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Occupational Exposure of Buffalo Gynaecologists to Zoonotic Bacterial Diseases

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Abstract: In the present study, gynaecological examinations were carried out on 916 buffaloes and samples of vaginal swabs, blood and milk were collected. Serum samples were checked for brucellosis and assayed for progesterone level. Vaginal swabs and milk samples were examined for zoonotic bacteria that may be transmitted to veterinarians during handling and examination of these animals during the different phases of the reproductive cycle. 1.09% of the serum samples were positive for brucella antibodies. Zoonotic bacteria were isolated from vaginal swabs (*E. coli*, *Y. enterocolitica*, *Klebsiella* sp., *E. faecalis*, *S. aureus* and *Bacillus* sp.) and milk samples (*E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Serratia marescens*, *S. aureus* and *Streptococcus agalactiae*). PCR analysis showed that *E. coli* O157 and O119 isolated from animal suffering from ovarian inactivity were positive for the toxigenic genes (*VT-II*, *stx-2* and *eae-A*). It can be concluded that risk of development of a zoonotic disease can be lessened by early recognition of infected animals, proper animal handling, basic biosecurity precautions and most importantly, personal hygiene.

Key words: Occupational, veterinary gynecologists, zoonotic diseases, buffaloes, PCR, toxigenic genes

INTRODUCTION

The world buffalo population is 160 million heads which represent an integral part of the agricultural economy in many developing countries (FAO, 2005). However, this species tends to suffer from a lot of reproductive disorders, mainly inactive ovaries and long calving interval (Ahmed *et al*., 2006) and subjected to gynaecological intervention in higher frequency than other species.

Injuries and other occupational hazards reported together with work days lost demonstrate a need for improving the working environment of veterinarians and their staff and the development of comprehensive health and safety programs in general. One of the inherent risks in the practice of veterinary medicine is exposure to zoonotic agents. In an Australian survey, 4% of veterinarians were reported to have acquired zoonotic diseases (Hill *et al*., 2000; Jeyaretnam *et al*., 2000). Also, a variety of zoonotic diseases may be encountered in animal practice, including, *S. aureus* infection, *Cl. difficile*-associated diarrhea, salmonellosis, campylobacteriosis, dermatophytosis and blastomycosis (Weese *et al*., 2002). Moreover, It was recorded that veterinary personnel can be infected with *L. interrogans* via contact by the urine or tissues from an infected animal with mucous membranes or skin lesions (Tan, 1997). This organism may cause human disease ranging from mild and self-limiting to fatal. Brucellosis is among zoonotic diseases which is associated with chronic serious sequel in humans. It is one of the most common occupational health hazard (Robichaud *et al*., 2004). Veterinary gynecologists may be infected during vaginal delivery, a cesarean delivery and a necropsy on a stillborn calf (Corbel, 1997).

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A lot of microorganisms were isolated from the genital tract of buffalo-cows during the different reproductive stages including enterohemorrhagic *E. coli*, *Y. enterocolitica*, *Salmonella* sp., *Klebsiella* sp., *Micrococcus* sp., *C. diversus*, *P. vulgaris*, *P. mirabilis*, *P. multocida*, *S. aureus*, *S. bovis*, *C. bovis* and *E. faecalis* (Abd El Moez, 2007). Most of these microorganisms have zoonotic importance.

There is a shortage in data concerning zoonotic diseases that can be transmitted to veterinarians dealing with buffalo's reproduction; therefore in this study light will be thrown on those diseases that may cause a risk for veterinarians carried out gynaecological examination for this species.

MATERIALS AND METHODS

The current research was carried out on 916 female buffaloes reared in form of small holder farms at Lower Egypt. Field trips were weekly carried out during the period from July 2004 to March 2007 as a part of the National Research Center Project No. 7120106. Animal's case history and general health status were recorded. Gynaecological examination was carried out by rectal palpation, vaginal inspection and udder examination. Blood (916 cases), milk (318 cases) and vaginal swab (916 cases) samples were collected.

Serum Examination

Serum samples were harvested from blood samples by centrifugation (1500 x g, for 15 min at 4°C). Samples were checked for brucella antibodies using Rose Bengal plate test (Alton *et al*., 1988) and progesterone level was assayed by micro ELISA method (Hubl *et al*., 1982) to confirm the results of the gynaecological examination.

Bacteriological Examination of Vaginal Swabs

Vagina was dry cleaned and vaginal swabs were collected under possible aseptic conditions from the anterior vagina using the rectovaginal technique (Youngquist, 1997). Swabs were inoculated into tryptic soy broth and incubated at 37°C for 24 h. Suspected colonies appearing on the different media were identified biochemically (Holt *et al*., 1994).

Bacteriological Examination of Milk Samples

Milk samples were aseptically collected from all lactating animals, either they are apparently healthy or suffering from clinical mastitis. Ten milliliter of each milk sample was centrifugated for 20 min at 3000 rpm. Sediment was seeded onto plates of nutrient agar, MacConkey agar and blood agar which were incubated at 37°C for 48 h. Suspected colonies appearing on the different media were identified (Holt *et al*., 1994). The recovered *Salmonella* isolates were serologically identified using the diagnostic polyvalent and monovalent antisera (Kauffmann, 1972).

Further Studies on *E. coli* Isolates

E. coli isolates were subjected to serological identification by the slide agglutination test (Edwards and Ewing, 1972) using standard polyvalent and monovalent *E. coli* antisera. DNA from *E. coli* isolates was extracted (Sritharan and Barker, 1991). Detection of Shiga toxin type 2 (*stx2* encoded by *stx2*) and intimin gene (encoded by *eae-A*) in the extracted DNA of *E. coli* (serotypes O28, O126, O157, O119 and O78) was carried out by multiplex PCR (Paton and Paton, 1998). The following primers were used; *stx2F* (GGCACTGTCTGAAACTGCTCC), *tx2R* (TCGCCAGTTATCTGACATTCT), *eaeAF* (GACCCGGC-ACAAGCATAAGC) and *eaeAR* (CCACCTGCAACAA-GAGG). Also, PCR amplification of the Verotoxin-II from the *E. coli* isolates was done (Ramotar *et al*., 1995) using the forward and reverse primers (*VT-IIF*-TTAACCACACCCACGGCAGT and *VT-IIR*-GCTCTGGATGCATCTCT-GGT). Agarose gel electrophoresis was carried out according to Sambrook *et al*. (1989).

Statistical Analysis

Statistical analysis was carried out using Student t-test and Analysis of Variance as outlined by Snedecor and Cochran (1980).

RESULTS

Serum Examination

Results showed that 10 out of 916 (1.09%) serum samples were positive for brucella antibodies. Regarding serum progesterone; the level (ng mL^{-1}) was significantly ($p<0.01$) higher during the luteal phase (4.62±0.95) as compared to the follicular phase (0.52±0.15) in normal cyclic animals. In pregnant buffalo-cows, the level was significantly varied ($p<0.01$) with the highest value during mid stages (7.36±0.31) and the lowest value during late stages (1.29±0.64). After calving, the level was the lowest during 2-4th week and the highest during 5-12th week post-partum. The level was undetectable in animals suffering from bilateral smooth inactive ovaries.

Bacteriological Examination of Vaginal Swabs

Zoonotic bacteria isolated from the vaginal swabs of 916 female buffaloes during normal estrous cycles, pregnancy, post partum periods and ovarian inactivity are shown in Table 1. It was evident that the most predominant isolates were *E. coli*, *Y. enterocolitica*, *Micrococcus* sp. and *E. faecalis* (normal estrous cycles and pregnancy), *E. coli*, *S. aureus* and *S. pyogenes* (post partum) and *E. coli*, *S. aureus*, *S. pyogenes* and *Klebsiella* sp. (ovarian inactivity). The rate of isolation in animals with ovarian inactivity was significantly ($p<0.001$) higher (3.48±0.25) as compared to the normal cyclic animals (2.70±0.33).

Bacteriological Examination of Milk Samples

One hundred and ninety-seven out of the examined 318 milk samples (61.95%) were positive for bacterial isolation. Table 2 shows the isolated bacteria from milk samples of apparently normal and mastitic buffalo-cows. The incidence of isolation was clearly high in the first (38.99%) as compared

Table 1: Bacteria isolated from the genital tract of buffalo-cows during the different reproductive stages (%)

Bacterial isolates	Normal cycle (N = 176)	Pregnancy (N = 204)	Post partum (N = 195)	Ovarian inactivity (N = 341)
Gram negative				
E. coli	73.21	78.58	83.09	80.00
Y. enterocolitica	38.88	27.98	12.15	19.35
Klebsiella sp.	20.40	16.29	16.85	30.00
Protus multocida	9.89	1.59	5.33	13.88
Protus mirabilis	4.85	1.35	7.57	6.00
Protus vulgaris	2.90	1.99	15.33	15.07
Salmonella sp.	-	5.06	-	6.00
Shigella sp.	-	-	-	2.55
Gram positive				
Micrococcus sp.	42.01	53.00	21.00	13.52
E. faecalis	39.19	37.20	14.00	12.00
S. epidermidis	7.00	19.98	10.45	5.40
S. aureus	3.95	8.00	31.90	46.70
S. pyogenes	1.79	-	22.55	31.40
S. bovis	-	2.88	15.09	3.00
C. bovis	4.15	-	-	5.26
Bacillus sp.	19.00	9.10	22.00	13.90

N = Number

Table 2: Bacteria isolated from milk samples obtained from apparently healthy and clinical mastitic buffalo-cows (%)

Bacterial isolates	Apparently healthy animals (N = 124)	Mastitic animals (N = 73)
Gram negative		
E. coli	54.84	36.90
Klebsiella pneumoniae	23.39	11.60
S. typhimurium	14.51	5.20
Serratia marescens	7.26	2.90
Gram positive		
S. aureus	-	38.00
S. agalactiae	-	5.40

N = Number

Table 3: Serotyping and toxigenic genes of *E. coli* strains isolated from the genital tract of buffalo-cows

		Toxigenic genes		
Animal condition	Serotyping (Monovalent)	*VT-II*	*stx-2*	*eae-A*
Luteal phase	O28	-	-	-
Luteal phase	Untypable	-	-	-
Luteal phase	O126	-	-	-
Pregnancy	Untypable	-	-	-
Pregnancy	O28	-	-	-
Inactive ovaries	O157	+	+	+
Inactive ovaries	O119	+	+	+
Inactive ovaries	O78	-	-	-

VT-II = Verotoxin-II, *Stx-2* = Shiga toxin type 2, *eae-A* = Intimin gene, + = Positive, - = Negative

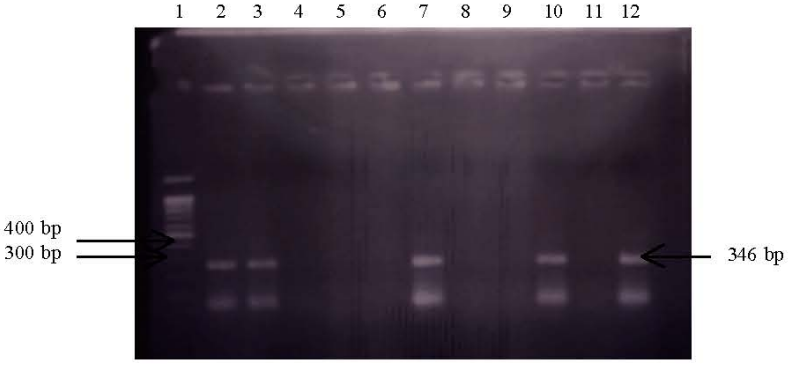

Fig. 1: Agarose gel electrophoresis showing amplification of 346 bp of *VT-II* gene lanes 2, 3, 7 and 10 while lanes 4, 5, 6, 8, 9 and 11 are negative. Lane 1 is DNA marker and lane 12 is a control positive

to the later (22.96%) group. *E. coli*, *K. pneumoniae*, *S. typhimurium* and *Serratia marescens* were isolated from apparently normal animals, while, *E. coli*, *K. pneumoniae*, *S. typhimurium*, *Serratia marescens*, *S. aureus* and *S. agalactiae* were isolated from mastitic animals.

Further Studies on *E. coli* Isolates

Serotyping of *E. coli* isolates recovered from the vaginal swabs of buffalo-cows using antisera against pathogenic strains indicated that serotypes O28 were prevailed in normal animals during luteal phase and pregnancy, O126 in normal animals during luteal phase and O78, O119 and O157 in animals suffering from ovarian inactivity (Table 3). PCR (Verotoxin II) and multiplex PCR (Shiga toxin-2 and intimin) revealed that *E. coli* of serotypes O157 and O119 were positive for the tested toxigenic genes (*VT-II*, *stx-2* and *eae-A*); while serotype O78 was negative for all the tested toxigenic genes, as shown in Table 3 and Fig. 1-2.

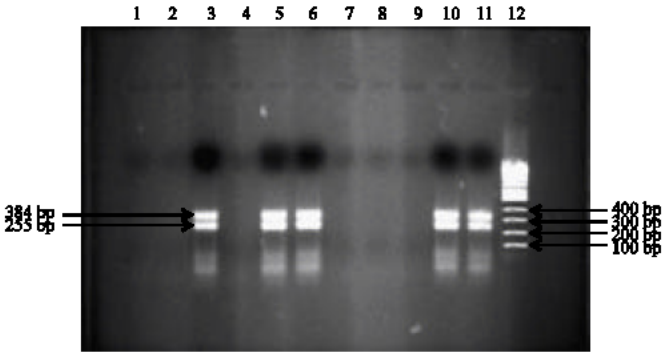

Fig. 2: Agarose gel electrophoresis showing multiplex PCR amplification of 384 and 255 bp of Intimin (*eae-A*) and shiga toxin-2 (*stx-2*) genes in lanes 3, 5, 6 and 10 while lanes 1, 2, 4, 7, 8 and 9 are negative. Lane 12 is 100 bp ladders and lane 11 is a control positive

DISCUSSION

The world population of buffaloes stands at approximately 160 millions and about 80% of these animals are located in India, China and Pakistan (FAO, 2005). There is also considerable number of buffaloes in Southeast Asian countries, Australia, North Africa and the Mediterranean countries, Italy and Bulgaria. Also, a sizeable population exists in South America, mainly in Brazil (Beg and Totey, 1999). Buffaloes contributes 10% of the world's total milk production, virtually all of which (>99%) is produced in developing countries (Shah, 1988). Regarding meat production, an estimated 1.6 million metric tons of buffalo meat is produced annually (Agarwal and Tomer, 1998). So there is an increasing worldwide interest for buffalo breeding, however, this species is reputed for high frequency of genital disorders and continuous genital intervention and there is an urgent need to know the zoonotic risk that might affect buffalo gynecologist.

In the present study, 1.09% of the examined serum samples were positive for brucella. In high risk occupations, living in Lebanon, Araj and Azzam (1996) recorded seroprevalence of Brucella-specific antibodies based on ELISA IgG, IgM and IgA in 57, 61 and 26%, respectively. Corbel (1997) reported nine persons participated in an attempted vaginal delivery, a cesarean delivery and a necropsy on a stillborn calf that died because of *Brucella abortus* infection. Omer *et al*. (2002) found that the highest prevalence of brucellosis among high risk occupational groups using Rose Bengal and complement fixation tests is among dairy farm workers and owners (7.1%), followed by veterinary personnel (4.5%). Mudaliar *et al*. (2003) recorded prevalence of brucellosis of 5.33% in animal handlers and advised that the clinician should keep in mind the possibility of an occupational or environmental exposure in cases of fever of unknown origin. Progesterone level is assayed in this study to confirm the present status of ovarian activity since it is secreted mainly from corpora lutea in buffaloes (Ahmed *et al*., 2006).

Microbiological examination of the vaginal swabs and milk samples of buffalo cows in this study indicated that most of the isolated bacteria have zoonotic importance. These included *E. coli*, *Y. enterocolitica*, *Klebsiella* sp., *Micrococcus* sp., *E. faecalis*, *S. aureus* and *Bacillus* sp. (vaginal isolates) and *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Serratia marescens*, *S. aureus* and *Streptococcus agalactiae* (milk samples). These microorganisms may cause serious diseases in buffalo's veterinary gynecologists particularly immunosuppressed personnel. In the same time, the incidence of isolation of these zoonotic pathogens is higher in animals suffering from genital disorders; represented by inactive ovaries which is the highest disorder that influence buffalo productivity and

needs more gynaecological intervention. Moreover, animals with inactive ovaries have great affinity for infection due to their lower immune response (Ahmed *et al*., 1993, 2006; Subandrio *et al*., 2000).

E. coli isolates were subjected to serotyping and PCR for diagnosis of toxigenic genes for three reasons. Firstly, *E. coli* was the most predominant organism among all the isolated and identified microorganisms. Secondly, human infection with shiga toxin-producing *E. coli* (STEC) is potentially fatal and may be associated with serious complications such as Hemolytic-Uremic Syndrome (HUS) and hemorrhagic colitis (Griffin, 1995). Thirdly, cattle have been implicated as a principle reservoir of STEC (Blanco *et al*., 1997).

PCR (Verotoxin II) and multiplex PCR (Shiga toxin-2 and intimin) revealed that *E. coli* isolated from animals with ovarian inactivity (serotypes O157 and O119) were positive for the tested toxigenic genes (*VT-II*, *stx-2* and *eae-A*). Wells *et al*. (1991) stated that the majority of outbreaks and/or sporadic cases of hemorrhagic colitis and HUS have been caused by members of only a few serogroups, such as O26, O111 and O157. The ability of STEC strains to cause serious disease in human is related to their ability to produce one or more shiga toxins (*stx1*, *stx2* and variants of *stx2*) (Boerlin *et al*., 1999).

It could be concluded that potential exposure to zoonotic diseases is an inherent risk in veterinary gynecologists. While, it is virtually impossible to completely prevent exposure to zoonotic agents, measures can be taken to protect veterinarians and staff from acquiring infections. If attention is paid to awareness of disease with zoonotic potential, early identification of infected animals, proper handling and housing and personal hygiene, the risks to veterinary personnel can be greatly reduced. Moreover, animals that appear healthy must not be dismissed as possible sources of zoonotic pathogens, as some animals may be asymptomatic carriers.

REFERENCES

Abd El Moez, S.I., 2007. Bacterial profile of the genital tract of female buffaloes during the different reproductive stages. Ph.D Thesis, Cairo University, Egypt.

Agarwal, S.K. and O.S. Tomar, 1998. Reproductive technologies in buffalo. Indian Veterinary Research Institute, Izatnagar, India.

Ahmed, W.M., A.R. Nada and S.T.A. Shalaby, 1993. Uterine, hormonal and cellular immune response in some cases of genital disorders in buffaloes. Reprod. Dom. Anim., 28: 298-301.

Ahmed, W.M., H.H. El-Khadrawy and Abd El-Hameed, 2006. Applied investigations on ovarian inactivity. Proc. The 3rd Int. Conf. Vet. Res. Div., NRC, Egypt, pp: 1-16.

Alton, G.G., L.M. Jones, R.D. Angus and J.M. Verger, 1988. Techniques for the Brucellosis Laboratory Institute. Institute Nationale de le Rech. Ayron, Paris, pp: 174.

Araj, G.F. and R.A. Azzam, 1996. Seroprevalence of brucella antibodies among persons in high-risk occupations in Lebanon. Epidemiol. Infect., 117: 281-288.

Beg, M.A. and S.M. Totey, 1999. The oestrous cycle, oestrous behaviour and the endocrinology of the oestrous cycle in the buffalo (*Bubalus bubalis*). Anim. Breed. Abst., 67: 5-11.

Blanco, M., J.E. Blanco, J. Blanco, A. Mora, C. Prado, M.P. Alonso, M. Mourino, C. Madrid, C. Balsalobere and A. Juarez, 1997. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet. Microbiol., 54: 309-319.

Boerlin, P., S.A. McEwen, E. Boerlin-Petzold, J.B. Wilson, R.P. Johonson and G.L. Gyles, 1999. Associations between virulence factor of shiga toxin-producing *Escherichia coli* and disease in humans. J. Clin. Microbiol., 37: 497-503.

Corbel, M.J., 1997. Brucellosis: An overview. Human exposure to *Brucella abortus* strain RB51-Kansas. Emerg. Infect. Dis., 3: 213-221.

Edwards, P.R. and W.H. Ewing, 1972. Identification of *Enterobacteriaceae*. 3rd Edn., Minneapolis, Burgess Cp. Atlanta, USA.

FAO, 2005. Food and Agriculture Organization, Year Book of Production, United Nation.

Griffin, P.M., 1995. *Escherichia coli* O157:H7 and Other Enterohemorrhagic *Escherichia coli*. In: Infections of Gastrointestinal Tract. Blaser, M.J., P.D. Smith, J.I. Ravdin, H.B. Greenberg and R.L. Guerrant (Eds.), Raven Press, New York, pp: 739-761.

Hill, S.L., J.M. Cheney, G.F. Taton-Allen, J.S. Reif, C. Bruns and M.R. Lappin, 2000. Prevalence of enteric zoonotic organisms in cats. JAVMA., 216: 687-692.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore.

Hubl, W., T. Fehert, W. Ronde, G. Domer, H. Taubert and E. Feymann, 1982. Determination of progesterone. Endocrinology, 79: 165-171.

Jeyaretnam, J., H. Jones and M. Phillips, 2000. Disease and injury among veterinarians. Aust. Vet. J., 78: 625-629.

Kauffmann, F., 1972. Serological Diagnosis of *Salmonella* Species. Kauffmann White Scheme Minkagaard, Copenhagen, Denmark.

Mudaliar, S., A. Bhore and D. Pandit, 2003. Detection of antibodies to *Brucella abortus* in animal handlers. Indian J. Med. Sci., 57: 181-186.

Omer, M.K., T. Assefaw, E. Skjerve, T. Tekleghiorghis and Z. Woldehiwet, 2002. Prevalence of antibodies to *Brucella* sp. and risk factors related to high-risk occupational groups in Eritrea. Epidemiol. Infect., 129: 85-91.

Paton, A.W. and J.C. Paton, 1998. Detection and characterization of shiga toxigenic *E. coli* by using multiplex PCR assays for *stx-1*, *stx-2*, *eae-A*, enterohemorrhagic *E. coli*, *hlyA*, *rfb* and *rfb*. J. Clin. Microbiol., 36: 598-602.

Ramotar, K., B. Waldhart, D. Church, R. Szumaki and T.J. Louie, 1995. Direct detection of verotoxin-producing *Escherichia coli* in stool samples by PCR. J. Clin. Microbiol., 33: 519-524.

Robichaud, S., M. Libman, M. Behr and E. Rubin, 2004. Prevention of Laboratory-acquired brucellosis. Clin. Infect. Dis., 15: 119-122.

Sambrook, J., E.F. Fritscgh and T. Meniates, 1989. Molecular Cloning. A Laboratory Manual. Vol. 1, Cold Spring Harbor Laboratory Press, New York.

Shah, S.N., 1988. Comparative studies of seasonal influence on breeding behaviour and conception rate of dairy buffalo and zebu cattle. Proc. the 11th Int. Congr. Anim. Reprod and AI, pp: 538-541.

Snedecor, G.R. and R.G. Cochran, 1980. Statistical Methods. 7th Edn., Iwoa State Univ. Press, USA.

Sritharan, V. and R.H. Barker, 1991. A simple method for diagnosing *M. tuberculosis* infection in clinical samples using PCR. Mol. Cell Probes, 5: 385-395.

Subandrio, A.L., I.M. Sheldon and D.E. Noakes, 2000. Peripheral and intrauterine neutrophil function in the cow: The influence of endogenous and exogenous sex hormones. Theriogenology, 53: 1591-1608.

Tan, J.S., 1997. Human zoonotic infections transmitted by dogs and cats. Arch. Int. Med., 157: 1933-1943.

Weese, J.S., A.S. Peregrine and J. Armstrong, 2002. Occupational health and safety in small animal veterinary practice: Part I-nonparasitic zoonotic diseases. Can. Vet. J., 43: 631-636.

Wells, J.G., L.D. Shipman, K.D. Greene, E.G. Sowers, G.H. Green, D.N. Cameron, F.P. Downes, M.L. Martin, P.M. Griffin, S.M. Ostroff, M.E. Potter, R.W. Tauxe and I.K. Wachsmuth, 1991. Isolation of *E. coli* serotype O157:H7 and other shiga like toxin producing *E. coli* from dairy cattle. J. Clin. Microbiol., 29: 985-989.

Youngquist, R.S., 1997. Current Therapy in Large Animals. Theriogenology, W.B. Sunders Co., USA.