Colibacillosis in Newly Born Buffalo Calves and Role of Lacteol Fort in Preventing Recurrence of Calf Diarrhea

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Abstract: *Escherichia coli* diarrhea (scours) of newborn calves is characterized by watery white or yellowish diarrhea, rapid onset, and high mortality. The objectives of this study were twofold. First, the role of *E. coli* infection in diarrheic newborn buffalo calves was assessed through analysis of clinical data, morbidity and mortality rates, presence of varying *E. coli* serotypes, and antimicrobial sensitivity of bacterial populations in diarrheic fecal samples. Second, we assessed the role of probiotic supplementation with Lacteol Fort in prevention of reoccurrence of calf diarrhea. Our study population consisted of 130 diarrheic newborn buffalo calves, aged 1–11 days, 35–45 kg in body weight, suckling normally during the winter season. The incidence of *E. coli* infection in diarrheic newborn calves was 39.23% (51 of 130 calves). The mortality rate in these infected calves was 25.49% (13 of 51 calves). Clinical findings in calves infected with *E. coli* varied, and included fever, growth depression, recumbency, dehydration, profuse watery yellowish diarrhea in some cases, profuse watery to pasty foul-smelling diarrhea in other cases, and occasional occurrence of yellowish to white diarrhea streaked with blood. The most common *E. coli* serotypes (with rate of detection) were O26 (23.52%), O103 (19.6%), and O119 (17.64%). Antimicrobial sensitivity tests on 51 fecal samples revealed that *E. coli* present in fecal samples was most sensitive (with rate of sensitivity) to marbofloxacin (96.07%), enrofloxacin (88.23%), cefotaxime (84.31%), amoxicillin (78.43%) and spectinomycin (33.33%) and more resistant to penicillin, neomycin, erythromycin, streptomycin, and chloramphenicol. The cure rate in calves supplemented with Lacteol Fort was 80% compared to 69.23% in calves in the unsupplemented group. The recurrence rate of diarrhea, assessed 15 days post treatment, was 8% in the Lacteol Fort group, compared with 26.92% in the unsupplemented group. We conclude that probiotic supplementation with Lacteol Fort can significantly reduce the recurrence of diarrhea in newborn buffalo calves.

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1. Introduction

Diarrhea is a major problem in livestock production in Egypt and throughout the world (**Farid *et al.*, 2001 and Ibrahim, 2007**). Enteritis in newborn calves causes high morbidity and mortality, leading to significant economic losses in Egypt (**Novert and Hammad, 2001 and Ashraf, 2007**). Diarrhea due to *Escherichia coli* (also known as scours) is one of the most common diseases of young calves, despite vaccination programs and management measures, necessitating treatment with antibiotics and fluid therapy (**Gyles, 1993**). *E. coli* diarrhea in newborn calves (9–10 days of age) is usually characterized by watery white or yellowish diarrhea, rapid onset and time course, and high mortality. In affected calves, diarrhea typically begins within 36–72 hours of birth, and affected calves die within 2–3 days. Some calves die several hours after appearing healthy and free of diarrhea.

Calf scours is not a single disease entity; it is a clinical syndrome associated with several diseases characterized by diarrhea. Regardless of the cause, absorption of fluids from the intestine is altered, leading to life-threatening electrolyte imbalances. The scouring calf loses fluids, rapidly dehydrates, and suffers from electrolyte loss and acidosis. Infectious agents may cause initial damage to the intestine, but death from scours usually results from dehydration, acidosis, and loss of electrolytes. Identification of infectious agents that cause scours is essential for implementation of effective preventive and treatment measures (**Radostits *et al.*, 2007**).

E. coli populations are divided into serotypes and serogroups on the basis of antigenic composition (somatic or O antigens, flagellar or H antigens, and capsular or K antigens; Campos ***et al.*, 2004**). In one study, the most common *E. coli* serotypes isolated from diarrheic fecal samples were O119, O111, O126, and O78 (**Tamaki *et al.* , 2005**). **Badouei *et al.* (2010)** isolated O157:H7, O111 and O26 serotypes of *E. coli* strains from 297 fecal samples, from 200 diarrheic and 97 non-diarrheic calves. The most predominant serogroup was O26 (18.4%).

Current treatment regimens for the treatment of neonatal calf diarrhea center on antimicrobial therapy and fluid therapy. Recently, there has been increased use of probiotics in treatment of neonatal calf diarrhea. Probiotics support the balance of intestinal flora, inhibit epithelial adhesion and invasion by pathogenic bacteria, and produce antimicrobial substances that are inhibitory to both gram positive and gram-negative bacteria (**Rolfe, 2000**). Probiotics are bacterial preparations that benefit the host by improving microbial balance and eliminating or reducing pathogenic microorganisms. In the case of diarrhea, a potential effect of probiotic bacteria is the production of enzymes that inactivate enterohemorrhagic *E. coli*, reducing its colonization of the gut and consequently lowering shedding of the pathogen (**Hughes *et al.,* 2010**).

The lactobacilli in Lacteol Fort are able to adhere to intestinal cells. By inhibiting the ability of pathogens to adhere to and invade intestinal cells, Lacteol Fort blocks the infectious process of diarrhea and stops symptoms rapidly (***Chauviere, 1992 and Coconnier, 1997***).

The aims of our study were twofold. First, we sought to determine the incidence of E. *coli* infection in diarrheic newborn buffalo calves and to examine clinical findings, morbidity and mortality rates, *E. coli* serotypes, and antimicrobial sensitivity of bacteria in diarrheic fecal samples. Second, we aimed to examine the role of Lacteol Fort in preventing the recurrence of calf diarrhea.

2. Material and Methods

A. Animals:

Our study population consisted of 130 buffalo calves from a dairy breeding buffalo farm at El-Bahiera Governorate, Egypt, aged 1–11 days, 35–45 kg in body weight, suckling normally during the winter season. Dams were not vaccinated against enteric infection. Of the 130 calves, 51 suffered from diarrhea.

B. Clinical examination and collection of samples:

Calves were examined clinically by veterinarian, and morbidity and mortality rates were documented. For assessment of colibacillosis, rectal swabs were taken from diarrheic calves using sterile cotton swabs (Boyd *et al.,* 1974) and kept on ice before storage in the laboratory.

C. Isolation and identification of *E. coli*:

Bacterial methods were previously described (Cruickshank *et al.,* 1975; Koneman *et al.,* 1992; and Quinn *et al.,* 1994).

D. Serological identification of *E. coli* isolates:

E. coli isolates from fecal samples were analyzed using monospecific antisera (SSI Diagnostica, Hillerød, Denmark) as previously described (Ewing, 1986). See Table 1 for a list of sera used in the analysis. Serological tests were performed by the Department of Clinical Microbiology, Central Health Laboratories, Ministry of Health, Cairo, Egypt.

Table 1. Monospecfic sera used in serological identification of *E. coli*

Anti-coli O25:(K11)	Anti-coli O114:(K90)
Anti-coli O26:(K60)	Anti-coli O118:(K-)
Anti-coli O44:(K74)	Anti-coli O119:(K69)
Anti-coli O55:(K59)	Anti-coli O124:(K72)
Anti-coli O78:(K80)	Anti-coli O125:(K70)
Anti-coli O86:(K61)	Anti-coli O126:(K71)
Anti-coli O91:(K-)	Anti-coli O127:(K63)
Anti-coli O25:(K11)	Anti-coli O128:(K67)
Anti-coli O103:(K-)	Anti-coli O142:(K86)
Anti-coli O111:(K58)	Anti-coli O157:(K-)

E. Antimicrobial sensitivity test:

Assessment of antimicrobial sensitivity in fecal isolates was performed as previously described (Quinn *et al.,* 1994). Results were interpreted according to Koneman *et al.,* 1992 (Table 2 for parameters).

Table 2. Parameters used in assessing antimicrobial sensitivity

Antimicrobial agent	Disc content	Inhibition zone diameter (mm)		
		Susceptible	Intermediate	Resistant
Marbofloxacin	10 µg	>30	20–24	<20
Enrofloxacin	10 µg	>25	14–15	<12
Gentamycin	10 µg	>15	13–14	<12
Erythromycin	15 µg	>18	14–17	<13
Cefotaxime	30 µg	>18	15–17	<14
Amoxicillin	10 µg	>29	21–28	<19
Penicillin	10 units	>29	20–28	<20
Tetracycline	30 µg	>19	15–18	<14
Chloramphenicol	30 µg	>18	13–17	<12
Streptomycin	10 µg	>15	12–14	<11
Trimeth/sulfa	1.25/23.75 µg	>16	11–15	<10
Spectinomycin	20 µg	>15	12–13	<10
Neomycin	20 µg	>18	15–17	<14

Antimicrobial sensitivity was assayed as described in (Koneman et al., 1992) and categorized as indicated in the table. Trimeth/sulfa: trimethoprim/sulfamethoxazole.

F. Treatment of *E. coli* diarrhea:

Calves were treated for diarrhea with the following reagents:

1. Antimicrobial therapy: Enroflox 10%, containing 10% enrofloxacin (El-Nasr, Egypt); and Finadyne, containing 50 mg/ml flunixin meglumine (Schering- Plough Animal Health, Germany).
2. Oral rehydration salts: Each sachet of 5.5 g contains glucose anhydrous 4.00 g, trisodium citrate anhydrous 0.51 g, sodium chloride 0.70 g, and
potassium chloride 0.30 g (Elamria Co., Egypt).
3. Probiotics: Lacteol Fort; each sachet contains 10 billion lactobacilli (*Lactobacillus delbruekii* and *Lactobacillus fermentum*; Rameda, Egypt).
4. Electrolyte therapy: Equal mixture of isotonic saline (85%), isotonic sodium bicarbonate (1.3%) and isotonic dextrose (5%).

The 51 diarrheic calves were classified into 2 treatments groups as follows.

1. Unsupplemented group (26 calves)

Enroflox 10%, 1 ml/20–40 kg body weight, i.m. for 3 successive days.

Oral rehydration therapy.

Finadyne, 2 ml/45 kg body weight, i.v. or i.m.

Fluid therapy: 1–3 liter fluid/calf, delivered 50–100 ml/kg body weight, i.v.

Milk intake reduced for up to 24 hours until clinical improvement.

2. Lacteol Fort group (25 calves)

Treatment was identical to that in the unsupplemented group, with the addition of probiotics: Lacteol Fort 3 sachets daily for 3 successive days.

3. Results

Table 3. Incidence of *E. coli* in diarrheic buffalo calves

Age	No. of calves examined	No. of calves infected with *E. coli*	% of calves with *E. coli* infection
3–4 days	46	14	30.43
5–7 days	32	21	65.62
8–11 days	52	16	30.76
Total	130	51	39.23

$\chi^2 = 5.85^{*}$ *Significant at $P = 0.05$

Table 4. Morbidity and mortality in calves infected with *E. coli*.

Age	No. of infected calves	Morbidity rate (%)	No. of dead calves	Mortality rate (%)
3–4 days	14	30.43	6	42.85
5–7 days	21	65.62	4	19.04
8–11 days	16	30.76	3	18.75
Total	51	39.23	13	25.49

$\chi^2 = 20.87^{***}$ *** Significant at $P < 0.001$

Morbidity and mortality are expressed as percentages of the total number of calves (diarrheic and unaffected) in each group.

Table 5. Clinical findings in diarrheic calves

Age (number of calves)	Clinical observations
3–4 days (14)	Six calves suffered from fever, growth depression, recumbency, dehydration, profuse watery yellowish diarrhea with foul odor, followed by death. Eight calves suffered from profuse watery to pasty diarrhea with foul odor, and refuse to suckle.
5–7 days (21)	Four calves suffered from profuse watery diarrhea, usually yellow to white color (sometimes streaked with blood) with foul odor, dehydration, recumbency, followed by death. Seventeen calves suffered from pasty diarrhea with foul odor.
8–11 days (16)	Three calves suffered from yellowish watery diarrhea, dehydration, recumbency with subnormal temperature, followed by death. Thirteen calves suffered from pasty diarrhea with foul odor.

Table 6. Prevalence of *E. coli* serotypes in fecal samples from diarrheic calves.

Serotype	Number of samples containing serotype (total 51)	Prevalence of serotype in diarrheic samples
O26	12	23.52
O44	2	3.92
O78	1	1.96
O86	3	5.88
O103	10	19.6
O111	3	5.88
O119	9	17.64
O125	2	3.92
O128	2	3.92
O157	3	3.92
Untyped	4	7.84

$\chi^2 = 22.55^{**}$ ** Significant at $P < 0.01$

Prevalence is expressed as a percentage of the total number of diarrheic calves.

Table 7. Antimicrobial sensitivity of bacteria in fecal samples from diarrheic calves.

Antimicrobial agent	Susceptible (S)		Moderately susceptible (M)		Resistant (R)	
	No. of samples	% of total	No. of samples	% of total	No. of samples	% of total
Marbofloxacin	49	96.07	2	3.92	0	0
Enrofloxacin	45	88.23	4	7.84	2	3.92
Gentamycin	9	17.64	25	49.01	17	33.33
Erythromycin	1	1.96	6	11.76	44	86.2
Cefotaxime	43	84.31	4	7.84	4	7.84
Amoxicillin	40	78.43	7	13.72	4	7.84
Penicillin	-	-	-	-	51	100
Tetracycline	4	7.84	19	37.25	28	54.90
Chloramphenicol	3	5.88	22	43.13	26	50.98
Streptomycin	3	5.88	13	25.49	35	68.62
Trimeth/sulfa	7	13.72	24	47.05	20	39.21
Spectinomycin	17	33.33	18	35.29	16	31.37
Neomycin	-	-	13	25.49	38	74.50

Sensitivity is expressed as a percentage of the total number of diarrheic calves.

Table 8. Cure rate and recurrence rate in diarrheic calves after different treatments.

Group	Cure rate from diarrhea		Recurrence of diarrhea	
	No.	%	No.	%
Unsupplemented group (26 calves)	18	69.23	7	26.92
Lacteol Fort group (25 calves)	20	80	2	8

$\chi^2 = 1.55^{***}$ *** Significant at $P < 0.001$

Rates are expressed as percentages of the total number of calves in each group.

4. Discussion

Incidence of *E. coli* in diarrheic newborn buffalo calves was 39.23% (51 of 130 calves). As shown in Table 3, incidence varied by age group, with calves aged 5–7 days showing much higher incidence (65.62%) than calves aged 3–4 days (30.43%) and 8–11 days (30.76%). Incidence of *E. coli* diarrhea in newly born calves was significantly different according to age at P = 0.05. These results suggest that calves aged 5–7 days were more susceptible to *E. coli* diarrhea than other age groups. Notably, *E. coli* was the most common bacteria isolated from fecal samples of diarrheic calves in previous studies (China *et al.,* 1998 and Harbby, 2002).

Morbidity rate and mortality rate in diarrheic calves infected with *E. coli* also showed age dependence. Overall morbidity was 39.23% (51 of 130 calves), and overall mortality was 25.49% (13 of 51 calves). As shown in Table 4, mortality rate was the highest (42.85%) in calves aged 3–4 days; mortality was much lower in calves aged 5–7 days (19.04%) and 8–11 days (18.75%). These results were significantly different according to age at P < 0.001. Enteritis in newborn calves is known cause high morbidity and mortality rates, leading to significant economical losses in Egypt (Novert and Hammad, 2001 and Ashraf, 2007).

Clinical findings in calves infected with *E. coli* are presented in Table 5. Our findings are consistent with previous reports on *E. coli* diarrhea (scours) in newborn calves (9–10 days of age). Scours is usually characterized by watery white or yellowish diarrhea, rapid onset and time course, and high mortality. In affected calves, diarrhea typically begins within 36 to 72 hours after birth, and the calves die within 2 to 3 days (El- Sawalhy, 1999, Radostits, *et al.,* 2007 and Mahmoud, 2009).

As shown in Table 6, the most common *E. coli* serotypes in isolated samples (and their prevalence) were O26 (23.52%), O103 (19.6%), and O119 (17.64%). Other serotypes detected (and their prevalence) were as follows: O86, O111, and O157 (5.88%); O44, O125, and O128 (3.92%); O78 (1.96%); and untyped *E. coli* (7.84%). There was a significant difference in the number of *E. coli* serotypes in fecal samples (P < 0.01). Our findings are similar to previous data that found the most common *E. coli* serotypes isolated from diarrheic fecal samples to be O119, O111, O126, and O78 (Tamaki, et al. 2005). Similarly, Badouei *et al.* isolated O157:H7, O111, and O26 serotypes from 297 fecal samples from 200 diarrheic and 97 non-diarrheic calves. The most common serogroup was O26 (18.4%).

Antimicrobial sensitivity tests showed that *E. coli* present in fecal samples were differentially sensitive to antimicrobial agents (Table 7). *E. coli* present in fecal samples were most sensitive to marbofloxacin (96.07%), enrofloxacin (88.23%), cefotaxime (84.31%), amoxicillin (78.43%), and spectinomycin (33.33%). The *E. coli* present in fecal samples were most resistant to penicillin, neomycin, erythromycin, streptomycin, tetracycline, and chloramphenicol. Our results agree with those of Sadiek and Sohair (1999) who studied antibiotic sensitivity of fecal samples from diarrheic calves and found that enrofloxacin was the antibiotic of choice for most bacterial isolates (including *E. coli*, *Salmonella* species, *Klebsiella* species, and *Proteus* species). In another study, *in vitro* sensitivity testing of isolated bacteria from the feces of diarrheic calves showed that ciprofloxacin, enrofloxacin, and gentamicin were the most effective drugs (El-Gaml *et al.,* 2001). Recent work has also shown that ceftiofur and enrofloxacin were highly efficient antibiotics in treatment of neonatal calf diarrhea, as indicated by antimicrobial sensitivity tests (Aba-Alkhalil and El-Naenaeey, 2003). In addition, most *E. coli* isolates from diarrheic calves have been shown to be resistant to kanamycin, gentamycin, chloramphenicol, tetracycline, and ampicillin (Sato *et al.,* 2005).

To assess the influence of probiotic supplementation on diarrhea in buffalo calves, we divided the 51 diarrheic calves into 2 groups: an unsupplemented group of 26 calves was treated with Enroflox 10%, oral rehydration therapy, Finadyne, fluid therapy, and milk intake reduction for up to 24 hours until clinical improvement; and a group of 25 calves was treated in the same way, but with Lacteol Fort supplementation. These lines of treatment for calf diarrhea have been previously described (El-Sawalhy, 1999, Radostits *et al.,* 2007 and Mahmoud, 2009).

Table 8 reveal the efficacy of Lacteol Fort (*lactobacillus* culture) in increasing cure rate and reducing recurrence rate of *E. coli* diarrhea. The cure rate in the Lacteol Fort group was 80%, compared to the 69.23% cure rate in the unsupplemented group. Importantly, the recurrence rate (defined as recurrence of diarrhea within 15 days post treatment) in treated calves was 8%, compared with 26.92% in the unsupplemented group. Differences in cure rate and recurrence rate were statistically significant at $P < 0.001$. Our results concur with those of Tkalcic *et al*. (2003) who found that diarrheic calves treated with probiotics experienced mild transient diarrhea and reduced fecal shedding of *E. coli* compared to control animals.

Probiotics exert beneficial effects through various means. Probiotics create conditions unfavorable for the growth of enteropathogens, reducing the occurrence of scour and restore balance of intestinal flora (El- Sawalhy, 1999). *Lactobacillus casei* plays an important role in the prevention of enteric infection by increasing IgA secretion in the intestinal lumen, providing a defense of the mucosal surface (Predigon *et al.,* 1991 and Predigon *et al.,* 1995). Probiotics prevent colonization and overgrowth of pathogenic bacteria by adherence to the intestinal wall (Drisko *et al.,* 2003). *Lactobacillus* species can prevent the adhesion of *E. coli* and *Salmonella typhimurium* to intestinal cells and induce the secretion of anti-inflammatory cytokines (Petros *et al.,* 2006). *Lactobacillus casei* has been shown to reduce some pathological outcomes (especially diarrhea) in calves (Erickson and Hubbard, 2000). These data are consistent with our findings that *E. coli* infection is prevalent and serotypically diverse in diarrheic buffalo calves and that probiotic treatment can substantially improve recovery from diarrhea by increasing cure rates and decreasing recurrence.

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References

Aba-Alkhalil, and El-Naenaeey, E.Y. (2003): Evaluation of antimicrobial action of ceftifour sodium (Excenel), *in vitro* and *vivo*. Suez canal Vet.Med. J., VI (2):42-51.

Ashraf, N.M.R. (2007): Enzootic gram negative bacteria associated with diarrhea in neonates in Egypt. Ph.D Thesis, Dept. of Microbiol., Fac. Vet. Med., Alex. Univ.

Badouei, M.A., Salehi, T.Z., Khorasgani, M.R., Tadjbakhsh, H., Brujeni, G.N. and Nadalian, M.G. (2010): Virulence gene profiles and intimin subtypes of Shiga toxin-producing Escherichia coli isolated from healthy and diarrhoeic calves. Vet Rec. 167(22):858-61.

Boyd, J.W., Walker, J.R., and Leyland, A. (1974): Neonatal diarrhea in calves. Vet. Rec., 95(14): 310-313.

Campos, L.C., Franzolin, M.R. and Trabulsi, L.R. (2004): Diarrheogenic Escherichia coli categories among the traditional enteropathogenic *Escherichia coli* O serogroups- a review Mem. Inst. Oswaldo Cruz.; 99(6): 545-552.

Chauviere, G. (1992): Competitive exclusion of diarrheagenic Escherichia coli (ETEC) from human enterocyte-like Caco-2 cells by heat-killed *lactobacillus*. FEMS Microbiology Letters. 81:213-218.

China, B., Pirson, V. and Mainil, J. (1998): Prevalence and molecular of attaching effacing E.coli among calf populations in Belgium. 63(2-4): 249-259.

Coconnier, M.H. (1997): Antibacterial effect of the adhering human *Lactobacillus acidophilus* Strain LB. Antimicrobial Agents and Chemotherapy, 41(5): 1046-1052.

Cruickshank, R., Duguid, J.P., Marion, B.P. and Swain, R.H.A. (1975): Medical Microbiology, Vol.

2, The Practice of Medical Microbiology. 12th Ed., Churchill Livingstone, Edinburgh, London.

Drisko, A.J., Giles, C.K. and Bischoff, B.J. (2003): Probiotics in health maintenance and disease prevention. Altern. Med.Rev., 8:143-155.

El- Sawalhy, A. A. (1999): "Veterinary Infectious Diseases" 2nd Edit. Pps. 329-336. Ahram Distribution Agency, Egypt.

El-Gaml, A.M., Hashem, M.EL. and Hatab, M. (2001): Diarrhea in kids attributed to enterobacteria and cryptosporidium. Assiut Vet. Med. J. ,45(89):132-141.

Erickson, K.L. and Hubbard, N.E. (2000): Probiotic immunomodulation in health and disease. J. Nutr., 130:403s-409s.

Ewing, W.H. (1986): Edwards and Ewing's identification of Enterobacteriaceae, 4th edition, pp, 135-172. New York: Elsevier.

Farid, A., Eid, G.E., Abdel- Mawla, Y.R. and Nagat, A.S. (2001): Evaluation of the efficacy of Escherichia coli (K99) vaccine on the incidence of *Escherichia coli* and immunity in buffaloes. Vet. Med. J., Giza, 49(3): 385-399.

Gyles, ,C.L. (1993): *Escherichia coli*. In: Gyles CL, Thoen CO, eds. Pathogenesis of Bacterial Infections in Animals, 2nd ed, Ames, Iowa: Iowa State Univ Pr, 164–187.

Harbby, H.A. (2002): Bacterial causes of diarrhea in small animals (Kids, lambs and calves) in Sultanate of Oman. J. Egypt. Vet. Med. Ass., 62(3): 227-235.

Hughes, D.T., Terekhova, D.A., Liou, L., Hovde, C.J., Sahl, J.W. and Patankar, A.V. (2010): Chemical sensing in mammalian host-bacterial commensal associations. Proc Natl Acad Sci USA.107:9831–9836.

Ibrahim, E.D. (2007): Studies on microbial causes of diarrhea in calves. M.V.Sc. Thesis, Fac. Vet. Med., Kafr El-Sheikh Univ.

Koneman, E.W.; Allen, S.D.; Dowell, V.R.; Janda, W.H. and Sommers, H.M. (1992): Color atlas and Textbook of Diagnostic Microbiology. 4TH Ed., J.B. Lippincott CO., New York.

Mahmoud, A.A. (2009): Animal infectious diseases. 7th Ed. PPs. 298-302. Faculty of Veterinary Medicine, Alexandria University, Egypt.

Novert, A. and Hammad, A.(2001): Studies on mycotic and bacterial enteritis. J. Egypt. Vet. Med. Assoc, 61(6 B): 190-201.

Oxoid (1987): Agents and main distributors, the manual, 6th Ed.

Petros, A. Maragkoudakis, Gaorgia Zoumpopouleu, Christes Mlaris, Gaorge Kaalantzepoules, Brune Pet and Effie Tsakalldeu (2006): Probiotic potential of *lactobacillus* strains isolated from dairy products. Int. Dairy J. ,16 (3): 189-194.

Predigon, G., Alvares, S. and Ruzi Holgado, A. (1991): Adjuvant activity of oral *Lactobacillus casei*: influence of dose on the secretary immune response and protective capacity in intestinal infections. J. Dairy Res., 58:485-496.

Predigon, G., Alvares, S., Rachid, M., Aguero, G. and Gobbato, N. (1995): Immune system stimulation of probiotics. J. Dairy Sci., 78:1597-1606.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology. Mosby. Yearbook Europe Limited.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007): Veterinary Medicine, 10th edition. PP. 847-888. Saunders, Philadelphia.

Rolfe, R.D. (2000): The role of probiotic cultures in the control of gastrointestinal health. J. Nutr., 130 (2 suppl): 396s- 402s.

Sadiek, A. and Sohair, Z.H. (1999): Acute enteritis in neonatal calves in Sohag governorate with special reference to aetiology, clinico-chemical aspects and therapy. Assuit Vet. Med. J., 42(83):200-212.

Sato, K., Baretlett, P.C. and Saeed, M.A. (2005): Antimicrobial susceptibility of *E. coli* isolates from dairy farms using organic versus conventional production methods. J. Am. Med. Assoc., 226 (4): 589-594.

Tamaki, Y., Narimatsu, H., Miyazato, T., Nakasone, N., Toma, C., and Iwanaga, M., (2005): The relationship between O antigens and pathogenic genes of diarrhea- associated *E. coli.* Jpn. J. Infect. Dis., 58: 65-69.

Tkalcic, S., Zhao, T., Harmon, B.G., Doyle, M.P., Brown, C,A. and Zhao, P.(2003): Fecal shedding of enterohemorrhagic *E. coli* in weaned calves following treatmrnt with probiotic *E. coli.* J. Food Prot. 66(7): 1184-9.

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