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Research in Veterinary Science 87 (2009) 373–379

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Molecular screening and risk factors of enterotoxigenic *Escherichia coli* and *Salmonella* spp. in diarrheic neonatal calves in Egypt

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ARTICLE INFO

Article history:
Accepted 7 April 2009

Keywords:
ETEC K99
Salmonella
Risk factors
Diarrhea
Calves
Egypt

ABSTRACT

The aim of the present study was to carry out molecular epidemiological investigation on enterotoxigenic *Escherichia coli* (ETEC) K99 and *Salmonella* spp. in diarrheic neonatal calves. Fecal samples were obtained from 220 diarrheic calves at 9 farms related to four governorates in central and northern Egypt. *E. coli* and *Salmonella* spp. isolates were examined for *E. coli* K99 and *Salmonella* spp. using PCR. ETEC K99 was recovered from 20 (10.36 %) out of 193 isolates, whereas *Salmonella* spp. was recovered from nine calves (4.09%).

Multivariable logistic regression was used to evaluate the risk factors associated with both infections. ETEC K99 was significantly affected by age ($P < 0.01$; OR: 1.812; CI 95%: 0.566–1.769), colostrum feeding practice ($P < 0.01$; OR: 5.525; CI 95%: 2.025–15.076), rotavirus infection ($P < 0.001$; OR: 2.220; CI 95%: 0.273–1.251), vaccination of pregnant dams with combined vaccine against rotavirus, coronavirus and *E. coli* (K99) ($P < 0.001$; OR: 4.753; CI 95%: 2.124–10.641), and vitamin E and selenium administration to the pregnant dam ($P < 0.01$; OR: 3.933; CI 95%: 0.703–1.248).

Infection with *Salmonella* spp. was found to be significantly affected by the animal age ($P < 0.05$; OR: 0.376; CI 95%: 0.511–1.369), Hygiene ($P < 0.05$; OR: 0.628; CI 95%: 1.729–5.612), and region ($P < 0.01$; OR: 0.970; CI 95%: 0.841–1.624).

The results of the present study indicate the importance of PCR as rapid, effective and reliable tool for screening of ETEC and *Salmonella* spp. when confronted with cases of undifferentiated calf diarrhea. Moreover, identification of the risk factors associated with the spreading of bacteria causing diarrhea may be helpful for construction of suitable methods for prevention and control.

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1. Introduction

Neonatal calf diarrhea remains an important cause of morbidity and mortality in young calves (Constable, 2004). Diarrhea is a multifactorial disease which despite decades of research on the topics, remains the most common cause of deaths in neonatal calves, even though major risk factors that have long been identified, numbers of calves losses due to diarrhea are not declining (Snodgrass et al., 1986). Several enteropathogens were recovered from neonatal calf with diarrhea, their relative prevalence varies geographically but the most common prevalent infections in most areas are *Escherichia coli,* rotavirus, and coronavirus, *C. Perfringens, Salmonella* spp. and *Cryptosporidium* spp. (Snodgrass et al., 1986; García et al., 2000).

Enterotoxigenic *E. coli* (ETEC) infection is the most common type of colibacillosis of young animals (primarily pigs and calves), and it is a significant cause of diarrhea among travelers and children in the developing world (Nagy and Fekete, 2005). Diarrhea-producing *E. coli* possess colonization antigens or adhesions that enable the bacteria to colonize the small intestines (Chakraborty et al., 2001). The expression of K99 fimbriae (or F5 ETEC) accounts for nearly all cases of ETEC infection found in newborn calves (Jay et al., 2004). A number of diagnostic tests are currently available for detecting ETEC including: Double-antibody enzyme-linked immunosorbent assay (ELISA) (Holley et al., 1984), DNA gene probes specific for genes encoding toxins and adhesions of ETEC (Woodward and Wray, 1990), multiplex polymerase chain reaction (PCR) for the rapid screening of ETEC toxins (Watterworth et al., 2005), and monoclonal antibody-based co-agglutination test (Varshney et al., 2007).

Salmonella infections also in calves continue to be a major worldwide problem. Substantial economic losses were manifested through mortality and poor growth of infected animals as well as the hazard of transmission to humans (Smith et al., 2004). While it may be convenient to focus on the principal infectious causes of calf diarrhea, remember that it is generally the result of interaction

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0034-5288/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2009.04.006

between a number of related risk factors, (Crouch et al., 2001; Langoni et al., 2004). Many risk factors are involved in etiology of neonatal calf diarrhea including management and environmental factors (Lundborg et al., 2005). The risk factors associated with *Salmonella* (Fossler et al., 2005; Davison et al., 2006), and *E. coli* (Schouten et al., 2004; Kuhnert et al., 2005) have been described.

In Egypt, little systematic work has been done to obviate the impact of enteric pathogens and diarrhea. Also, little attention was undertaken to study the epidemiology and risk factors of specific etiology of neonatal calf diarrhea (El-Khodery and Osman, 2008). To the authors' knowledge, molecular screening of bacterial causes of neonatal calf diarrhea and the associated risk factors have not been described not only in Egypt but also in Middle East and Africa. Consequently, the aim of the present study was to carry out molecular screening of ETEC K99 and *Salmonella* spp. in diarrheic neonatal calves. Furthermore, to study the risk factors associated with these infections.

2. Materials and methods

2.1. Calves and collection of data

A total of 220 diarrheic neonatal calves at 1–30 days of age were studied during one year. These calves were raised in nine farms belonging to Dakahlia, Kafr El-Sheikh, Damietta and Behera governorates of central and northern Egypt. These farms were visited once per month. The animals' identification, age, gender, and number of animals per herd were recorded. The constant clinical signs observed in the examined calves were sudden onset of profuse yellow/white diarrhea causing rapid and severe dehydration. Competent clinical examination of each calf was performed and the clinical parameters related to diarrhea were recorded. A questionnaire was done about the housing conditions, hygienic measures, source of drinking water, preventive measures, mastitis, vaccination of dams with combined vaccine against rotavirus, coronavirus and ETEC K99 and parity. Furthermore, there was a series of questions about the management and raising of the newborn calves.

2.2. Sampling, isolation and identification procedures

Individual fecal sample was collected from each calf, transported to the laboratory on ice and processed in the same day. Bacteriological examination was carried out according to the method described by Cruickshank et al. (1975). Briefly, swabs from these samples were inoculated in peptone water broth and Rappaport Vassiliadis broth (Difco) and incubated at 37 °C for 18 h. Then, subcultured on MacConky, XLD (xylose lysine deoxycholate) and EMB (eosin methylene blue) agar plates and incubated at 37 °C for 24–48 h. Regarding to isolation of *E. coli*, three blue–black colonies (presumptive *E. coli*) with metallic sheen growing on EMB agar plates were randomly selected from each plate. Regarding to *Salmonella* spp. all isolates was identified as *Salmonella* spp. based on their colony morphology on selective media, and the biochemical testing using TSI agar, Urea agar (Christensen), L-lysine decarboxylase, β-galactosidase (ONPG), Voges Proskauer and Indole tests (Edwards and Ewing, 1986). Also, both *E. coli* and *Salmonella* spp. were confirmed biochemically by using API 20E system (BioMérieux, Marcy-l'Étoile, France).

2.3. Bacterial DNA preparation for PCR

An overnight bacterial culture (200 μl) was mixed with 800 μl of distilled water and boiled for 10 min. The resulting solution was centrifuged and the supernatant used as the DNA template. Amplification reactions were carried out with 10 μl of boiled bacterial suspensions, 250 mM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 50 pmol of primers and 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Roche, NJ, USA). Distilled water was added to bring the final volume to 50 μl. After PCR reactions, the reaction products were subjected to electrophoresis in a 1.0% agarose gel, stained with ethidium bromide and visualized under UV light.

2.4. PCR screening for K99

E. coli isolates were screened for the presence of K99 coding gene by using the primers K99-F and K99-R as previously described (Table 1) (DebRoy and Maddox, 2001). Briefly, the PCR cycling conditions consisted of initial denaturation at 94 °C for 5 min, followed by 30 cycles each of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, and extension at 72 °C for 45 s. The amplified PCR product was electrophoresed on a 2% agarose gel in Tris–acetate–EDTA buffer. A 100-bp DNA ladder (Invitrogen, Carlsbad, CA) was used as a molecular weight marker. A K99 serologically-positive *E. coli* strain and water were used as positive and negative controls, respectively throughout the PCR-based assays.

2.5. Salmonella serotyping by using multiplex PCR

Multiplex PCR was used for serotyping of suspected *Salmonella* isolates. Many sets of primers were used for PCR as described previously (Table 1) (Alvarez et al., 2004).

2.6. Parasitological examinations

Fecal smears were prepared from the fecal samples and examined for the presence of *Cryptosporidium* spp. oocysts after staining using modified Ziehl-Neelsen stain (Henricksen and Pohlenz, 1981).

2.7. Detection of rotavirus and coronavirus

Fecal samples were examined for presence of viral antigens to rotavirus and or coronavirus using virus neutralization test according to Robson et al. (1960).

2.8. Statistical analysis

All data analyses were carried out using the statistical software program (SPSS for Windows, Version 15.0, USA). Association between the occurrence of infection and the potential risk factors were studied using logistic regression. At first step, a univariate logistic regression was carried out. In this method, the dependent dichotomous variable was the status of the calves (infected or non-infected). However, the independent variables were the hypothesized risk factors. Variables with significance at $P < 0.1$ were selected for further multivariate logistic regression model. Hosmer and Lemeshow's goodness of fit statistic test greater than 0.05 was used to imply that the model's estimates fit the data at an acceptable level in multivariate analysis. The results were each expressed as P value and odds ratio (OR) with a 95% confidence interval (CI 95%). Result was considered to be significant at $P < 0.05$.

3. Results

In the present study, out of the examined 220 diarrheic calves, 193 *E. coli* isolates were identified, 20 of them were ETEC K99 (10.36%), (Table 2; Fig. 1). *Salmonella* spp. were identified using PCR in nine cases (4.09); six isolates were *S. enterica* serovar Typhimurium and two isolates were *S. enterica* serovar Enteritidis

Table 1
Primers used for PCR screening.

Primer	Sequence (5′–3′)	Amplicon size (bp)	Target	Reference
Salmonella serotyping				
OMPCF	ATCGCTGACTTATGCAATCG	204	*Salmonella*	Alvarez et al. (2004)
OMPCR	CGGGTTGCGTTATAGGTCTG			
ENTF	TGTGTTTTATCTGATGCAAGAGG	304	Enteritidis	Alvarez et al. (2004)
ENTR	TGAACTACGTTCGTTCTTCTGG			
TYPHF	TTGTTCACTTTTTACCCCTGA A	401	Typhimurium	Alvarez et al. (2004)
TYPHR	CCCTGACAGCCGTTAGATATT			
HADF	ACCGAGCCAACGATTATCAA	502	Serogroup	Alvarez et al. (2004)
HADR	AATAGGCCGAAACAACATCG		C2	
4512F	CGCTGTGGTGTAGCTGTTTC	705	Serotype 4,5,12:i:–	Alvarez et al. (2004)
4512R	TCTGCCACTTCTTCACGTTG			
K99				
K99-F	TGGGACTACCAATGCTTCTG	450	K99 coding gene	DebRoy and Maddox (2001)
K99-R	TATCCACCATTAGACGGAGC			

Table 2
PCR screening results for *E. coli* K99 and *Salmonella* serovars.

	Number	Mixed infections
E. coli	193	
ETEC K99	20	3 with *S. enterica* serovar Typhimurium
Salmonella spp.	9	
S. enterica serovar Typhimurium	6	3 with ETEC K99
S. enterica serovar Enteritidis	2	
Non Typhimurium		
Non enteritidis	1	

whereas one isolate was not typed (Table 2 and Figs. 2 and 3). Three cases were confirmed to have mixed infection with ETEC K99 and *S. enterica* serovar Typhimurium.

After the construction of a multivariable model, Hosmer and Lemeshow's goodness of fit test statistic revealed that the model adequately fit the data for the risk factors associated with *E. coli* ($\chi 2 = 7.911$; $P = 0.261$) and *Salmonella* spp. ($\chi 2 = 4.521$; $P = 0.17$) infection. Age, vaccination of dams with combined vaccine against rotavirus, coronavirus and ETEC K99 (Scour Guard 3), colostrum feeding practice, rotavirus infection, and administration of vitamin E/selenium to pregnant dams were found to be significantly associated with ETEC K99 infection in diarrheic calves (Table 3).

Animal age was found to affect significantly the prevalence ($P < 0.01$; OR: 1.812; CI 95%: 0.566–1.769). Thus, 100% of the cases were found to be at the first week of age. Vaccination of dams with Scour Guard 3 (Pfizer, Egypt) significantly reduce the infection rate with ETEC K99 ($P < 0.001$; OR: 4.753; CI 95%: 2.124–10.641). Thus, 14 out of 20 infected calves were born from non-vaccinated dams.

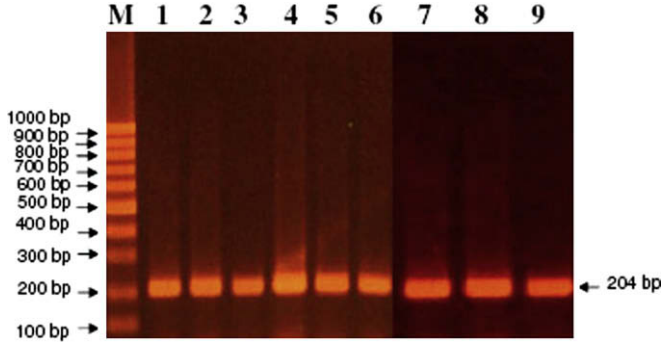

Fig. 2. PCR identification of genus *Salmonella*. The target size is 204 bp. *M* = 100 bp ladder size marker.

The prevalence of ETEC K99 was also found to be significantly affected by the hand feeding of colostrums ($P < 0.01$; OR: 5.525; CI 95%: 2.025–15.076); 16 infected calves were fed manually, whereas 4 cases only were naturally fed. Infection with rotavirus recorded significant association with infection by ETEC K99 ($P < 0.001$; OR: 2.220; CI 95%: 0.273–1.251). Thus, 13 infected calves with ETEC K99 were also found to be infected by rotavirus. Vitamin E and selenium supplementation of pregnant dams significantly affected the prevalence of ETEC K99 ($P < 0.01$; OR: 3.933; CI 95%: 0.703–1.248). 16 of infected calves were found to born from dams those did not receive combination of vitamin and selenium injection. On the contrary, Mastitis, season, *Cryptosporidium* spp. infection, hygiene, herd size, parity, and coronavirus infection showed no significant effect on the prevalence of ETEC K99 infection.

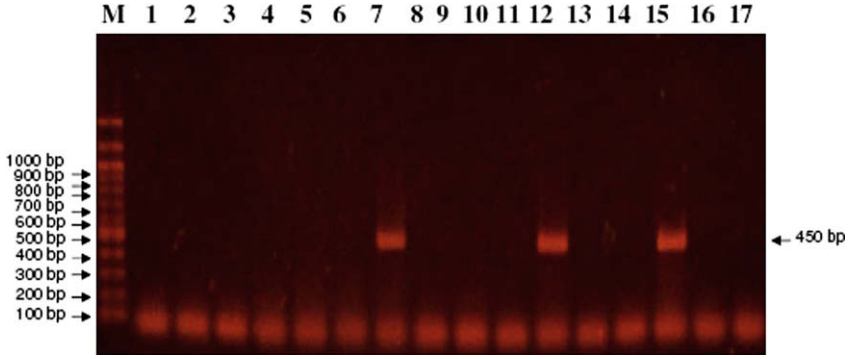

Fig. 1. Example of PCR identification of *E. coli* K99. The target size is 450 bp. *M* = 100 bp ladder size marker.

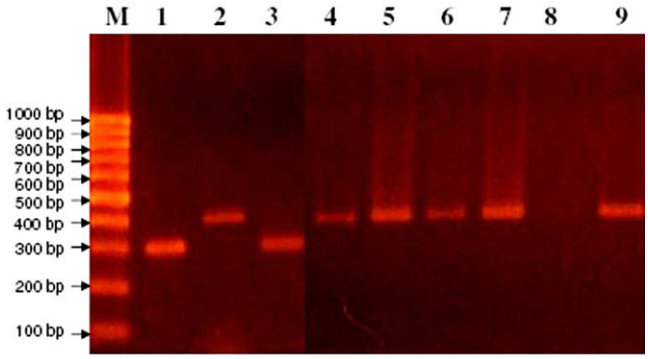

Fig. 3. PCR serotyping for *Salmonella* isolates. No. 2, 4, 5, 6, 7, 9 and 2 are *S. enterica* serovar Typhimurium = 401. No. 1 and 3 are *S. enterica* serovar Enteritidis = 304. No. 8 neither *S. enterica* serovar Typhimurium nor *S. enterica* serovar Enteritidis. *M* = 100 bp ladder size marker.

Infection with *Salmonella* spp. was found to be significantly affected by the animal age ($P < 0.05$; OR: 0.376; CI 95%: 0.511–1.369) (Table 4). Thus, seven cases were found at the fourth week of age, whereas two cases were at the first week. Significant association was also found between the hygiene and infection with *Salmonella* ($P < 0.05$; OR: 0.628; CI 95%: 1.729–5.612); six calves infected with *Salmonella* spp. were found to be raised in unhygienic places. Also, the region of the farm was found to affect significantly on the prevalence of infection with *Salmonella* spp. ($P < 0.01$; OR: 0.970; CI 95%: 0.841–1.624). Thus, seven cases were isolated from farms in governorates lie in Delta of Nile River (Kafr El-Sheikh, Dakahlia and Damietta) versus two cases in farms lie in semi arid area of Behera governorate. On the other hand, including mastitis, season, herd size, *E. coli, Cryptosporidium* spp. infection, parity, rotavirus infection, and coronavirus infection significantly did not affect the *Salmonella* spp. infection in the present investigation.

4. Discussion

The intent of this study was to describe the prevalence and risk factors associated with ETEC K99 and *Salmonella* spp. infections in neonatal diarrheic calves. The prevalence of ETEC K99 was 10.36%. Similar result was reported previously by Wang et al. (2006). However, higher prevalence was reported by Bendali et al. (1999) and Achá et al. (2004) who reported a prevalence rate of 20.3% and 16%, respectively. Moreover, in India, PCR could identify higher prevalence (20%) in buffalo calves (Singh et al., 2007). On the contrary, lower prevalence (4.7%, 3.86% and 5.8%) of ETEC K99 was recorded by Akam et al. (2004), Kanwar et al. (2007) and Oliveira Filho et al. (2007), respectively. In a study carried out in Egypt and Israel, higher prevalence (23%) was also recorded (Perka et al., 2000). The differences in the prevalence from those previously recorded may be due to variations in region, management conditions and hygienic measures. Diarrhea caused by ETEC is considered the main infectious disease of newborn calves (Martín et al., 2003); however, bovine *E. coli* F5 (K99) seemed to be of minor importance in the investigated population compared with cryptosporidiosis and rotavirus infection (Luginbühl et al., 2005).

Multivariate logistic regression model enabled to identify the significant risk factors associated with examined bacteria. Age, vaccination of the pregnant dams with Scour Guard 3, colostrum feeding practice, rotavirus infection, and administration of vitamin E/selenium to pregnant dams were found to affect significantly the prevalence of ETEC K99 in diarrheic calves. Calf age significantly affect the prevalence ($P < 0.01$; OR: 1.812; CI 95%: 0.566–1.769). Thus first week of life is the main age for occurrence. This finding came in accordance with those previously recorded (Bendali et al., 1999; Radostits et al., 2007; Wieler et al., 2007; Güler et al., 2008). This finding was also supported by the result recorded by Akam et al. (2004) who found that the susceptibility was higher during the first week of life to *E. coli* K99 (66.6%). It is suggested that the age-dependent shedding dynamic of the ETEC has to be considered regarding prophylaxis as well as planning intervention studies for calves.

Vaccination of the pregnant dams significantly caused minimization of the occurrence of ETEC in their calves ($P < 0.001$; OR: 4.753; CI 95%: 2.124–10.641). This result was in agreement with those described by Ganaba et al. (1995) and Crouch et al. (2001) who found that there was a significant increase in the mean specific antibody titre against all three K99, rotavirus, and coronavirus antigens in the serum of the vaccinated animals (even in the presence of pre-existing antibody) which was accompanied by increased levels of protective antibodies to rotavirus, coronavirus and *E. coli* F5 (K99) in their colostrum and milk for at least 28 days. In a field study, the results of application of Nobi-vac vaccine containing K99 adhesive antigen denoted a significant decrease in the percentage of diarrhea to 5.05% in calves born from vaccinated dams. Furthermore, the K99 antibody titres in the sera of newborn calves differed significantly ($P < 0.05$) and mean antibody titres increased (Farid et al., 2001).

Table 4
Final logistic regression model for positive risk factors associated with *Salmonella* spp. in diarrheic calves.

Variable	β	SE	*P*	OR	CI
Age	−.978	0. 466	0.036	0.376	0.511–1.369
Hygiene	1.891	0.976	0.05	0.628	1.729–5.612
Region	−3.636	1.163	0.002	0.970	0.841–1.624
Constant	−1.709	1.922	0.191	0.81	–

β: Regression coefficient.
SE: Standard error.
OR: Odds ratio.
CI: Confidence interval.

Table 3
Final logistic regression model for positive risk factors associated with *E. coli* K99 in diarrheic calves.

Variable	β	SE	*P*	OR	CI
Age	−0.794	0.271	0.003	1.812	0.566–1.769
Vaccination	1.559	0.411	0.000	4.753	2.124–10.641
Colostrum feeding practice	1.709	0.512	0.001	5.525	2.025–15.076
Rota virus infection	1.129	0.426	0.000	2.220	0.273–1.251
Vitamin E/Selenium injection in pregnant dams	1.369	0.517	0.007	3.933	0.003–0.248
Constant	−0.535	0.698	0.048	0.749	–

β: Regression coefficient.
SE: Standard error.
OR: Odds ratio.
CI: Confidence interval.

It was evident that calves in farms, which received colostrum directly from their dames were less frequent to be infected with ETEC ($P < 0.01$; OR: 5.525; CI 95%: 2.025–15.076) than those hand fed calves. This result coincided with that previously recorded by Barrington et al. (2002) who reported that passively acquired immunity through colostrum, is the major risk factor related to the calf and the occurrence of diarrhea. It was also found that colostral leukocytes obviously contribute to the passive immunity and resistance of the newborn calf against experimental infection by ETEC (Riedel-Caspari, 1993). The sera of the colostrums fed calves had significantly higher concentrations of antibodies against ETEC mainly of IgG1 specificity on the second day of life as compared to those of the milk substitutes. The sera of the colostrums fed calves contained significantly more IgM on days 2 and 5, and slightly more IgA during the first week (Riedel-Caspari and Schmidt, 1991). However, colostral leukocytes in the absence of humoral components of the colostrum were not able to prevent fatal losses in the calves due to natural infection, although their influence on immune responses of the calves was detectable in vitro (Riedel-Caspari et al., 1991). Moreover, the antibody independent complement activities of serum can be increased substantially by feeding colostral whey concentrate to calves during their first days of life (Rokka et al., 2001).

Infection with rotavirus recorded significant association with infection by ETEC K99 ($P < 0.001$; OR: 2.220; CI 95%: 0.273–1.251). This result coincided with that reported by Miraglia et al. (2001) who recorded an outbreak caused by *E. coli* and rotavirus in 216 calves in Brazil. Moreover, *E. coli* STX infection was found a significant risk factor that could induce rotavirus infection in neonatal calves in Mexico (Anda et al., 2000). However, the detection rates of the other enteropathogens considered in calves with rotavirus infection were 20.4% for coronavirus, 85.2% for *Cryptosporidium*, 16.7% for $F5^+$ *E. coli* and 1.8% for *Salmonella* spp. (García et al., 2000).

Vitamin E/selenium injection in the pregnant dams significantly affected the prevalence of ETEC K99. Thus, the prevalence was increased in calves born from dams those did not receive vitamin E/selenium in the late stage of pregnancy. Although there are no previous studies on this respect, it is generally found that the frequency of respiratory diseases in calves born from dams received selenium during pregnancy is low (Radostits et al., 2007).

Mastitis, season, *Cryptosporidium* spp. infection, hygiene, herd size, parity, and coronavirus infection showed no significant effect on the prevalence of ETEC K99 infection. There was a controversy about the association of mastitis and season with the infection. In the present study, the prevalence of ETEC K99 in calves with mastitic dams did not significantly differ from those with non mastitic ones ($P = 0.12$). This result is supported by Riedel-Caspari (1993) who reported that in calves experimentally infected with ETEC, colostral leukocytes obtained from cows which developed clinical mastitis, showed a marked reduction in the number of shed bacteria. On the other hand, calves directly fed colostrum of mother cows, which had shown infectious mastitis during the dry period before delivery, suffered from diarrhea possibly induced by a pathogenic strain of *E. coli* (K99). Also, season was not found to be significantly associated with ETEC K99. This finding may be due to presence of narrow variations in the climatic conditions in the examined areas. On contrary, December and March were recorded to have the highest prevalence (Bendali et al., 1999).

Salmonella spp. were isolated from nine calves (4.09%); six of them were typed as *S. enterica* serovar Typhimurium and two of them were *S. enterica* serovar Enteritidis, whereas one case was non- *S. enterica* serovar Enteritidis and non- *S. enterica* serovar Typhimurium. Three cases have mixed infection by *S. enterica* serovar Typhimurium and ETEC K99. In the present study, the prevalence of *Salmonella* spp. were higher than that previously reported (Achá et al., 2004) who recorded 2% infection rate. On contrary, Langoni et al. (2004) recorded *S. enterica* serovar Typhimurium in 6.1% of the fecal samples. Moreover, Akam et al. (2004) reported that the susceptibility was higher during the end of the first month to *Salmonella* (66.6%). In this study, *Salmonella* spp. were examined only in diarrheic calves; however, subclinical fecal *Salmonella* shedding can persist in dairy herds for up to 18 months with no measurable effects on health or production of individual cows (Huston et al., 2002).

Final multivariate logistic regression model showed that age, hygiene and region were significantly associated with *Salmonella* spp. Shedding. Calf age was significantly associated with *Salmonella* spp. ($P < 0.05$; OR: 0.376; CI 95%: 0.511–1.369). On the contrary, calf age was not associated with *Salmonella* shedding (Fossler et al., 2005).

Hygiene recorded significant association with *Salmonella* infections in diarrheic calves ($P < 0.05$; OR: 0.628; CI 95%: 1.729–5.612). Farms related to governorates of Delta of River Nile had more infection rate than that of semi arid area of Behera governorate. This may be attributed to hygienic measures, which represented by infrequent cleaning of the boxes and transmission of infection from the neighboring farms by carriers. However, in farms located in Behera, the calves were reared on sandy area and boxes were cleaned daily. The low number of farms in this area and the far distance between farms may explain the lower infection rate. It was reported that the primary risk factors associated with the increased prevalence of *Salmonella* in water offered to weaned dairy calves were continuous water tank-filling method compared with a valve (Kirk et al., 2002). Moreover, disposal of manure in liquid form on owned or rented land was reported as a risk facto (Fossler et al., 2005).

Region was significantly associated with the prevalence of *Salmonella* spp. ($P < 0.01$; OR: 0.970; CI 95%: 0.841–1.624). Thus, seven cases with *Salmonella* spp. infection were recorded governorates of river Nile delta region. However, two cases only were recovered from calves raised in semiarid region of Behrra governorate. This result could be due to the system of management of calves, and the nature of environment. The farms located in Behera governorate is a semi arid region. Moreover, the farms were away from other human and animal buildings. This result is supported by that described by Davison et al. (2006) who recorded significant association between the region and prevalence of *Salmonella* infection in cattle. In a study carried out by Sidhu et al. (2008) it was found that viability of *S. enterica* serovar Typhimurium was affected by direct exposure to the sunlight, especially during summer. It is suggested that interaction of more than one factor could contribute for the occurrence of *Salmonella* spp. infection in newborn calves.

Mastitis, season, herd size, *E. coli, Cryptosporidium* spp. infection, parity, rotavirus infection, and coronavirus infection showed none significant association with *Salmonella* spp. infection in the present investigation. Although season had no significant association with *Salmonella* infection, previous reports recorded significant effect of the season on the prevalence of *Salmonella* infection in both calves and adult cattle where summer recorded the highest prevalence (Fossler et al., 2004, 2005) or during summer and autumn (Davison et al., 2006). Also, herd size not significantly affected the prevalence of *Salmonella* in diarrheic calves. This result coincides with that reported by Fossler et al. (2005). On contrast, Herd size was recorded to affect significantly on the occurrence of diarrhea (Warnick et al., 2003). Parity showed no significant association with *Salmonella* infection in the newborn calves. It has been reported that parity was not associated with *Salmonella* shedding (Fossler et al., 2005).

In the present study, PCR enabled identification of ETEC K99 and *Salmonella* spp. in neonatal calves. Although there are available

many diagnostic tests, PCR was found specific and convenient for large-scale screening of ETEC K99 (Franck et al., 1998; Cesaris et al., 2007). To the best of our knowledge, this is the first report for molecular screening of ETEC K99 and *Salmonella* spp. not only in Egypt but also in the Middle East and Africa. The results of the present study indicate that molecular screening with PCR would be helpful for rapid and accurate tool for identification of ETEC K99 and *Salmonella* spp. in diarrheic calves. Moreover, identification of the risk factors associated with the spreading of bacteria causing diarrhea, may be helpful for construction of suitable methods for prevention and control.

Acknowledgement

Authors are grateful to Professor Tadashi Shimamoto, Laboratory of Food Microbiology and Hygiene, Graduate school of Biosphere Science, Hiroshima University, Japan for providing us with all molecular technique facilities, reagent and kits used in this study.

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