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Risk factors of diarrhea in small ruminants in Kuwait

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Abstract

Background: Diarrhea is the most severe disease affecting small ruminant farms, even though there is continuous progress in the control and prevention strategies. **Aims:** This study aimed to identify some of the risk factors associated with diarrhea in small ruminants in Kuwait. **Methods:** Faecal samples from 556 animals (334 sheep and 222 goats) were collected and examined for different enteropathogens. The collected data from animals and herd management were finally analysed. **Results:** Statistical analysis identified that age (pre-weaned; P=0.022, OR=2.38), herd size (large; P=0.037, OR=1.7), and season (dry; P=0.022, OR=2.39) were as non-infectious risk factors of the diarrhea occurrence. It was found that *Escherichia coli* K99 (P=0.000, OR=7.7), *Cryptosporidium* spp. (P=0.000, OR=586), and *Eimeria* spp. (P=0.000, OR=2.97) were reported more frequently in diarrheic animals than non-diarrheic ones. **Conclusion:** Pre-weaned small ruminants reared in the large herd were more susceptible to diarrhea. *Escherichia coli* K99, *Cryptosporidium* spp., and *Eimeria* spp. were the most predominant enteropathogens causing diarrhea in small ruminants in Kuwait. Diarrhea was more common in dry season than in wet season.

Key words: *Cryptosporidium*, Diarrhea, *E. coli*, Goats, Sheep

Introduction

Sheep and goats constitute the majority of livestock in Kuwait, as they are adapted to geographic and topographic conditions prevailing in this country. FAOSTAT has estimated sheep and goat populations in Kuwait with 684,231 sheep and 209,686 goats in 2018 (FAO, 2018). Diarrhea is a multi-factorial severe malady in small ruminants. It causes severe financial losses, especially in neonatal animals resulting in weight losses and deaths. Diarrhea accounted for 46% of lamb mortalities in the USA (Schoenian, 2007) and 51.6% of lambs and kids' neonatal fatalities in Jordan (Aldomy and Abu Zeid, 2007).

The etiology of diarrhea in sheep and goats includes non-infectious and infectious causes. The non-infectious causes are the animal, environmental and nutritional factors (Skirnisson and Hansson, 2006; Schoenian, 2007). The infectious agents frequently identified in diarrheic small ruminants include *Escherichia coli* K99 (Shabana *et al*., 2017), rotavirus A (RVA) (Singh *et al*., 2017), bovine coronavirus (BCoV) (Amer, 2018), *Cryptosporidium* spp. (Majeed *et al*., 2018), and *Eimeria*

spp. (Keeton and Navarre, 2018).

To the best of our knowledge, this is the first study to investigate some risk factors of diarrhea in small ruminants in Kuwait.

Materials and Methods

Study design

A cross-sectional study was conducted from October 2014 to September 2015 to identify the risk factors of diarrhea in small ruminants. The farms were visited once to collect faecal samples and data on herd management. There were two major housing systems in the period of the study: the closed system in which the animals were reared indoors in partially roofed yards (sheds), and the open system in which the animals were kept in fenced yards in desert with small barns for shelter. Forty-seven farms were visited in different regions. The total number of the farm animals was 9,057, and the herd size ranged from 7 to 1,210 heads (median 100; mean 198). Herds containing more than 100 animals were considered as large size herds. The Ethics Committee of Kuwait Foundation for the Advancement

of Sciences (KFAS-Award No. 2012-1207-04) approved this field study. Permission was obtained from farms' owners before sample collection.

Sampling

The sample size was calculated using EpiInfo™ sample size calculator depending on the total number of animals and the prevalence of diarrhea in each farm. Systematic random sampling was used to select animals for the study. A total of 556 animals (334 sheep and 222 goats) with different ages and sexes were selected for the collection of their faecal samples. In a sterile screw-capped bottle, 5-10 g of the faeces were collected directly from the rectum or immediately after defecation, then labeled with the animal's information. The samples were transferred to the laboratory at the earliest in an icebox and were refrigerated at 4°C to be processed within two days.

Laboratory diagnosis

The samples were divided into two portions.

Rapid immunochromatography (IC) test BoviD-4

The first portion of the faecal samples was used for the detection of *Cryptosporidium* spp., RVA, BCoV, and *E. coli* K99 antigens by rapid IC kit (BoviD-4, BioNote, Gyeonggi-do, Republic of Korea). The rapid test was used as described by the manufacturer. The BoviD-4 was used for diagnosis of diarrhea in small ruminants as a rapid pen-side test for *Cryptosporidium* spp., *E. coli* K99, and RVA in the field with a good performance, but care must be taken while interpreting BCoV positive results of the kit (Majeed, 2016).

Floatation technique for detection of Eimeria spp.

The second portion of the faecal samples was examined for detection of *Eimeria* spp. oocysts. It was performed as described by Soulsby (1982).

Statistical analysis

To identify different risk factors of diarrhea in small ruminants, a univariate analysis (Chi-square test, χ^2) and a multivariate stepwise logistic regression was applied. The P-values and odds ratios (OR) were calculated using Statistix 10© Analytical Software.

Results

Single and mixed infections of different enteropathogens detected in this study showed that most of the cases were single infections (Table 1). *Cryptosporidium* spp., *E. coli* K99, RVA, BCoV, and *Eimeria* spp. were diagnosed in 7.7%, 4.1%, 1.3%, 0.4%, and 15.8% of samples, respectively. Mixed infections with two, three, and four enteropathogens are reported in Table 1.

Pre-weaned lambs and kids were more susceptible to have diarrhea than post-weaned animals (45.2% vs. 25.6%; P=0.022; OR=2.38). Diarrhea in sheep and goats was higher during the dry season compared to the wet

season, and also was higher in those reared in large herds than in small ones in Kuwait (P=0.022, OR=2.39; P=0.037, OR=1.7, respectively; Table 2).

Table 1: Prevalence of single and mixed infections in small ruminants (n=556)

Enteropathogens	Positive	(%)
Cryptosporidium	43	7.7
E. coli K99	23	4.1
RVA	7	1.3
BCoV	2	0.4
Eimeria	88	15.8
E. coli K99 + BCoV	2	0.4
Cryptosporidium + *E. coli* K99	1	0.2
E. coli K99 + *Eimeria*	2	0.4
E. coli K99 + *Eimeria* + RVA	1	0.2
Cryptosporidium + BCoV + *E. coli* K99	2	0.4
Cryptosporidium + RVA + BCoV + *E. coli* K99	1	0.2

RVA: Rotavirus A, and BCoV: Bovine coronavirus

The frequency of diarrhea was higher in female animals (47.2%) than males (30.2%). Statistical analysis of this result using univariate analysis (χ^2) showed a significant relationship (P=0.000), although the stepwise logistic regression revealed that the result was not statistically significant. Animal species (sheep vs. goats) and housing systems (closed vs. open) were not significantly different with diarrhea causes.

Statistical analysis identified *E. coli* K99, *Cryptosporidium* spp., and *Eimeria* spp. as risk factors for diarrhea in sheep and goats in Kuwait (P=0.000, OR=7.70; P=0.000, OR=5.86; P=0.000, OR=2.97, respectively; Table 2). There was no statistically significant association between the presence of RVA and BCoV in faeces and diarrhea in this study, even though they were detected in the faeces of diarrheic animals (22.2% and 71.4%, respectively).

Discussion

Enteric pathogen infections are of interest to farmers, veterinarians, and public health officers. Such agents are considerable for producers' economic loss due to decreased production efficiency, high morbidity and mortality rates, and therapy-related costs. Additionally, diarrheic animals can harbor these pathogens and serve as sources of infection to healthy animals and humans (Holland, 1990).

In the present study, age, season, sex, and herd size were identified as risk factors for the occurrence of diarrhea in small ruminant farms. Pre-weaned lambs and kids less than three months of age were the most vulnerable age group, showing signs of diarrhea 2.38 times more (45.2%; 112/248; OR=2.38) than post-weaned animals (25.6%; 79/308). Previous studies observed a high frequency of the diarrhea occurrence in lambs and kids due to infectious and non-infectious causes (Schoenian, 2007; Shabana *et al.*, 2017). *Cryptosporidium* spp., *E. coli* K99, and *Eimeria* spp. were the most common diarrhea-causing pathogens in this age group in this study as well as in studies of Shabana *et al.* (2017) in Saudi Arabia and Majeed *et al.* (2018) in

Table 2: Risk factors associated with diarrhea in small ruminants using univariate (χ^2) and multivariate analysis (stepwise logistic regression)

Risk factors		No. of samples	Diarrhea cases (n=191)		P-value (univariate analysis)	P-value (multivariate analysis)	Odds ratio (95% CI)[b]
			No.	%			
Age group	Pre-weaned	248	112	45.2	0.000	0.022	2.38 (1.66-3.41)
	Post-weaned	308	79	25.6			
Sex[a]	Male	367	111	30.2	0.000	–[c]	–
	Female	163	77	47.2			
Species	Sheep	334	117	35.0	0.680	–	–
	Goat	222	74	33.3			
Season	Wet	295	68	23.0	0.000	0.022	2.39 (1.50-3.75)
	Dry	261	123	47.1			
Herd size	Small	176	46	26.1	0.006	0.037	1.7 (1.17-2.58)
	Large	380	145	38.1			
Housing system	Open	234	78	35.1	0.666	–	–
	Closed	322	113	33.3			
Cryptosporidium[d]	Positive	47	34	72.3	0.000	0.000	5.86 (3.01-11.41)
	Negative	509	157	31.0			
RVA[d]	Positive	9	2	22.2	0.440	–	–
	Negative	547	189	34.5			
BCoV[d]	Positive	7	5	71.4	0.038	–	–
	Negative	549	186	34.0			
E. coli K99[d]	Positive	32	25	78.1	0.000	0.000	7.70 (3.2-18.16)[d]
	Negative	524	166	31.6			
Eimeria spp.[e]	Positive	91	47	51.6	0.000	0.000	2.97 (2.06-4.28)
	Negative	465	144	31.0			

[a] Data not available from 26 animals, [b] 95% confidence interval of the estimated odds ratio, [c] No significant association was observed, [d] Detected by BoviD-4 kit, and [e] Detected by direct flotation technique. RVA: Rotavirus A, and BCoV: Bovine coronavirus

Kuwait. Improper management (e.g. inadequate milk production by the dam, failure to suck or lack of supplementary feeding, and non-specific management-related diseases), cannot be excluded as non-infectious risk factors of diarrhea in this age group (Shabana *et al*., 2017).

A higher frequency of diarrhea was observed in large herds in comparison to the small ones. Large herds with overcrowding of animals concentrate the host and diarrhea-causing pathogens within a restricted area and therefore increases the risk of infection (Kusiluka and Kambarage, 1996); also, animals in large herds need more efforts for caring. The univariate analysis (χ^2) showed that there is a relationship between sex and diarrhea, but this was not significant using stepwise logistic regression, as a large number of the examined females were young. The effect of sex, therefore, could be resulted from the confounding influence of age and was not indeed a risk factor of diarrhea. Unexpectedly, diarrhea was more frequent in the dry/hot season than in the wet/cold season. In other studies, diarrheal episodes were mostly observed in the wet season with the increase of detection of enteropathogens (Skirnisson and Hansson, 2006). In this study, the prevalence of *Eimeria* spp. and *E. coli* K99 was higher in the dry season, while *Cryptosporidium* spp. was detected more frequently in the wet season.

The OR of *Cryptosporidium* spp., *E. coli* K99, and

Eimeria spp. were 5.86, 7.7, and 2.97, respectively, showing higher occerence in diarrheic animals compared to non-diarrheic animals. These pathogens, identified in the present study, considered as risk factors of diarrhea in small ruminants in Kuwait. This observation is compatible with previous studies (Schoenian, 2007; Engidaw *et al*., 2015). In this study, *E. coli* K99 was the most predominant enteropathogen responsible for diarrhea in sheep and goats in agreement with previous reports (Shabana *et al*., 2017).

In regards to the effect of viral agents, the detection of RVA and BCoV was too low (9 and 7 positive samples, respectively) to be interpreted as possible risk factors of diarrhea in small ruminants in Kuwait. Although infection rates of the both viruses are generally low in small ruminants around the world (Ozmen *et al*., 2006), these results may be due to several factors such as the stages of infection, the viral loads in each sample, as well as the diagnostic test used (Cho *et al*., 2001). Out of seven positive cases of BCoV (71.4%), five animals showed diarrhea signs (two sheep and three goats). Though this result was statistically significant using univariate χ^2 test, the multivariate analysis stepwise logistic regression showed no statistically significant effect of BCoV in diarrhea in small ruminants. Diarrhea in these five animals could be due to the other mixed infections, especially *E. coli* and/or *Cryptosporidium* species.

Escherichia coli K99, *Cryptosporidium* spp., and *Eimeria* spp. were the most predominant enteric pathogens causing diarrhea in sheep and goats in Kuwait. Young pre-weaned animals in intensive rearing farms were more susceptible to diarrhea.

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Conflict of interest

There was no conflict of interest.

References

Aldomy, F and Abu Zeid, N (2007). Neonatal mortality of small ruminants in Jordan. Bulg. J. Vet. Med., 10: 195-199.

Amer, HM (2018). Bovine-like coronaviruses in domestic and wild ruminants. Anim. Health Res. Rev., 19: 113-124.

Cho, KO; Hasoksuz, M; Nielsen, PR; Chang, KO; Lathrop, S and Saif, LJ (2001). Cross-protection studies between respiratory and calf diarrhea and winter dysentery coronavirus strains in calves and RT-PCR and nested PCR for their detection. Arch. Virol., 146: 2401-2419.

Engidaw, S; Anteneh, M and Demis, C (2015). Coccidiosis in small ruminants. Afr. J. Basic Appl. Sci., 7: 311-319.

FAO (2018). Food and Agriculture Organization of the United Nations statistics division. FAO-STAT. Rome, Italy. http://www.fao.org/faostat/en/#data/QA.

Holland, RE (1990). Some infectious causes of diarrhea in young farm animals. Clin. Microbiol. Rev., 3: 345-375.

Keeton, STN and Navarre, CB (2018). Coccidiosis in large and small ruminants. Vet. Clin. North Am. Food Anim. Pract., 34: 201-208.

Kusiluka, L and Kambarage, D (1996). System of small ruminants management in sub-sahran Africa. In: *Diseases of small ruminants: a handbook: common diseases of sheep and goats in sub-saharan Africa*. Easter Bush, Scotland, VETAID, Centre for Tropical Veterinary Medicine. PP: 1-7.

Majeed, QAH (2016). Epidemiological studies on cryptosporidiosis and rotavirus infection in livestock in Kuwait. KFAS Reports. 2: 1-19.

Majeed, QAH; El-Azazy, OME; Abdou, N-EMI; Al-Aal, ZA; El-Kabbany, AI; Tahrani, L; AlAzemi, MS; Wang, Y; Feng, Y and Xiao, L (2018). Epidemiological observations on cryptosporidiosis and molecular characterization of *Cryptosporidium* spp. in sheep and goats in Kuwait. Parasitol. Res., 117: 1631-1636.

Ozmen, O; Yukari, BA; Haligur, M and Sahinduran, S (2006). Observations and immunehistochemical detection of Coronavirus, *Cryptosporidium parvum* and *Giardia intestinalis* in neonatal diarrhoea in lambs and kids. Schweiz. Arch. Tierheilk. 148: 357-364.

Schoenian, S (2007). Diarrhea (scours) in small ruminants. Maryland Small Ruminant Page, https://www.sheepandgoat.com/scours.

Shabana, I; Bouqellah, N and Zaraket, H (2017). Investigation of viral and bacterial enteropathogens of diarrheic sheep and goats in Medina, Saudi Arabia. Trop. Biomed., 34: 944-955.

Singh, U; Singh, R; Singh, AP; Yadav, SK; Sircar, S and Malik, YS (2017). Detection and characterization of caprine and ovine rotaviruses, India. Indian J. Anim. Sci., 87: 1358-1361.

Skirnisson, K and Hansson, H (2006). Causes of diarrhoea in lambs during autumn and early winter in an Icelandic flock of sheep. Icel. Agric. Sci., 19: 43-57.

Soulsby, EJL (1982). *Helminths, arthropods and protozoa of domesticated animals*. 7th Edn., London, UK, Baillière Tindall. PP: 809-827.