PUBLIC HEALTH IMPORTANCE OF CERTAIN BACTERIA ISOLATED FROM CALVES AND SMALL RUMINANTS

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SUMMARY

A total of 173 samples from different sources (104 rectal swab from diarrhoeic calves, lambs and kids, and 69 nasal swabs from calves and lambs having respiratory manifestations) were examined bacteriologically. The bacteriological examination of rectal swab from diarrhoeic calves revealed the isolation of E. coli (82.0%), Campylobacer jejuni (16.0%), Salmonella typhimurium (4.0%), Proteus spp. (30%) and Pseudomonas aeruginosa (4.0%). In diarrhoeic lambs the most common bacteria isolated were E. coli (80.95%), Campylobacer jejuni (14.29%), Salmonella typhimurium (2.38%), Proteus spp. (23.81%) and Pseudomonas aeruginosa (7.14%). In addition, E. coli (83.33%), Proteus spp (8.33%) and Pseudomonas aeruginosa (16.67%) were isolated from rectal swab of diarrhoeic kids. Moreover, the bacteriological examination of nasal swabs revealed the isolation of E. coli (15.15%), Pasteurella multocida (9.09%), Pasteurella haemolytica (9.09%), Pseudomonas aeruginosa (3.03%) and Staphylococcus aerus (6.06%) from examined calves samples. Also, isolation of E. coli (16.67%), Pasteurella multocida (2.78%), Pasteurella haemolytica (8.33%), Pseudomonas aeruginosa (16.67%) and Staphylococcus aerus (5.56%) from examined lamb samples. The virulence factors of isolated E. coli including verotoxin and enterotoxin production were detected. Verotoxin was detected in 31.71, 44.12 and 25.0% of E. coli isolated from diarrhoeic calves, lambs and kids respectively, and in 20.0 and 33.33% from calves and lambs suffering from respiratory manifestations respectively. In addition, detection of enterotoxin production by PCR revealed that 7.32% of E. coli were positive in diarrheic calves, 17.65% in diarrheic lambs and 8.33% in diarrhoeic kids. The public health importance of each isolated bacteria were discussed.

INTRODUCTION

A number of infectious agents are implicated as a cause of infections among farm animals and human beings. Among the most important bacterial causes of disease in animals and man are E. coli, Salmonella spp., Campylobacter spp. Pseudomonas aeruginosa, Pasteurella spp. and Staph. aureus (Holland, 1990, Acha and Szyfers, 1991 and Khan and Khan, 1997).

Animal can transmit many pathogenic and potentially pathogenic bacteria to human beings through contamination of human food with secretions and excretions of such animals, handling animal carcasses during slaughtering and skinning or as a result of ingestion of meat of infected animals and/or contact with diseased animals (Acha and Szyfers, 1991).

Although E. coli belongs to the normal flora present in the gastrointestinal tract, certain E. coli strains have been associated with diarrhoea in man and animals (Oswald et al., 1994). There are four major categories of diarrhoeagenic E. coli, namely: enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC) (levine, 1987). ETEC was well recognized as an important cause of diarrhoea among calves, lambs and man (Levine, 1987 and Quinn et al., 1994). In addition, verocytotoxigenic E. coli (VTEC) was isolated from cattle and sheep with and without diarrhoea (Burnens et al., 1995), and from human beings suffering from diarrhoea (Sobieszczanska et al., 1999). Moreover, the occurrence of VTEC in young farm animals makes them candidate reservoirs of zoonotic agents (Holland, 1990).

The aim of the present study was to investigate certain bacteria of public health importance isolated from calves, lambs and kids suffering from diarrhoea or respiratory manifestations, detection of verocytotoxin by using Vero cell line and enterotoxigenic E. coli by polymerase chain reaction (PCR) and conclude the preventive measures available to over come such hazards.

MATERIAL AND METHODS

1-Collection of samples:

1.1. Rectal swabs:
A total of 104 rectal swab samples were collected from calves (50), lambs (42) and kids (12), clinically suffering from diarrhoea. The samples were collected from Alexandria Province.

1.2. Nasal swabs:
69 deep nasal swabs (33 from calves and 36 from lambs) were collected from living animals clinically suffering from respiratory manifestations.

2. Vero cell lines (African green monkey kidney cells):
Vero cells were kindly obtained from Veterinary Serum and Vaccine research Institute, Abbassia, Cairo. It was used for detection of verotoxin produced by E. coli.

3. Bacteriological examination of rectal and nasal swabs:
Rectal and nasal swabs were cultivated on nutrient broth, selenite F. broth and thioglycolate broth. A loopful from the incubated broth was streaked on different solid culture media (Koneman et al., 1988). Then the bacterial isolates were purified and identified by studying colonial, morphological and biochemical characteristics. The isolated Salmonellae were further identified by specific antisera (Quinn et al., 1994).

4. Detection of verocytotoxin-producing E. coli (VTEC):
Detection of verotoxin was done according to Brooks et al. (1997). A heavy growth of E. coli from MacConkey's agar medium was inoculated into 20 ml of trypticase soya broth and incubated at 37 °C for 5 hours with shaking. The broth was centrifuged at 10,000 xg for 10 minutes and the supernatant fluid was discarded.

The bacterial pellet was suspended in 1 ml of phosphate buffered saline containing 0.1 mg of polymyxin B and incubated at 37 °C for 30 minutes to release cell bound verocytotoxin. Then the suspension was centrifuged again and the supernatant was tested for verocytotoxin. A 25 ul of the suspected verocytotoxin extract were added to duplicate wells of the monolayers of vero cells. Uninoculated wells were used as a negative control. The plates were incubated at 37 °C in a humidified incubator containing 5% Co_2 and examined daily using an inverted microscopy for detection of cell lysis, rounding and cell detachment. If at least 50% of the Vero cells were rounded and detached from the bottom of the well at the end of the test period, the isolate was considered positive.

5. Detection of enterotoxigenic E. coli by PCR:
5.1. Extraction of DNA from E. coli:
DNA was extracted from E. coli according to Lou et al., (1997). E. coli isolates were grown in Luria Bertani (LB) broth overnight at 37 °C. 100 ul of broth culture were centrifuged and the pellet was resuspended in distilled water. The genomic DNA was extracted by boiling of the bacterial suspension for 10 minutes and the supernatant was used as a template for PCR.

5.2. Primers:
The primers were synthesized and supplied by Amersham Pharmacia Biotech. Inc.. The primers were designed to specifically amplify E. coli stable enterotoxin (STa) gene (Ojeniyi et al., 1994).
Two primers were used (i) upstream primer with a sequence of: 5' TCC GTG AAA CAA CAT GAC GG 3' and (ii) downstream primer with a sequence of: 5' ATT TCA TCC AGC ACA GGC AG 3'. The primers were dissolved in nuclease-free water to obtain 50 - 100 pmol concentrations (Baumforth et al., 1999).

5.3. Polymerase chain reaction (PCR) technique:

The reaction was conducted in a total volume of 25 ul in 0.5 ml microfuge tubes Ready-To-Go PCR beads contain all the components of PCR except primers and DNA template. The reaction was carried out by adding 12 ul of nuclease-free water, five ul of each primer and three ul of the template DNA to the bead in the microfuge tube. The reaction mixture was overlaid with 40 ul of nuclease-free mineral oil to prevent evaporation. The tubes were then put in the thermal cycler (Biometra, personal cycler) programmed according to Baumforth et al. (1999) as follows: one cycle for 5 minutes at 94 °C to denature the template DNA followed by 30 cycles of denaturation, annealing and extension at 94 °C for one minute, 57 °C for one minute and 72 °C for 2 minutes, respectively. The 30 cycles were followed by a final cycle of extension at 72 °C for 10 minutes to ensure that the entire PCR product is double stranded DNA. At the end of cycling the tubes were stored at -20 °C till needed.

5.4. Detection of PCR products:

5 ul of PCR product were run on 1% agarose at 100 voltage for 45 minutes. The gel was stained with 0.5 ug/ml ethidium bromide for 30 - 45 minutes and examined by UV- transilluminator for the presence of predictable bands.

RESULTS

Result presented in Table (1) indicated bacteria isolated from rectal swabs collected from diarrhoeic calves, lambs and kids. E. coli was the most prevalent organism followed by Proteus spp., Campylobacter jejuni, Salmonella typhimurium and Pseudomonas aeruginosa.

E. coli, Pasteurella multocida, Pasteurella haemolytica, Pseudomonas aerogenosa and Staph. aureus were isolated from nasal swabs collected from calves and lambs showing respiratory manifestations (Table, 2).

Verotoxin production was detected in E. coli isolated from diarrhoeic calves, lambs and kids at a percentage of 31.7, 44.1 and 25.0% respectively (Table, 3).

The result of detection of enterotoxigenic E. coli isolated from animals suffering from diarrhoea or respiratory manifestations by using PCR were presented in Table (4) and Figure (1).

DISCUSSION

In this study some bacterial agents of zoonotic importance associated with diarrhoea and respiratory manifestations among calves, lambs and kids were reported. As E. coli is the major cause of diseases among young animals, it was further studied for some virulence characters of the isolated E. coli.

The results illustrated in Table (1) showed that E. coli was isolated at a percentage of 82.0, 80.95 and 83.33% from examined rectal swab of calves, lambs and kids respectively. These results revealed that E. coli was the most predominant bacterial pathogens associated with diarrhoea among young animals, and nearly similar results obtained by Ibrahim (1995) and Aly et al. (1996). Holland (1990) mentioned that most strains of E. coli are commensal inhabitants of gastrointestinal tracts but some strains express virulence factors that enhance the ability of the organism to cause a variety of intestinal affections and diarrhoeal syndromes among young animals. So, the isolated E. coli was further characterized for production of virulence factors.

Table (2) showed that E. coli could be isolated from nasal swabs collected from calves and lambs with respiratory manifestations at a percentage of 15.15 and 16.67% respectively. The result of calves is agreement with that obtained by Mahmoud (1993) (12.5%) and higher than the result recorded by Selim et al. (1998) (2.5%). Sayed, (1996) reported high incidence of E. coli isolates from nasal swabs of lambs.

VTEC were detected from 13 (31.7%) of E. coli strains isolated from diarrhoeic calves (Table, 3). This result is lower than that recorded by Orden et al (1998) (69.8%), and higher than that reported by Caprioli et al. (1993) (7.7%), Wray et al. (1993) (2.8%) and Ibrahim (1995) (3.5%). These variations in results might be attributed to immunostatus, age and breed of animals and geographical area (Burnens et al., 1995). VTEC responsible for diarrhoea and haemorrhagic colitis with characteristic lesions in small and large intestines of calves (Holland, 1990). In addition, VTEC were detected from 1 (20.0%) of E. coli strains isolated from nasal swabs of affected calves (Table, 3). The result of this study and the previous investigations (Holland, 1990; Caprioli et al., 1993; and Burnens et al., 1995) indicated that cattle are an important source of verotoxin-producing strains of E. coli for human beings.

The data presented in Table (3) revealed that VTEC were detected from 15 (44.12%) and 2 (33.33%) of E. coli strains isolated from rectal and nasal swabs of affected lambs. Wray et al. (1993) reported the occurrence of VT-producing E. coli among ovine isolates at a rate of 6.1%. Some reports indicated that sheep are natural reservoir for potentially virulent E. coli O157 and other VTEC (Holland, 1990 and Wray et al., 1993). Moreover, VTEC were detected from 3 (25.0%) of E. coli strain isolated from diarrhoeic kids. Duhamel et al., (1992) reported the occurrence of enteric colibacillosis by shiga-like toxin producing E. coli in goats.

Polymerase chain reaction (PCR) – the nucleic acid-based technique - that enable the rapid and sensitive detection of specific microorganisms (genes) was used in this study to detect gene coding for enterotoxin production by E. coli isolates. ETEC were found in 3 out of 41 E. coli isolate (7.32%) from diarrhoeic calves (Table, 4).

This result is lower than that reported by Abraham et al. (1992) (11.1%) and higher than that obtained by Wray et al. (1993) (4.4%). In addition, Table (4) revealed that ETEC were detected in 6 (17.65%) of E. coli strains isolated from diarrhoeic lambs. This result is lower than that recorded by Abo El-Hassan (1996) (43.5%) and higher than the result obtained by Wray et al. (1993) (1.5%). Moreover, only one isolate of diarrhoeic kids was ETEC (8.33%). This result is lower than that reported by Abo El-Hassan (1996). These difference in the prevalence of ETEC among diarrhoeic animals compared with the previous studies might be attributed to the methods of assessment of enterotoxigenicity, the age of the animals, locality, immunostatus of the animals , hygienic measures and history of the herds.

E. coli is an important cause of diseases among animals and human beings especially VTEC and ETEC which are implicated as a cause of diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome in man (Sobieszczanska et al., 1999 and Keskimaki et al., 2000). In addition, E. coli can be transmitted to man either directly through contact with diseased animals resulting contamination of their fingers or indirectly by the consumption of food contaminated with secretion and excretions of infected animals (Acha and Szyfers, 1991).

Campylobacter jejuni was isolated from diarrhoeic calves samples at a percentage of 16.0% (Table, 1). This result is lower than that reported by Stanley et al. (1998) (69.9%) and higher than that recorded by Sadiek and Hussein (1999) (6.66%). This difference may be attributed to the seasonal variations and the degree of contamination of calf environment by this organism (Stanley et al., 1998). In addition, the result presented in Table (1) illustrated that C. jejuni could be isolated from diarrhoeic lamb samples at a percentage of 14.29%. C. jejuni has been implicated as a cause of enteritis in calves and human beings (Acha and Szyfers, 1991). It is assumed that man may be infected by direct contact with diseased animals suffering from campylobacter diarrhoea or by consumption of food or water contaminated by secretions and excretion of diseased animals.

The data present in Table (1) showed that Salmonella typhimurium was isolated from diarrhoeic calves at a percentage of 4.0%. This result is in accordance with that reported by Sadiek and Hussein (1999) and lower than that recorded by Khan and Khan (1997) (14.6%). In addition, S. tytphimurium could be isolated from diarrhoeic lamb samples at a percentage of 2.38%. This result is lower than that recorded by Abo El-Hassan (1996) (5.5%). The variation in results may be due to the condition of specimen as it allow contaminating organisms to inhibit salmonella isolation, Salmonella organisms may be shed only periodically and low numbers (Stone et al., 1994). Salmonellosis is perhaps the most widespread zoonotic disease in the world, and human salmonella infection are most commonly caused by ingestion of food contaminated by secretion and excretion of diseased animals. Moreover, S. typhimurium continues to be an important cause of human gastroenteritis (Acha and Szyfers, 1991).

Proteus spp. Could be isolated from diarrhoeic calves at a percentage of 30.0% (Pr. mirabilis 26% and Pr. vulgaris 4.0%) (Table, 1). This result is higher than that recorded by Sadiek and Hussein (1999) (6.66%) and Ibrahim (1995) (19.4%). In addition, Proteus spp. was isolated from diarrhoeic lamb and kid samples at a percentage of 23.81% (pr. mirabilis 19.05% and Pr. vulgaris 4.76%) and 8.33% (Pr. mirabils) respectively, (Table, 1). Proteus spp. are opportunistic pathogens and known occasionally to cause diarrhoea among animals and human beings and associated with urinary tract infection in man (Koneman et al., 1988 and Quinn et al., 1994).

The data given in Table (1) illustrate that Pseudomonas aeruginosa could be isolated from diarrhoeic calves at a percentage of 4.0%. This result is in accordance with Sadiek and Hussein (1999), but its rate is very low than that recorded by Aly et al. (1996) (40.0%). In addition, Pseud. aeruginosa was isolated from diarrhoeic lambs and kids at a percentage of 7.14 and 16.67% respectively (Table, 1).

Moreover, Pseud. aeruginosa was isolated from nasal swabs collected from calves and lambs at a percentage of 3.03 and 16.67% respectively (Table, 2). Several studies indicated the high incidence of Pseud. aeruginosa in nasal swab collected from lambs (Sayed, 1996). Pseud. aeruginosa is considered as an opportunistic pathogens and are known occasionally to cause infections among animals and human beings such as wound infection, urinary tract infection, pneumonia and gastroenteritis (Koneman et al., 1988 and Quinn et al., 1994).

The results presented in Table (2) showed that Pasteurella multocida and Pasteurella haemolytica were isolated from nasal swabs of affected calves at a percentage of 9.09 and 9.09% respectively. The result of P. multocida is in agreement with that recorded by Khan and Khan (1997) (8.8%) and Selim et al. (1998) (9.16%), while the result of P. haemolytica is lower than that obtained by Khan and Khan (1997) (20%) and Selim et al. (1998) (14.1%). In addition, P. multocida and P. haemolytica were isolated from affected lambs at a percentage of 2.78 and 8.33% respectively (Table, 2).

The result of P. multocida is lower than that recorded by Sayed (1996) (15.4%). The difference in the isolation rate of Pasteurella spp. may be attributed to the immunological status of the animals, age, environmental temperature and relative humidity (Woldehiwet et al., 1990). P. multocida and P. haemolytica are an important causes of pneumonia in cattle and sheep, that particularly affect calves and lambs. In addition, it can be transmitted to man through animal scratch or through respiratory and digestive tract leading to bronchitis and pneumonia and/or localized infections in different organs and tissues (Acha and Szyfers, 1991 and Quinn et al., 1994).

Staph. aureus was isolated from nasal swabs of affected calves and lambs at a percentage of 6.06 and 5.56% respectively (Table, 2). Concerning with the result of calves is agree with that recorded by Selim et al. (1998) and lower than that reported by Khan and Khan (1997). Sayed (1996) isolated Staph. aureus from sheep suffering from pneumonia. From public health point of view, Staph. aureus was always incriminated in cases of suppurative diseases, pyogenic lesions on the skin, septicaemia, food poisoning and pneumonia (Acha and Szyfers, 1991).

From this study it can be concluded that, calves, lambs and kids suffering from diarrhoea or respiratory manifestations act as a source of certain bacterial agents of public health importance, which can be transmitted to man contact. So, the following hygienic measures should be adapted including, periodical cleaning and disinfection of animal houses, hand washing and immersion in mild antiseptic after handling infected animals, hygienic disposal of animal excreta, avoid any environmental and nutritional distress, and attention should be paid to foods and drinking water given to animals.

REFERENCES

Abou El-Hassan, D.G. (1996): "Neonatal diarrhoea in lambs and goats kids" 4th Sc. Cong. Proc., Vet. Med. J., Giza 44(2): 371 – 380.

Abraham, C.; Roder, D. and Zewdu, R. (1992): "Agents associated with neonatal diarrhoea in Ethiopian dairy calves". Trop. Anim. Health. Prod., 24(2): 74 – 80.

Acha, P.N. and Szyfers, B. (1991): "Zoonoses and communicable diseases common to man and animals". 2nd Ed. Pan American Health Organization, Washington D.C.

Aly, A.O.; Abd El-Wahed, Z.H.; Kahilo, K. and El-Sheikh, A.R. (1996): "Some studies on clinical, haematological and biochemical changes in diarrhoeic neonatal buffalo calves with reference to hygienic condition". Assiut Vet. Med. J., 35(69): 91 – 104.

Baumforth, K.R.; Nelson, P.N.; O'Neil, J.D. and Murraym D.G. (1999): "The polymerase chain reaction". J. Clin. Mol. Pathol., 25: 1 – 10.

Brooks, H.L.; Bettelheim, K.; Todd, B. and Holdway, D. (1997): "Non-O157 Vero cytotoxin producing E. coli: etiological agents of diarrhea in children in dunedein, New Zealand". Comp. Immunol. Microbiol. Infect. Dis., 20(2): 163-170.

Burnens, A.; Frey, A., Lior, H. and Nicolet, J. (1995): "Prevalence and clinical significance of vero-cytotoxin producing Escherichia coli (VTEC) isolated from cattle herds with and without calf diarrhoea". J. Vet. Med. B., 42" 311 – 318.

Caprioli, A.; Flabo, V.; Roda, L.G.; Rugger, F.M. and Zona, C. (1993): "Partial purification and characterization of an E. coli toxic factor that induces morphological cell alterations" Infect. Immun., 39: 1300 – 1306.

Duhamel, G.E.; Moxley, R.A.; Maddox, C.W. and Erickson, M. E.D. (1992): "Enteric infection of goat with enterohaemorrhagic Escherichia coli". J. Vet. Diag. Invest., 4: 197 – 2000.

Holland, R.E. (1990): "Some infectious causes of diarrhoea in young farm animals". Clin. Microbiol. Rev., 3(4): 345 – 375.

Ibraheim, A.M. (1995): "Sanitary studies on newly born calves". Ph. D. Thesis, Fac. Vet. Med., Suez Canal Univ.

Keskimaki, M.; Mattila, L.; Peltola, H. and Siitonem, A. (2000): "Prevalence of diarrhogenic Escherichia coli in finns with or without diarrhoea during a round the world trip". J. Clin. Microbiol., 38(12): 4425 – 4429.

Khan, A. And Khan, M. (1997): "Bacteria isolated from natural cases of buffalo and bovune neonatal calf diarrhoea, pneumonia and pneumoenteritis". Vet. ARHIV., 67(4): 161 – 167.

Koneman, E.W.; Allen, S.D.; Dowell, V.R.; Janda, W.M; Sommers, H.M. and Winn, W.C. (1988): "Colour atlas and textbook of diagnostic microbiology". 3 rd Ed. T.B. Lippencott, Co., New York, London

Levine, M.M. (1987): "Escherichia coli that cause diarrhoea: Enterotoxigenic, enteropathogenic, enteroinvasive and enteroadherent". J. infect. Dis, 155(3): 337 – 389.

Lou, Q.; Chong, S.F.; Fitzgerald, J.F.; Siders, J.A.; Allen, S.D. and Lee, C. (1997): "Rapid and effective method for preparation of faecal specimens for PCR assays". J. Clin. Microbiol., 35: 281 – 283.

Mahmoud, A.R. (1993): "Clinicopathological studies on some diseases of cattle calves and buffalo calves". Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.

Ojeniyi, B.; Ahrens, P. and Meyling, A. (1994): "Detection of fimbrial and toxin genes in E. coli and their prevalence in piglets with diarrhoea, the application of colony hybridization assay, polymerase chain reaction and phenotypic assays". J. Vet. Med. B., 41: 49 – 59.

Orden, J.A.; Cid, D.; Garcia, S.; Sanz, R.; and de la Fuente, R. (1998): Verotoxin producing E. coli (VTEC) and eae-positive non-VTEC in 1-30 days old diarrheic dairy calves. Vet. Microbiol., 63:239-248.

Oswald, E.; Pohl, P.; Jacquemin, E.; Linterman. P.; VanMuyleum, K.; O'Brien, A.D. and Mainil, J. (1994): "Specific DNA probes to detect Escherichia coli strains producing cytotoxic necrotizing factor type 1 or type 2". J. Med. Microbiol., 40: 428 – 443.

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

Sadiek, A. and Hussein , S.Z. (1999): "Acute enteritis in neonate Fresian calves in Sohag Governorate with special reference to aetiology, clinicobiochemical aspects and therapy". Assiut Vet. Med. J., 42(83): 200 – 215.

Sayed, A. M. (1996): "Some bacteriological and mycological studies on sheep pneumonia at Assiut governorates". Assiut Vet. Med. J., 36(71): 68 – 73.

Selim, A.M.; El-Shaheedy, M.; Zaki, M.; Al-Atrash, S. and Abaza, F. (1998): "Observation on an outbreak of respiratory distress in feedlot calves, epidemiological, clinical and microbiological findings:. Assiut Vet. Med. J., 38(76): 98 – 110.

Sobieszczanska, B.; Gryko, R.; Franiczek, R.; Gosciniak, G. and Grazes, I. (1999): "Characteristic of verotoxigenic strains of Escherichia coli". Przegl. Epidemiol., 53(3-4): 375 – 383.

Stanley, K.N.; Wallace, J.S.; Currie, J.E.; Diggle, P.J. and Jones, K. (1998): "The seasonal variation of thermophilic campylobacteriosis in beef cattle, dairy cattle and calves". J. App. Microbiol., 85: 472 – 480.

Stone, G.G.; Oberstm R.D.; Hays, M.,; McVeys, S. and Chengappa, M.M. (1994): "Detection of Salmonella serovars from clinical samples by enrichment broth cultivation –PCR procedure". J. Clin. Microbiol., 32(7): 1742 – 1749.

Woldeheiwet, Z.; Mamache, B. and Rowan, T.G. (1990): "The effect of age, environmental temperature and relative humidity on the bacterial flora of the upper respiratory tract in calves". Br. Vet. J., 146: 211 – 218.

Wray, C.; Maclaren, I. and Carroll, P.J. (1993): "Escherichia coli isolated from farm animals in England and Wales between 1986 and 1991". Vet. Rec., 13(30): 439 – 442.

Table (1): Bacteria isolates from rectal swab samples collected from diarrhoeic calves, lambs and kids:

Bacterial isolates	Calves (N= 50)		Lambs (N = 42)		Kids (N =12)	
	+ ve	%	+ ve	%	+ ve	%
Escherichia coli	41	82.0	34	80.95	10	83.33
Campylobacter jejuni	8	16.0	6	14.29	0	0.00
Salmonella typhimurium	2	4.0	1	2.38	0	0.00
Proteus spp.:	15	30.0	10	23.81	1	8.33
Proteus mirabilis	13	26.0	8	19.05	1	8.33
Proteus vulgaris	2	4.0	2	4.76	0	0.00
Pseudomonas aeruginosa	2	4.0	3	7.14	2	16.67

N = No. of examined samples

Table (2): Bacteria isolated from nasal swab samples collected from calves and lambs showing respiratory manifestations:

Bacterial isolates	Calves (N= 33)		Lambs (N = 36)	
	+ ve	%	+ ve	%
Escherichia coli	5	15.15	6	16.67
Pasteurella multocida	3	9.09	1	2.78
Pasteurella haemolytica	3	9.09	3	8.33
Pseudomonas aeruginosa	1	3.03	6	16.67
Staphylococcus aureus	2	6.06	2	5.56

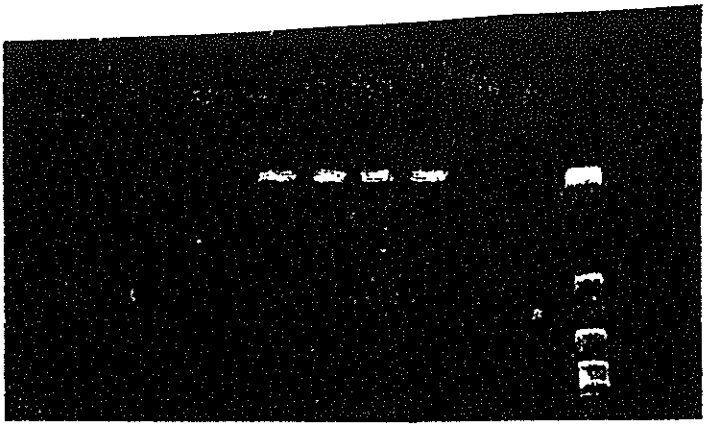
N = No. of examined samples

Table (3): Results of verocytotoxin production by E. coli isolated from calves, lambs and kids:

Origin of E. coli isolate	No. tested isolates	No. of verocytotoxigenic E. coli	%
Calves			
Rectal samples	41	13	31.71
Nasal swabs	5	1	20.0
Lambs			
Rectal samples	34	15	44.12
Nasal swabs	6	2	33.33
Kids			
Rectal samples	12	3	25.0
Total	98	34	34.69

Table (4): Results of Polymerase chain reaction for detection of enterotoxigenic E. coli isolated from calves, lambs and kids:

Origin of E. coli isolate	No. tested isolates	No. of enterotoxigenic E. coli	%
Calves			
Rectal samples	41	3	7.32
Nasal swabs	5	0	0.00
Lambs			
Rectal samples	34	6	17.65
Nasal swabs	6	0	0.00
Kids			
Rectal samples	12	1	8.33
Total	98	10	10.20



7 6 5 4 3 2 1 M

Figure (1): Electrophoretic analysis of PCR amplified DNA of Sta. M = 100 bp ladder DNA, lanes from 1 – 3 negative results and from 4 – 7 positive results.

الملخص العربى

الأهمية الصحية العامة لبعض البكتيريا المعزولة من العجول و المجترات الصغير

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تم جمع عدد ١٠٤ مسحة شرجية من العجول و الحملان و صغار الماعز المصابة بالإسهال و ٦٩ مسحة أنفية من العجول و الحملان التي تعانى من اضطرابات تنفسية و تم فحص هذه العينات بكتريولوجيا. أسفر الفحص البكتريولوجى لعينات المسحات الشرجية عن عزل كل من الأيشريشيا كولاى (٨٢%)، الكامبيلوباكتر جيجونى (١٦%)، سالمونيلا تيفيميوريم (٤%)، البروتيس (٣٠%) و سيدوموناس أيروجينوزا (٤%) من العجول و عزل كل من الأيشريشيا كولاى (٨٠,٩٥%)، الكامبيلوباكتر جيجونى (١٤,٢٩%)، سالمونيلا تيفيميوريم (٢,٣٨%)، البروتيس (٢٣,٨١%) و سيدوموناس أيروجينوزا (٧,١٤%) من الحملان و عزل كل من الأيشريشيا كولاى (٨٣,٣٣%)، البروتيس (٨,٣٣%) و سيدوموناس أيروجينوزا (١٦,٦٧%) من صغار الماعز. كما أسفر الفحص البكتريولوجى للمسحات الأنفية عن عزل كل من الأيشريشيا كولاى (١٥.١٥%)، الباستيرلا مالتوسيدا (٩,٠٩%)، الباستيرلا هيموليتكا (٩,٠٩%)، السيدومونس أيروجينوزا (٣,٠٣%) و الميكروب العنقودي الذهبى (٦,٠٦%) من العجول و عزل كل من الأيشريشيا كولاى (١٦,٦٧%)، الباستيرلا مالتوسيدا (٢,٧٨%)، الباستيرلا هيموليتكا (٨,٣٣%)، السيدومونس أيروجينوزا (١٦,٦٧%) و الميكروب العنقودى الذهبى (٥,٥٦%) من الحملان. كما تم دراسة مقدرة ميكروب الأيشريشيا كولاى المعزولة من الحيوانات المصابة على إفراز الفيروتوكسين و الأنتيروتوكسين و وجدت المقدرة على إنتاج الفيروتوكسين بنسبة ٣١,٧١، ٤٤,١٢ و ٢٥% من العترات المعزولة من العجول و الحملان و صغار الماعز المصابة بالإسهال على التوالي، و من ٢٠ و ٣٣.٣٣% من العترات المعزولة من العجول و الحملان التي تعانى اضطرابات تنفسية على التوالي. و استخدم تفاعل البلمرة المتسلسل (PCR) للكشف عن إنتاج الأنتيروتوكسين من الأيشريشيا كولاى المعزولة و قد وجد بنسبة ٧,٣٢% ، ١٧,٧٥% و٨,٣٣% في العجول و الحملان و صغار الماعز المصابة بالإسهال على التوالي. هذا و قد تم مناقشة تأثير البكتيريا المعزولة على الصحة العامة و طرق الوقاية منها.