Japan) according to the manufacturer instructions. The flagellar antigen was determined using the standard Craigie tube technique by passage through semi-solid agar with the proper flagellar antisera (Davies & Wray, 1997).

Detection of virulence factors of *E. coli* by PCR

Bacterial DNA was extracted from overnight *E. coli* cultures at 37°C. The colonies were suspended in 200 µl of sterile distilled water, and boiled for 10 min, then directly put on ice and centrifuged in a refrigerated centrifuge at 15000 rpm for 5 minutes (Usein *et al.*, 2009). Virulence factors were detected by using conventional PCR. Primer sequences and PCR conditions are listed in Table 1. PCR was performed in a Bio-Rad thermal cycler (Bio-Rad, USA) and the PCR products were visualized on 1.5% agarose by gel electrophoresis.

Isolation and identification of *Salmonella* species

Salmonella species isolates were enriched, isolated, biochemically identified according to the Standard Operating Procedure (SOP, 2004). Fecal swabs were inoculated into Buffered Peptone Water (Oxoid) and incubated overnight at 37ºC. A 100ul aliquot was then streaked on modified semi-solid Rappaport Vassiliadis medium (Sigma) plates and incubated overnight at 42ºC. Presumptive *salmonella* colonies then plated on Xylose Lysine Desoxycholate agar (Merck)and incubated overnight at 37ºC. For biochemical confirmation, colonies showing typical *salmonella* appearance were then inoculated in TSI agar (Oxoid), Urea agar (Oxoid), l-Lysine decarboxylation medium (HiMedia). On TSI, typical *salmonella* reaction was black colored but with the appearance of bubbles. Red to purple color on urea agar and l-Lysine decarboxylation medium.

Isolation and identification of *Klebsiella* species

Fecal samples were cultivated on MacConkey agar (Difco, Sparks, Md.) plates and incubated at 37°Cfor 24 h. Typical mucoid, lactose fermenting colonies were picked and tested by Enterotube II test (Biomerieux Vitek, Inc.) according to the instructions of the manufacturers.

Table 1. PCR primers and cycling conditions for amplification of virulence genes in enterotoxigenic *Escherichia coli* (ETEC) strains

Target gene	Primer designation	Primer sequence (5'-3')	PCR conditions*	PCR product length (bp)	Reference
(*LT*)Heat-labile enterotoxin	*elt1* *elt2*	ATTTACGGCGTTACTATCCTC TTTTGGTCTCGGTCAGATATG	95°C,30s; 58°C,30s; 72°C,30s	281	Vu-Khac *et al.* (2007)
(*St*)Heat-stable enterotoxin	*Sta5* *Sta3*	ATTTTTCTTTCTGTATTGTCTT CACCCGGTACAAGCAGGATT	95°C,30s; 52°C,30s; 72°C,30s	244	Al-Gallas *et al.* (2007)
F41 fimbrial adhesion	*F41-F* *F41-R*	GAGGGACTTTCATCTTTTAG AGTCCATTCCATTTATAGGC	95°C,30s; 52°C,30s; 72°C,30s	431	Vu-Khac *et al.* (2007)
F4 (*K88*) fimbrial adhesion	*F4-F* *F4-R*	GCTGCATCTGCTGCATCTGGTATG CCACTGAGTGCTGGTAGTTACAGCC	95°C,30s; 62°C,30s; 72°C,30s	792	Vu-Khac *et al.* (2007)
F18 fimbrial adhesion	*F18-F* *F18-R*	GTGAAAAGACTAGTGTTTATTTC CTTGTAAGTAACCGCGTAAGC	95°C,30s; 52°C,30s; 72°C,30s	510	Vu-Khac *et al.* (2007)

* 35 cycles of amplification and a final extension step of 72°C for 5 min were performed for all of the PCR protocols described.

Viral examination using Enzyme-Linked Immunosorbent Assay (ELISA)

Feces were examined for rotavirus and bovine coronaviruses using a Sandwish ELISA kit (Bio-X Diagnostics – Belgium) according to the manufacturer's instructions. Briefly, microtiter plates were coated with antirotavirus and antibovine coronavirus IgG as capturing antibodies. 100 µl aliquot of the diluted fecal samples was added to the wells and incubated at 21°C for 1 h. After washing, 100 µl of the conjugate was added to each well and the plate was incubated at 21°C for 1 h. Next, 100 µl of the chromogen were added to each well and the plate was incubated at 21°C for 1 h. Finally, 50 µl of stop solution were added to stop the reaction. The net optical density of each was analyzed and calculated with an automated ELISA reader at 450 nm (SIRIOS Elisa Reader, Indonesia).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc. Chicago, USA). Pearson correlation coefficient was used to determine the association between the prevalence of the tested enteropathogens and age, sex and/or source of the examined animals. In addition, it was used to assess the association between bacterial and viral infections. A p-value of 0.05 or less was considered as statistically significant.

RESULTS

The demographic characteristics of the animals

Sheep and goats with diarrhea were examined for the presence of selected bacterial and viral enteropathogens. The demographic characteristics of the examined animals are summarized in Table 2. Out of 193 sheep, 73 (37.8%) were females and 120 (62.2%) were males, while out of 117 goats, 43 (36.8%) were females and 74 (63.2%) were males. Animals were categorized into three age groups: Group I, includes animals from birth till 12 months age; Group II, includes animals from 1 to 2 years; and Group III, includes animals from 2 to 3 years. Group I

Table 2. Basic demographic data of sheep and goats

	Characteristics	Sheep (n=193)	Goats (n=117)
Sex	Female	73 (37.8%)	43 (36.8%)
	Male	120 (62.2%)	74 (63.2%)
Age	Group I (0-12 months)	98 (50.8%)	69 (58.9%)
	Group II (1-2 years)	43 (22.3%)	21 (17.9%)
	Group III (2-3 years)	52 (26.9%)	27 (23.1%)
Source	Imported	69 (35.8%)	49 (41.9%)
	Local	124 (64.2%)	68 (58.1%)

Table 3. The prevalence of viral enteropathogens among sheep and goats

Host	Demographic factor		Rotavirus	Coronavirus
Sheep (193)	Sex	Male (n=120)	(22.3%) 43	(10.9%) 21
		Female (n=73)	(9.3%) 18	(8.8%) 17
	Age**	Group I (n=98)	(19.7%) 38	(9.8%) 19
		Group II (n=43)	(5.2%) 10	(4.9%) 9
		Group III (n=52)	(6.7%) 13	(5.2%) 10
	Source	Imported (n=69)	(23.8%) 46	(10.9%) 21
		Local (n=124)	(7.8%) 15	(8.8%) 17
	Total		31.6%	19.6%
Goat (117)	Sex	Male (n=74)	(19.7%) 23	(9.4%) 11
		Female (n=43)	(7.7%) 9	(6.8%) 8
	Age**	Group I (n=69)	(14.5%) 17	(6.8%) 8
		Group II (n=21)	(5.9%) 7	(4.3%) 5
		Group III (n=27)	(6.8%) 8	(5.1%) 6
	Source	Imported (n=49)	(17.9%) 21	(10.3%) 12
		Local (n=68)	(9.4%) 11	(5.9%) 7
	Total		27.4%	16.2%

** a highly significant relation between the prevalence of enteropathogens and the young animals.

accounted for 50.8% (98/193) of the sheep and 58.9% (69/117) of the goats. Group II included 22.3% (43/193) of the sheep and 17.9% (21/117) of the goats, while group III included 26.9% (52/193) of the sheep and 23.1% (27/117) of the goats. Local animals accounted for 64.2% and 58.1% of the sheep and goats, respectively, and the rest were imported.

Prevalence of rotavirus and coronavirus among sheep and goats

The prevalence of rotavirus and coronavirus in sheep and goats using ELISA is presented in Table 3. Overall, rotavirus was more prevalent than coronavirus among the surveyed animals, at a rate of (30% and 18.4%, respectively). Regarding the animal species, rotavirus and coronavirus were detected at higher, but not statistically significant frequencies in sheep (31.6% and 19.7%, respectively) than in goats (27.4% and 16.2%, respectively). Rotavirus infections were more frequent in male sheep and goats (22.3% and 19.7%, respectively) than in females (9.3% and 7.7%, respectively). Coronavirus was also more frequent in male sheep and goats (10.9% and 9.4%, respectively) than in females (8.8% and 6.8%, respectively). Rotavirus and coronavirus infections were more common among imported sheep and goats (23.8% and 17.9%, respectively) than

local ones (7.8% and 9.4%, respectively). Significant differences in the prevalence of rotavirus and coronavirus were found among the different age groups. The prevalence of rotavirus and coronavirus in age group I (0-12 months) of sheep (19.7% and 9.8%, respectively) were significantly higher than their prevalence in the older age groups II and III (p-values<0.05). Similarly in goats, the prevalence of rotavirus and coronavirus were also significantly higher in the age group I (14.5% and 6.8%, respectively) compared to the older age groups.

Prevalence of bacterial enteropathogens in sheep and goats

The prevalence of *E. coli*, *Salmonella* species and *Klebsiella* species in diarrheic sheep and goats are presented in Table 4. Out of 193 diarrheic sheep sampled, 34.7% had *E. coli*, 3.6% had *Salmonella* species, and 1.6% had *Klebsiella* species. Among the 117 diarrheic sheep 30.8% yielded *E. coli*, 2.6% had *Salmonella* species, and none had *Klebsiella* species. The frequencies of bacterial enteropathogens were higher, albeit not statistically significant, in sheep than in goats and in males than in females. Significantly higher detection rates of *E. coli*, *Salmonella* species and *Klebsiella* species were observed among age group I (0-12 months) animals compared to the older age groups (p-values <0.05). Bacterial enteropathogens were more frequently detected in imported animals compared to local animals. Of the193 diarrheic sheep, 23.8% and 10.9% bacterial enteropathogens were recovered from imported and local animals, respectively. In goats, the detection rates were 20.5% in imported and 10.3% in local animals.

Distribution of virulence genes among *E. coli* serotypes

The occurrence and profiles of virulence genes among the different *E. coli* serotypes are summarized in Table 5. Eight *E. coli* serotypes were detected among sheep with O74: H6 serotype being the most common, and 6 serotypes were detected among goats with O25:H40 serotypes being most prevalent. *E. coli* strains showed different virulence profiles with Heat-labile enterotoxin (*LT*) and Heat-stable enterotoxin (*ST*) being the most common among sheep (n=7; 10.4%) and goats (n=4; 11.1%). In sheep, fimbrial genes, *F41*, *F18* and *F4* (*K88*) were expressed by serotypes O165:H21, O126:H28 and O142:H2,

Table 4. Prevalence of bacterial enteropathogens among sheep and goats

Host	Demographic factor		(ETEC)	*Salmonella* spp.	*Klebsiella* spp.
Sheep (193)	Sex	Male (n=120)	(22.3%) 43	(2.3%) 5	(1.1%) 2
		Female (n=73)	(12.4%) 24	(1.1%) 2	(0.5%) 1
	Age**	Group I (n= 98)	(20.2%) 39	(2.1%) 4	(1.1%) 2
		Group II (n=43)	(4.7%) 9	(0.5%) 1	(0.0%) 0
		Group III (n=52)	(9.8%) 19	(1.1%) 2	(0.5%) 1
	Source	Imported (n=69)	(23.8%) 46	(2.1%) 4	(1.1%) 2
		Local (n=124)	(10.9%) 21	(1.6%) 3	(0.5%) 1
	Total		34.7%	3.6%	1.6%
Goat (117)	Sex	Male (n=74)	(20.5%) 24	(2.6%) 3	(0.0%) 0
		Female (n=43)	(10.3%) 12	(0.0%) 0	(0.0%) 0
	Age**	Group I (n=69)	(17.9%) 21	(1.7%) 2	(0.0%) 0
		Group II (n=21)	(5.1%) 6	(0.0%) 0	(0.0%) 0
		Group III (n=27)	(7.7%) 9	(0.9%) 1	(0.0%) 0
	Source	Imported (n=49)	(20.5%) 24	(2.6%) 3	(0.0%) 0
		Local (n=68)	(10.3%) 12	(0.0%) 0	(0.0%) 0
	Total		30.8%	2.6%	0.0%

** a highly significant relation between the prevalence of enteropathogens and the young animals.

Table 5. The distribution of virulence genes among the identified *E. coli* serotypes

Animal	Virulence profile	Serotypes	No. of strains (%)
Sheep (n=67)	*ST, LT*	O74:H6 (n=4), O25:H40 (n=3)	7 (10.4%)
	LT	O8:H4 (n=2), O142:HUT(n=1)	3 (4.55)
	F41	O165:H21	4 (5.9%)
	F18	O126:H28	4 (5.9%)
	ST	O128:H21	1 (1.55)
	F4 (*K88*)	O142:H2	4 (5.9%)
Total			23 (34.3%)
Goat (n=36)	*ST, LT*	O25:H40 (n=3), OUT:H2 (n=1)	4 (11.1%)
	LT	O128:H21	1 (2.8%)
	F4 (*K88*)	O8:H2	3 (8.3%)
	F41	O42:H2	2 (5.6%)
	F41, ST	OUT:HUT	1 (2.8%)
Total			11 (30.6%)

LT, Heat-labile enterotoxin; *St*, Heat-stable enterotoxin; *F41*, fimbrial adhesion; *F4* (*K88*), fimbrial adhesion; *F18*, fimbrial adhesion.

respectively. While in goats, *F4* and *F41* were possessed byO8:H2 and O42:H2 serotypes respectively.

Infection patterns of bacterial and /or viral infections

Mixed infections with viral and/or bacterial enteropathogens were detected in 53% of the infected animals as shown in Figure 1. The double infection pattern was the most common (53%), followed by single and triple infections (37% and 9.5%, respectively). The double infection pattern presented as a combination between two enteropathogens such as rotavirus and *E. coli*, coronavirus and *E. coli*, *Salmonella* species and *E. coli*, and *Salmonella* species and rotavirus. Triple infection pattern included combination between rotavirus, *E. coli* and *Salmonella* species Table 6. Rotavirus and ETEC mixed infections were the most common double infections with a highly significant correlation (*P* value <0.001) Table 7.

DISCUSSION

The present study was designed to determine the prevalence of viral and bacterial enteropathogens in diarrheic sheep and goats in Medina, Saudi Arabia. The prevalence of rotavirus was 31.6% and 27.4% in sheep and goats, respectively. *Bovine coronavirus* had lower frequency than rotavirus in sheep (19.6%) and goats (16.2%). *E. coli* was the most prevalent agent in both sheep and goats (34.7% and 30.7%). *Salmonella* species was detected in 3.6% of sheep and 2.6% of goats, while *Klebsiella* species was detected in 1.6% of sheep but not in goats. A study in the North Province of Saudi Arabia reported the detection of the same pathogens in diarrheic calf camel (Al-Ruwaili *et al.*, 2012). Rotavirus, enterotoxogenic *E. coli* and *Salmonella* spp. were detected in 14.7%,

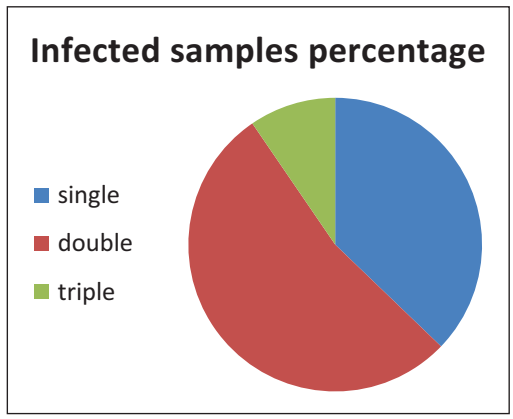

Figure 1. The infection pattern of bacterial and/or viral infections.

Table 6. Enteropathogens combinations

Infection pattern	Pattern percentage	Double* combination	Enteropathogens combinations
Single	37.5%		
Double	53%	77%	Rotavirus, *E. coli***
		9%	Coronavirus, *E. coli*
		7%	*E. coli*, Salmonella
		7%	Rotavirus, Salmonella
Triple	9.5%		Rotavirus, *E. coli*, Salmonella

* Percentage of the double pattern.
** A highly significant corelation between Rotavirus and *E. coli* infections.

Table 7. The association between Rotavirus and *Escherichia coli*

E. coli		Rotavirus		Total
		negative	positive	
Negative	goat	81		81
	sheep	126		126
	Total	207		207
Positive	goat	12	24	36**
	sheep	22	45	67**
	Total	34	69	103

** a highly significant association between Rotavirus and *E. coli* infection in double pattern infected animals.

58.2% and 12%, respectively, of diarrheic calf camels. Interestingly, rotavirus, *E. coli* and *salmonella* species were also reported to be associated with diarrhea among children in Jeddah, Saudi Arabia (el-Sheikh & el-Assouli, 2001). The high prevalence of these pathogens in food animals suggest the risk for potential zoonotic infections. Further genetic studies are required to compare the strains detected in human and animals and to determine the zoonotic potential of these pathogens.

Several reports have shown that rotavirus is a major cause of gastroenteritis in animals (Do *et al.*, 2015; Louge Uriarte *et al.*, 2014; Matthijnssens *et al.*, 2009). In the present study, rotavirus was the most prevalent viral enteroopathogen among the surveyed diarrheic sheep (31.6%) and goats (27.4%), especially among lambs and goat kids aged 0-12 months. These findings are in agreement with a previous study in Trinidad by Adesiyun *et al.* (2001). Although limited reports on the prevalence of rotavirus among animals in the kingdom, so many reports on its prevalence among diarrheic children are available (Tayeb, 2011; Khalil *et al.*, 2015; Kheyami *et al.*, 2008). In agreement with previous reports on the detection of *BCoV* among young diarrheic lambs and goat (Naylor, 1990; Eisa & Mohamed, 2004; Ozmen *et al.*, 2006), the present study showed that *BCoV* is an important cause of diarrhea in sheep and goats at a prevalence of 19.6% and 16.2%, respectively.

E. coli was the most frequently detected enteropathogen among the investigated sheep (34.7%) and goats (30.7%), which was in accordance with previous reports (Osman *et al.*, 2013; Adesiyun *et al.*, 2001). In absence of any epidemiological studies on the prevalence of *E. coli* among animals in the kingdom, only single study was carried out on children Jeddah (el-Sheikh & el-Assouli, 2001). A variety of serotypes was recovered from the diarrheic animals as show in Table 5, similar serotypes were recorded by several reports (Johura *et al.*, 2017; Osman *et al.*, 2013; Shabana *et al.*, 2013). 34.3% of sheep and 30.6% of goats isolates were categorized as Enterotoxigenic *E. coli* (ETEC) according to the expression of virulence genes. ETEC with at least one virulence factor was isolated from the examined animals. Fimbria (F41, F18, F4) were the most frequent virulence factor

detected among the isolated *E. coli* from sheep (17.9%) and goats (16.7%). Their prevalence is similar to that described by other reports on the prevalence of F5 and F41 (Muñoz *et al.*, 1996) and F17 (Cid *et al.*, 1993) in diarrheic sheep and goats. In addition, the present study reported a high prevalence of F5 and F18 among the diarrheic animals. There are limited studies on the production of heat-stable enterotoxins by caprine and ovine *E. coli* strains. The prevalence of heat-stable and heat-labile enterotoxins expression among the examined strains was low, which was in accordance with Cid *et al.* (1991). None of the fimbriated strains elaborated enterotoxins, except in a single strain isolated from the goats, and these findings agreed with Muñoz *et al.* (1996).

The overall prevalence of *Salmonella* was low in both sheep (3.6%) and goats (2.6%). Similar findings have been reported by Adesiyun *et al.* (2001). *Salmonella* species seem to have little role in diarrhea in goats and sheep (Muñoz *et al.*, 1996). *Klebsiella* species was reported only among diarrheic sheep (1.6%), its prevalence was agreed with what was reported by Herrera-Luna *et al.* (2009) among diarrheic calves. *Klebsiella* species seems to have minor role as a cause of diarrhea among small ruminants, as no reports on its prevalence in small ruminants.

Regardless of the type of enteropathogens and animal species, age seemed to be a major factor affecting the occurrence of diarrhea. The frequency of diarrhea and the prevalence of enteropathogens was higher among the young than in older animals. This results in morbidity and mortality burdens being highest among the young animals (Adesiyun *et al.*, 2001).

The present study reported a significant synergistic infection between rotavirus and *E. coli*. Mixed viral-bacterial infections are common in gastroenteritis and this combination increases the disease severity (Marshall, 2002). Viral infection may facilitate bacterial adhesion and invasion, consequently increase its pathogenicity (Albert *et al.*, 1999). The exact interactions and the possible synergistic mechanisms between bacteria and viruses in the gastrointestinal tract infection are still poorly understood. Asynergistic effect between rotavirus and ETEC in infected animals may occur, causing higher mortality than those infected with either rotavirus or ETEC alone (Oyofo *et al.*, 1999). Several studies tried to illustrate such synergism, briefly, it may occur due to breakdown in the normal balance between intestinal secretion and absorption, ETEC enterotoxin A increase the intestinal secretion and this could not be neutralized because of the villus damage by rotavirus (Newsome and Coney, 1985). Another hypothesis stated that, the more severe effect of the combined pathogens was due the independent action of the two agents rather than the interaction between them (Benfield *et al.*, 1988).

Foodborne illnesses are responsible for high morbidity and mortality in developing and industrialized countries (Amézquita-Montes *et al.*, 2015). Bacterial pathogens including *Salmonella* and *E. coli* are considered as the main causes of foodborne illnesses in Saudi Arabia (Al Mazrou, 2004). *E. coli* frequently contaminate undercooked ground beef and unpasteurized (raw) milk (Gould *et al.*, 2011). Detection of *E. coli* in meat and milk products indicates fecal contamination that may have taken place during production, storage, packaging, or transport (Polañski *et al.*, 2016).

Conclusion and Recommendation

We report a high prevalence of viral and bacterial enteropathogens among diarrheic sheep and goats. Therefore, there is need to strengthen the control strategies to minimize the burden of these pathogens and their risk to cause zoonotic infections and foodborne illness. Medina hosts pilgrims from all around the world during the Umrah and Hajj, and the presence of foodborne pathogens in the food supply represents a threat for the potential spread of these pathogens not only to the local community but also to visitor, who can carry them back to their countries.

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