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ORIGINAL ARTICLE

Viral and bacterial infections associated with camel (*Camelus dromedarius*) calf diarrhea in North Province, Saudi Arabia

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Abstract Diarrhea and deaths in new-born camel calves were noticed by veterinary investigators and pastoralist in Saudi Arabia to be very high. Hence, it is thought to be necessary to investigate this problem from the virological and bacteriological point of view. The role of pathogenic bacteria and viruses in six different towns of North Province (Al-Assafia, Arar, Domat Aljandal, Hail, Skaka and Khoa) in Saudi Arabia was studied. Survey was conducted in diarrheic camel calves aged 12 months or younger. In our study calf diarrhea was reported in 184 out of 2308 camels examined clinically during one year, the prevalence of diarrhea was found to be 8.0% in calves ranging from one month to one year. In the present study group A rotavirus and *Brucella abortus* were detected in 14.7% and 8.98%, respectively, using ELISA technique. *Escherichia coli* was isolated from diarrheic calf camel (58.2%) 99/170 samples during dry and wet season. *Salmonella* spp. and *Enterococcus* spp. were detected in 12% and 8.8% of the specimens, respectively. In this study enterotoxogenic *E. coli* (ET *E. coli*) was isolated from 7% of diarrheic camel, which indicates the strong correlation between the camel calf diarrhea and the detection of enterotoxogenic *E. coli*. This study represented the first report for the detection of group A rotavirus and *B. abortus* antigen and antibodies in calf

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camels in Saudi Arabia. It is recommended that the disease should be controlled by vaccination in calf camels.

1. Introduction

The camel has played such an important role in Arab culture that there are over 160 words for camel in the Arabic language. There were 11.24 million camels in the Arab world which represent 61% of camel numbers in the world (Farid, 1990) and 15% of the total number of animal units. They produce about 9%, 24% and 8% of the total meat, milk, and wool, respectively, in the Arab world. The amount of meat and milk produced from camels is 289.2 and 213 thousand tons, respectively (Wardeh, 1990). Hamam (1993) reported that camel meat constituted 30% of the meat produced in the Kingdom of Saudi Arabia. The camel plays an effective and primary role in the history of the Kingdom of Saudi Arabia and represents a national wealth and source of income to the majority of citizens particularly in desert areas. Improvement of camel breeds production and health would preserve the recent increasing demand for camel meat and milk of distinguished quality (Wernery and Kaaden, 1995).

Concerning camel disease, camels were formerly considered resistant to most of the diseases commonly affecting livestock, but as more research was conducted, camels were found to be susceptible to a large number of pathogenic agents. Many factors contribute to calf mortality, among which is calf diarrhea (Agab, 1993). Neonatal camel calf diarrhea is an economically important disease causing great losses in camel calves all over the world (Mohammed et al., 2003). High calf mortality is considered one of the major constraints to higher productivity in camels in which calf diarrhea is regarded the major cause (Salih et al., 1998). Mortality in camel populations was found to be higher in camels less than six months of age (Khanna et al., 1992). Ali et al. (2005) reported a morality of 39.9% in Sudan due to calf-camel diarrhea. Most of the fatal diarrhea cases among newly born camel calves are suspected to be caused by namely viruses, bacteria and protozoa.

Viruses can be primary pathogens in the neonatal calf diarrhea and the most common viruses causing diarrhea found throughout the world are group A rotavirus and corona virus. Primary infection of newborn calves with these viruses can cause severe intestinal alterations and diarrhea. Abubaker et al. (2006) reported that the main etiological agents in camel calf diarrhea are bacterial agents. Earlier, neonatal calf diarrhea was attributed to *Escherichia coli* and was designated calf scour (Moore, 1989). Concerning the bacterial infection Fouda and Al Mujalii (2007) in a bacteriological examination revealed that *E. coli* and *Proteus* spp. were the incriminated microorganisms causing diarrhea and *Staphylococcus aureus* was the causative agent of respiratory troubles in diseased calves. Salih et al. (1998) mentioned that bacteriological examination of fecal sample collected from diarrhetic camel calves revealed that 69 (66%) out of 121 yielded *E. coli*. Furthermore, Salwa (2004) isolated 81 *E. coli* of 100 fecal specimens collected from diarrheic calves. Zakia (2004) examined 71 fecal specimens collected from diarrhetic camel calves. The results revealed the detection of *Clostridium perfringens* in 27, *E. coli* in 9 and both *E. coli* and *Clostridium* in 7 samples. More over Abubaker et al. (2006) isolated 52 (27.3%) *E. coli* from 190 diarrheic specimens collected from young camels in Saudi Arabia. Al Afaleq et al. (2007) conducted a Serosurveillance of camels (*Camelus dromedarius*) to detect antibodies against viral diseases in camels. The overall results indicated that out of 2472 examined sera samples, 10.6% had antibodies against the viruses investigated in the study.

The incidence of infection was 18% for bovine viral diarrhea. To study the risk factors associated with some camel viral diseases, Khalafalla and Ali (2007) detected group A rotavirus in 20% of diarrheic camels in Sudan. The main age group affected was 0–3 months. Higher prevalence of group A rotavirus infection was noticed during wet season than dry and winter seasons. Risk factors for these viral diseases contributing to disease transmission in free ranging camels are identified and discussed. Agab (2006) reported the diseases and causes of mortality in intensively kept dromedary camels in a dairy camel farm in Al-Qassim region, central Saudi Arabia. Out of 2316 adults and weaned calves and 126 suckling calves, 942 camels were affected with one or more disease conditions, giving a crude morbidity rate of 38.6%. The most common diseases encountered among the camels of the farm were (22.6%) mastitis, (20.9%) camel dermatophilosis, and (10%) calf diarrhea.

Our investigation sought to determine the epidemiology of viral and bacterial infection in several cities in North Province, Saudi Arabia by using different serologic tests, as well as bacteriologic tests, to identify viral and bacterial organisms isolated from serum and feces specimens of camel calf diarrhea.

2. Material and methods

2.1. Areas of study

The present study was conducted in North Province, Saudi Arabia. Six areas (Al-Assafia, Arar, Domat Aljandal, Hail, Skaka and Khoa) of study have been selected which are rich in camel population that represents all camel breeds and various tribes with those rear camels. Each of times areas had been visited twice to collect data, fecal and serum samples from diarrheic, and healthy camel.

2.2. Data collection

Data about the incidence of camel calf diarrhea, age and sex of the affected calves were collected. A total of 308 camels of different sex age and health status were investigated for bacterial and viral carriage and disease. The camel owners in the areas of study were interviewed. Data about the incidence of camel calf diarrhea, the morbidity and mortality rates of the disease were collected and analyzed.

2.3. Sample collection and preparation

A total of 280 faecal and 308 serum samples from diarrheic and healthy calves and camels were collected. Blood was

withdrawn from the jugular vein and serum was separated by centrifugation. Fecal samples were collected in sterile plastic bags and kept on ice till reaching the lab. Serum and feacal samples were stored at −20 °C until tested.

2.4. Viral detection of group A rotavirus in stool and serum

Fecal samples (280) were diluted 10% in phosphate buffer saline (PBS), vortexed and centrifuged at 5000 rpm for 20 min. The supernatants were taken for detection of group A rotavirus antigen. Competitive ELISA, Latex agglutination and an immuno chromatographic test (rota-strip) kits for group A rotavirus antibody detection in stool and serum, from Bio-X diagnostics-Belgium were used. The test was performed according to the instructions of the manufacturer.

2.5. Serological test of Brucella

All sera were screened for *Brucella* antibodies using both enzyme linked immunosorbent assay (ELISA) and Rose Bengal plate-agglutination (RBPT). The ELISA test was carried out according to Alton et al. (1988), using commercially coated plates supplied by IDEXX company, ELISA Staph. Serum samples with both positive RBPT and ELISA results were considered *Brucella* sero-positive camels. The RBPT was performed as follows, 30 μL of test serum was added to 30 μL of the in-house or commercial rose Bengal antigen on a white porcelain plate and mixed thoroughly with a clean toothpick to produce a zone approximately 2 cm in diameter. The plate was rocked slowly for 3 min. The test was read and scored as positive if any degree of agglutination was observed.

2.6. Detection of enterotoxogenic E. coli antibodies

Enterotoxogenic *E. coli* antibodies were detected by ELISA. Briefly, wells of polystyrene microtiter plates were coated with 100 μl of purified fimbrial antigen (1 μg/ml in PBS) and kept at 37 °C overnight. After blocking with 0.1% BSA in PBS, serial dilutions of antisera or MAbs in PBS containing 0.1% BSA and 0.05% Tween 20 were added and incubated for 90 min at room temperature. The plates were washed, and horseradish peroxidase-labeled anti-mouse IgG (H + L) antibodies (Jackson Immuno Research Laboratories, West Grove, PA) were added followed by *o*-phenylenediamine. Within 15–20 min, the optical density of the developed color was read with an ELISA reader (ELx800 Absorbance Microplate Reader, BioTek Instruments, California, USA) at 450 nm.

2.7. Bacteriological analysis of fecal samples

One loop of stool sample was taken aseptically from each sample, streaked on blood agar and incubated aerobically overnight to obtain discrete colonies. Discrete colonies were subcultured on MaCconkey and Xylose lysine deoxycholate agar (XLD), pure cultures were preserved on brain heart infusion broth under −20 °C. Identification of *Enterococci* used standard conventional and commercial tests. These included the Gram stain reaction, growth on bile-aesculin agar, growth in the presence of 6.5% NaCl and absence of catalase. The identification to species level used API 20E Strep system (bio Merieux, Cedex, France) and the software supplied by the manufacturer.

2.8. Statistical analysis

The data were statistically analyzed using SPSS statistical package. The A p-value of <0.05 was considered significant.

3. Results and discussion

Neonatal calf diarrhea is considered one of the most serious constraints of animal production. The incidence of calf diarrhea occurs all over the year with some increase in calving seasons. Schwartz and Dioli (1992) reported that morbidity and mortality rates due to camel calf diarrhea could reach up to 30% and 100%, respectively. Abbas et al. (1992) reported that camel calf diarrhea affects about 33% of the neonates causing 23% mortality in Sudan. In our study calf diarrhea was reported in 184 out of 2308 camels examined clinically during one year, the prevalence of diarrhea was found to be 8.0% in calves ranging from one month to one year. This was in agreement with Agab (2006) who reported an incidence of 10% diarrhea in a dairy camel farm in Al-Qassim region, central Saudi Arabia. The aim of the present study was to elucidate the epidemiology of camel calf diarrhea with emphasis on group A rotavirus and bacterial infections at six different areas in Saudi Arabia. Epidemiological data were collected either from camel owners or the pastoralists. It was noticed that there was no significant difference in morbidity rate among the six different areas of study as shown in Fig. 1. A total of 308 camel calves were selected for laboratory investigations of which 184/308 (60%) were diarrheic calves. It was noticed that camel calf diarrhea is considered one of the main killing diseases of camel calves up to 6 month of age. The analysis of the data collected revealed that the occurrence of the disease was reported in all areas of the study and the statistical analysis using SPSS program showed that there was no significant difference in morbidity rates in the different areas of study as well as in the two seasons studied (Table 1). The results obtained were in disagreement with the previous reports that the incidence of calf diarrhea increases during the calving seasons due to the increase in susceptible individuals with the persistence of the causative agent in the environment (Babuik et al., 1985).

In the present study three different techniques were used, latex agglutination and immuno chromatographic technique for the detection of group A rotavirus antigen in fecal

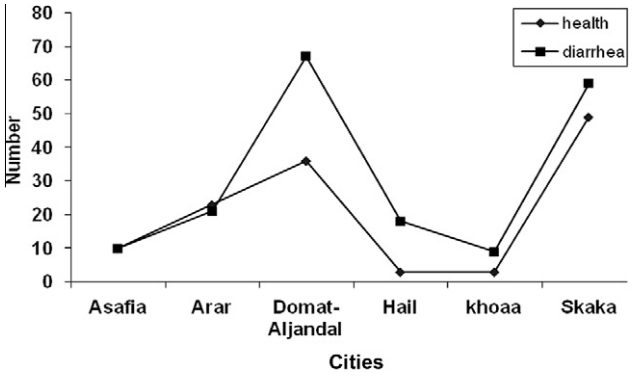

Figure 1 Frequency of camel calf diarrhea among different studied areas.

Table 1 Epidemiology of camel calf diarrhea during dry and wet season.

Status of camel calf	Season						Total
	Dry season			Wet season			
	July	June	May	October	November	December	
Diarrhea	6	13	9	15	38	43	124
Healthy	7	17	6	31	57	66	184
Total	13	30	15	46	95	109	308

specimens and ELISA technique for group A rotavirus antibody in serum. ELISA was and still considered as one of the main techniques that is used for the detection of group A rotavirus antigen in feces. Mohamed et al. (1998) was the first to report the detection of group A rotavirus in 11 out of 117 (9.4%) samples from 1 to 3 month old diarrheic camel. In our study group A rotavirus was detected in 48/256 (18.7%), 41/278 (14.7%) and 34/255 (13.3%) samples from one month to one year old diarrheic and healthy calves using latex agglutination, ELISA and immuno chromatographic techniques, respectively (Fig. 2). Concerning the latex agglutination technique Hughes et al. (1984) described the use of latex agglutination test (LA) for the detection of group A rotavirus antigen in human stool. The high specificity and low sensitivity of LA was described by Al-Yousif et al. (2001). In this work 28/169 (11.7%) positives have been detected in total specimen examined while 34/157 (21.7%) positives were detected in diarrheic specimens. It was noticed that most of LA positive samples (21 out of 28) were highly positive with ELISA. All latex positive as well as doubtful samples were from diarrheic calves, which may indicate that this test can detect group A rotavirus antigen only in high concentration. The test can be used to screen diarrheic fecal samples for group A rotavirus antigen, but negative samples have either to be tested by other technique or re-tested after being concentrated. The use of the immuno chromatographic test (IC) for the detection of group A rotavirus was evaluated by De Verdier and Esfandiari (1996). IC was compared with ELISA for group A rotavirus antigen in 161 bovine, porcine and equine fecal samples. Eighty-nine percent sensitivity and 99% specificity of the IC test were found. In the present study IC test detected 23 out of 157 (14.6%) group A rotavirus antigens in diarrheic camel calf samples. The results showed a comparable sensitivity of IC and latex agglutination test although the results of both tests revealed some

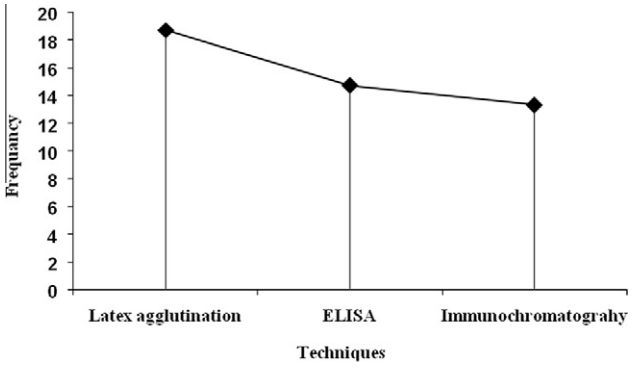

Figure 2 Frequency of group A rotavirus in diarrheic camel calves using different techniques.

differences with the ELISA test. It was noticed that most of the camel owners ignore the microbial causation and believes that calf suckling during the hot weather mainly causes camel calf diarrhea naturally and they usually stop calf suckling when diarrhea is observed. This practice in fact is valuable in case of group A rotavirus induced diarrhea, as it was proved that the main cause of diarrhea is the persistence of lactose in the lumen causing osmotic drain attracting body fluids into the lumen (Flewett and Wood, 1978). But there is usually no compensation by fluid therapy which explains the high mortalities observed that is most probably due to dehydration.

On the other hand asymptomatic group A rotavirus infections detected in this study were prevalent infections, not incident infections The findings from this study suggest that asymptomatic group A rotavirus infections are transmitted through the same routes as group A rotavirus is associated It is therefore likely that host immunity, rather than infection route or dose, determines whether disease develops after infection or not. ELISA was successfully used to detect antibodies against group A rotavirus in human and various species including bovine, chicken and swine (Corthier and Franz, 1981). In these works antibodies to group A rotavirus were found to be existing in camel sera from all areas of study as detected using ELISA with an overall percentage of 15%. The results of antibody ELISA obtained in this study indicated the low prevalence of group A rotavirus infection in the six areas of study which is completely different from that obtained by (Khatter Pandey, 1990) who reported antibody titers in more than 50% of the positive samples, which may indicate that the animal had been exposed to group A rotavirus infection several times, and most of the positive results were found in more than one year age group thus strengthening the hypothesis that those animals had been exposed to group A rotavirus several times. The first group A rotavirus infection was most likely to occur during the first month of age and at this age most of the calves receive group A rotavirus antibodies through colostrums. Maternal antibodies may neutralize the virus leading to absence or low titers of antibodies in serum, which then increases due to subsequent exposures. Positive group A rotavirus antibodies were detected in healthy camel calves and adult camels and then in diarrheic. This may be attributed to the fact that usually the antibody titers during the infection are low and increase to reach high level in recovered and clinically healthy animals in which previous infections were more likely to be occurred specially in endemic areas.

Concerning the sex distribution of positive sampled animals for detection of rotavirus antibody, females were found to possess slightly higher positives than males in the different age groups tested. Generally the prevalence of camel calf diarrhea in this study is not high (8.0%) but it was at an alarming rate. Therefore attention should be focused on this problem. Since

Table 2 Frequency of serological diagnosis of brucellosis using enzyme linked immunosorbent assay (ELISA) and Rose Bengal plate-agglutination (RBPT) techniques at different locations, North Province, Saudi Arabia.

Location	ELISA (%)	RBTP (%)
Al-Assafia	2 (18.18)	1 (9.1)
Arar	3 (11.53)	2 (7.7)
Domat-Aljandal	6 (8.21)	4 (5.5)
Hail	0 (0)	1 (6.7)
Skaka	4 (9.52)	1 (2.4)
Total	15 (8.98)	9 (5.4)

the etiology of camel calf diarrhea is caused by many pathogens, bacteria (Zakia, 2004; Abubaker et al., 2006), virus (Abbas and Omer, 2005) and parasites (Tzipori, 1981). Thus this work is considered as a screening test for this problem which should be considered by a team and should be well funded to cover all aspects of the problem.

Brucella infection in farm animals is considered a great problem in most countries of the world. Thus, the early detection of *Brucella* infection in a herd or flock is a pre-requisite for the successful control and elimination of one of the major problems considered to be a predisposing factor leading to infertility and sterility along with the possible transmission of infection to man (Wasseif, 1992). Camels are not known to be primary hosts for any of the *Brucella* organisms, but they are susceptible to both *Brucella melitensis* and *Brucella abortus* and the infection rate depends upon the infection rate in primary hosts animals in contact with them, this may further suggest the role of small ruminants in the occurrence of camel brucellosis (Agab et al., 1994). The results of serological diagnosis of brucellosis in camels at different locations are summarized in (Table 2). Most positive cases (18.18%) were from Al Assafia location. Camels were tested serologically for *Brucella* antibodies, by ELISA and RBTP, positive result for the disease was found to be (8.98%) and (5.4%), respectively. Brucellosis in camels seems to display less clinical signs than in other ruminant animals. The survey of our study confirms the existence of brucellosis among camels in Saudi Arabia. The health authorities should recognize this and apply intervention strategies in order to prevent and control brucellosis in the future. The ELISA method used in this study detected higher reactors than RBTP, this may be ascribed to the fact that the test is more sensitive in detecting IgM as well as IgG immunoglobulin (Stemshorn et al., 1985). Although *Brucella* spp. organisms were not isolated in this study because of inadequate facilities, the evidence showed clearly the occurrence of the disease.

Prevalence of *B. abortus* antibodies and pathogenic bacteria in all age groups of camels showed that infection in the

Table 4 Frequency of bacteria isolated from diarrheic camel calves specimens.

Bacterial isolate	No. of isolates from diarrheic camel calves (%)
E. coli	99 (58.2)
Salmonella spp.	25 (14.7)
Enterococcus spp.	15 (8.8)
Enterotoxogenic *E. coli*	12 (7.0)

animals may have started early in life probably through suckling and persisted into adulthood (Table 3). This is in agreement with Oloffs et al. (1998), who reported that 30% of the sero positive animals in Uganda were younger than three years of age and among them was a 2-year-old bull. In this study *E. coli* was isolated from 99/170 (58.2%) samples during dry and wet season (Table 4). This may confirm the significance of *E. coli* in the causation of diarrhea in camel calves. These findings were in agreement to other investigators Salih et al. (1998), Mohamed et al. (1998), Zakia (2004) and Abubaker et al. (2006) who isolated 66%, 40.04%, 22.55% and 27.3% *E. coli,* respectively. Isolation of *E. coli* does not necessarily mean disease unless virulence factors are identified such as toxins and/ or fimbriae (Yang et al., 2011). In this study toxin producing *E. coli* strains (ET *E. coli*) were isolated from 7% of diarrheic camels (Table 4), which indicates the strong correlation between the camel calf diarrhea and detection of enterotoxogenic *E. coli.* Previous reports from various countries have reported Salmonellosis in camels, in Egypt (Osman, 1995), UAE (Wernery, 1992), and Ethiopia (Molla et al., 2004). Moreover, Selim (1990) reported that healthy camels can be carriers of *Salmonella* species and *Salmonella* have been isolated from feces and lymph. Camels that are chronic carriers of *Salmonella* species may present a human health hazard through consumption of camel products (Matofari et al., 2007). Continuous surveillance studies for *Salmonella* in human and animals are important, since new *Salmonella* serovars are emerging yearly and serotyping is very important to the epidemiology study (Kim, 2010). In the present study, *Salmonella* species were isolated from 35/280 camel feces with the percentage of 14.7% as shown in Table 4. The recorded infection rate of *Salmonella* species in camel feces is near to those reported by Molla et al. (2004) whose result was 15.1% and *Salmonella* species were isolated from camel-calf diarrhea (13%) in eastern Sudan (Salih et al., 1998). On the other hand, a higher infection rate with *Salmonellosis* in calf camel (13%) was recorded by Salih et al. (1998). The variation of *Salmonella* species incidence could be attributed to the overcrowding and transportation stresses which increase the excretion of *Salmonella* species.

Table 3 The distribution of brucellosis (ELISA detection) and pathogenic bacteria among different age groups of calf camels.

Age groups (months)	Brucellosis	Pathogenic bacteria			
		E. coli	*Salmonella*	*Enterococcus*	ET *E. coli*
1–3	7.5	27	9	4	3
4–6	6.8	13	7	2	2
7–9	15	8	5	2	0
10–12	0.0	3	0	2	1
>12	27	5	0	3	0

4. Recommendations

It is recommended that the disease should be controlled by vaccination in camels and primary hosts as there are uncontrolled movements of different animals (camels, sheep and goats) through the borders between Saudi Arabia and surrounding countries. It is also recommended to vaccinate the animals in Saudi Arabia at regular intervals especially along the border regions and adequate *Brucella* control programs in small ruminants may contribute to the reduction in the prevalence of this disease in calf camel. For animals that will be remaining on our property a minimum of 30-day quarantine is recommended before introducing new animals to our herd. Pregnant she-camel should not be moved within the last two weeks before calving. Moreover, feeding colostrums during the first day is strongly advised, other management factors should also be adopted. Vaccination through feeding edible transgenic plants that contain genes coding for cholera toxin B subunit should be adopted. Further studies are needed to identify other causes of camel calf diarrhea, study the role and to study the role of *E. coli* heat-stable (STb) enterotoxin, verotoxins (VTs) and cytotoxic necrotizing factors (CNF).

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