Diarrhoeagenic *Escherichia coli* and salmonellae in calves and lambs in Kashmir: absence, prevalence and antibiogram

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Summary

Polymerase chain reaction assays and culture were used to investigate 728 faecal samples from 404 calves (286 diarrhoeic, 118 healthy) and 324 lambs (230 diarrhoeic, 94 healthy) in Kashmir, India, for the presence of enterotoxigenic *Escherichia coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and salmonellae. Antimicrobial sensitivity patterns were also investigated. In total, 23 ETEC isolates were obtained from the diarrhoeic calves and 12 from diarrhoeic lambs. Most (74%) of the isolates from calves harboured the gene encoding heat-labile enterotoxin I, whereas 75% of the isolates from lambs possessed only the gene encoding for heat-stable enterotoxin a. The ETEC isolates belonged to 20 serogroups, among which serogroups O15 (five

isolates) and O8 (four isolates) were most the frequent. *Salmonella* Typhimurium or *S.* Enteritidis was identified in three samples from diarrhoeic lambs. The ETEC isolates and the salmonellae showed multidrug resistance. No EAEC or DAEC was detected in any of the samples.

Keywords

Calf – Diffusely adherent *Escherichia coli* – Diarrhoea – Enteroaggregative *Escherichia coli* – Enterotoxigenic *Escherichia coli* – India – Kashmir – Lamb – *Salmonella*.

Introduction

Escherichia coli (*E. coli*) and salmonellae are among the most important causes of diarrhoea in both animals and humans. To date, diarrhoeagenic *E. coli* strains have been divided into six pathotypes, based on the mechanisms by which they produce disease (1):

- enterotoxigenic (ETEC)
- enteropathogenic
- entero-invasive
- Shiga toxin-producing/enterohaemorrhagic
- enteroaggregative (EAEC), and
- diffusely adherent (DAEC).

Among diarrhoeagenic strains of *E. coli*, ETEC is most commonly associated with diarrhoea in calves and lambs (2, 3). These strains produce plasmid-mediated enterotoxins, namely heat-labile and heat-stable enterotoxins, encoded by the genes *elt* and *est*, respectively. There are two major subtypes of heat-labile enterotoxin, designated as LT-I and LT-II, with no cross-reactivity. Strains that express LT-I are pathogenic to both humans and animals, whereas LT-II is found primarily in animal isolates (4). Similarly, heat-stable enterotoxin has two subtypes: STa and STb. Bovine and ovine ETEC isolates usually produce STa toxin (5).

The EAEC and DAEC are increasingly recognised as emerging pathotypes responsible for acute and persistent diarrhoea in humans

(6), but their reservoirs are unknown. A fragment of a 60–65 MDa virulence plasmid, referred to as DNA probe pCVD432, has been used to identify EAEC in polymerase chain reaction (PCR) assays (7). Similarly, DAEC strains can be identified in PCR, based on their afimbrial adhesive sheaths (Afas), which are encoded by gene clusters comprising *afaA*, *afaB*, *afaC*, *afaD* and *afaE* (8). Whether or not animals serve as reservoirs of EAEC and DAEC remains to be determined.

The virulence of salmonellae depends upon an array of factors. For example, the chromosomally located invasion gene *invA* is thought to trigger the invasion of salmonellae into cultured epithelial cells (9), and the genes for *Salmonella* Enteritidis fimbriae (*sefA*) and enterotoxin of salmonellae (*stn*) are responsible for colonisation and secretory diarrhoea, respectively (10).

At present, antimicrobial therapy is one of the primary control measures for reducing morbidity and mortality in animals infected with diarrhoeagenic bacteria. However, prescription of antimicrobials precedes the antimicrobial sensitivity test and the indiscriminate and widespread use of these drugs, in Kashmir as elsewhere, leads to the development of resistance. It is therefore necessary to monitor the drug resistance pattern of these bacteria, both for effective treatment and to prevent the emergence of drug resistance.

Shiga toxin-producing and enteropathogenic *E. coli* in calves and lambs in Kashmir, India, have been isolated and characterised (11). The present study was undertaken to investigate the prevalence of ETEC and salmonellae serotypes in calves and lambs in Kashmir and to find out whether these animals serve as reservoirs for EAEC and DAEC. The antimicrobial sensitivity patterns of the isolates were also investigated.

Materials and methods

Samples

A total of 728 faecal samples from 286 calves and 230 lambs with diarrhoea and 118 calves and 94 lambs that were apparently healthy were collected between 2006 and 2009. The age of the animals ranged from newborn to three months. The calf samples came from the Cattle Research Station at Manasbal, the Veterinary Clinical Complex, Faculty of Veterinary Sciences and Animal Husbandry, at Shuhama, the Military Dairy Farm at Qamarwari and the Central Veterinary Hospital in Srinagar. The lamb samples came from the Sheep Research Station at Shuhama and two government sheep breeding farms at Goabal (Ganderbal district) and Dachigam (Srinagar district). The samples were collected in sterile, screw-capped vials and transported to the laboratory on ice.

The farms from which the samples were collected are the organised farms in Kashmir. However, the sheep migrate to highland pastures for grazing during the summer and, during migration, they come into contact with other sheep flocks in the valley, including those from the Jammu region. Furthermore, these farms supply rams to other farmers who hold 20 to 50 animals for breeding.

Isolation of *Escherichia coli* and salmonellae

Strains of *E. coli* were isolated from the faecal samples and identified as described (12). Briefly, samples were immediately inoculated into EC broth (HiMedia, Mumbai, India). After overnight incubation at 37°C, 1 ml of each culture was processed for extraction of DNA to detect the genes *elt*, *est*, *pCVD432* and *afaBC* in PCR assay. All broth cultures that tested positive by PCR for at least one virulence gene were inoculated onto MacConkey agar (HiMedia) to isolate *E. coli*. After overnight incubation at 37°C, at least five *E. coli*-like colonies were randomly picked and subcultured on eosin methylene blue agar to observe the characteristic metallic sheen of the *E. coli*. Well-separated colonies were transferred onto nutrient agar slants as pure cultures and subjected to standard morphological and biochemical

tests. All *E. coli* isolates were confirmed for the presence of corresponding virulence gene/s using the PCR protocols described below.

To isolate salmonellae, 1 g to 2 g of faecal material was inoculated into 10 ml of tetrathionate broth (HiMedia) for selective enrichment of salmonellae and incubated at 42°C for 48 h before being subcultured on brilliant green agar and Salmonella Shigella agar (HiMedia). Suspected colonies of salmonellae were purified by subculture. The isolates were then subjected to standard morphological and biochemical tests to confirm their identity as salmonellae.

Polymerase chain reaction

The procedure for extracting bacterial DNA was as described previously (12). The PCR assays were carried out in 25 µl reaction volumes containing 1 U of Taq polymerase, 200 µmol of each dNTP and 2.5 µl of 10× PCR buffer. Reactions were performed in a GeneAmp® PCR System 2400 Thermal Cycler (Applied Biosystems, Foster City, California) and a FlexiGene® thermal cycler (Techne Inc., Princeton, New Jersey). Oligonucleotide primers were obtained from M⁄S Sigma Genosys (Ishikaria, Hokkaido, Japan), and details of the primer sequences are given in Table I. The DNA extracted from ETEC strain V27, EAEC strain JM042 and DAEC strain V64 served as positive controls for their respective genes. Sterile distilled water was used as the negative control.

Detection of the *Escherichia coli* genes *elt*, *est*, *pCVD432* and *afaBC*

All *E. coli* isolates were subjected to PCR assay to detect the genes *elt* (LT-I) and *est* (STa), as described previously (13). The pCVD432 probe was detected using specific primers (7). Each faecal sample was also screened for the gene *afaBC* (8) using a specific primer pair, namely, afa1 and afa2. Positive control DNA samples were incorporated into each reaction, as above.

Molecular characterisation of salmonellae

All salmonellae isolates were tested by genus-specific PCR assay, as described previously (14), with 16S rRNA primers, as shown in Table I. The serotype-specific PCR assay for *S.* Typhimurium was similar to the protocol described by Alveraz *et al.* (15), whereas, for *S.* Enteritidis, the protocol (15) was used with minor modifications to the PCR conditions as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of 95°C for 1 min, 57°C for 1 min and 72°C for 2 min. The final extension was at 72°C for 5 min.

To detect the virulence genes *invA*, *sefA* and *stn*, different PCR protocols were followed, as described (16, 17). The strains *S.* Typhimurium S-B-10829 and *S.* Enteritidis S-AV-11 were used as positive controls.

Serogrouping/serotyping and antibacterial sensitivity of *Escherichia coli* and salmonellae isolates

Three isolates of salmonellae and all the isolates of *E. coli* (one to three isolates per sample) that carried at least one virulence gene were referred to the National Salmonella and Escherichia Centre, Kasauli-173204, Himachal Pradesh, India for serogrouping (*E. coli*) or serotyping (salmonellae).

The disc diffusion technique was used to test all the ETEC and salmonellae isolates for susceptibility to 14 antimicrobial agents (HiMedia) (18). The results were recorded as 'sensitive' or 'resistant', in accordance with the Performance Standards for Antimicrobial Disk Susceptibility Tests, Clinical and Laboratory Standard Institute Guidelines. *Escherichia coli* strain ATCC 25922 and *Staphylococcus aureus* strain ATCC 25923 were used as controls.

Results

From a total of 286 samples from calves with diarrhoea, 23 ETEC isolates were obtained from 23 animals. Seventeen isolates carried the gene for LT-I alone, four carried the gene for STa, and the remaining two isolates carried genes for both LT-I and STa (Table II). The

ETEC isolates belonged to 16 serogroups, two isolates were rough and one was untypeable (Table II). Serogroup O8 was predominant, with four isolates.

Similarly, 12 ETEC isolates were recovered from 12 lambs with diarrhoea. Ten isolates belonged to serogroups O15, O33, O49 and O78 and the remaining two were untypeable (Table II). Serogroup O15 was the most common with five isolates, followed by O78 with three. Two isolates carried only the gene *elt* for LT-I, nine carried only the gene *est* for STa, and the remaining O15 isolate carried both genes (Table II).

No ETEC was detected in the samples from healthy calves and lambs. Similarly, none of the samples screened in this investigation revealed the presence of EAEC or DAEC.

Three isolates of salmonellae were recovered from three other diarrhoeic lambs: two isolates were *S.* Typhimurium (4,12:i:1,2), the third was *S.* Enteritidis (9,12:g,m). None of the calf samples yielded any salmonellae.

The antibacterial sensitivity patterns of the ETEC isolates are shown in Table III. All the ETEC isolates from calves were sensitive to amikacin (100%), followed by gentamicin (83%), enrofloxacin and ciprofloxacin (74%), norfloxacin (70%), streptomycin (61%), chloramphenicol and oxytetracycline (57%), and cefotaxime and ceftriaxone (56%). Most (91%) of the isolates were resistant to co-trimoxazole, followed by ampicillin (78%), cefalexin and co-amoxiclav (amoxicillin + clavulanic acid) (74%).

Similarly, all 12 ETEC isolates from the diarrhoeic lambs were sensitive to amikacin. The majority (83%) were sensitive to gentamicin, followed by enrofloxacin and ciprofloxacin (75%), norfloxacin and ceftriaxone (67%), chloramphenicol and streptomycin (58%) and cefotaxime and oxytetracycline (50%). Most (83%) of the isolates were resistant to ampicillin and cefalexin, followed by co-trimoxazole (75%) and co-amoxiclav (67%).

The salmonellae isolates were sensitive to all antibacterials tested except ampicillin, co-trimoxazole, oxytetracycline and streptomycin. One isolate of *S.* Enteritidis was also sensitive to streptomycin.

Discussion

In the present study, ETEC was isolated from 8% of the diarrhoeic calves. The presence of the gene for LT-I in most (74%) of the bovine ETEC isolates is in contrast to the findings of other investigators (19), who observed that such isolates mostly produced the STa toxin. Similarly, among 25 ETEC isolates from diarrhoeic calves in Brazil, eight (32%) carried the gene encoding STa and 17 (68%) the gene encoding LT-II (20). Recently, ETEC isolates with the gene encoding STa were detected in 16% of diarrhoeic calves in Turkey (21).

The isolation of ETEC of serogroups O1, O8, O18, O21, O55, O101, O117 and O119 is in agreement with another report from India, in which these serogroups were isolated from diarrhoeic calves in Assam (22). The ETEC isolates belonging to serogroups O26 and O55 were also isolated from diarrhoeic mithun calves (the domesticated form of wild Gaur, *Bos frontalis*) in India (23). More recently, *E. coli* serogroups O5, O8, O20, O25 and O76 were detected in cow and buffalo calves with diarrhoea in Gujarat, India (24). Strains of ETEC belonging to serogroups O8, O20 and O25 have also been isolated from humans with diarrhoea (5), and strains belonging to serogroups O5 and O9 have been isolated from children with diarrhoea in Kashmir (12). The observed predominance of serogroup O8 among calf ETEC in is agreement with other reports (22, 25).

In the present study, ETEC were also isolated from 5% of diarrhoeic lambs. The presence of the gene encoding STa in the majority (75%) of the lamb isolates is in agreement with other reports (5), although there is very little information on the isolation of ETEC from diarrhoeic lambs in India. In contrast, in a recent report from Arunachal Pradesh, only 21.73% of ETEC isolates from diarrhoeic lambs were found to carry the gene for STa (26). Furthermore, *E. coli* strains isolated from diarrhoeic lambs in Spain were usually non-toxigenic and belonged to a broad range of serogroups (27). The lamb

ETEC isolates in the present investigation belonged to serogroups O15, O33, O49 and O78, of which serogroup O15 was the most common. Isolates of ETEC from diarrhoeic lambs in India have been reported as belonging to 14 different serogroups (26); however, none of them matched the serogroups detected in the present study. Thus, it seems that a wide range of ETEC serogroups are prevalent in lambs in India. Serogroups O15 and O78 have been reported as the most common among ETEC isolates from humans in India (28).

No ETEC was isolated from healthy calves or lambs in this study. This finding is in agreement with a report from Turkey (21), where all 45 isolates of *E. coli* from healthy calves tested negative for the gene encoding STa. Similarly, 390 *E. coli* strains from healthy calves in Poland were found not to contain the genes for heat-stable or heat-labile toxins by PCR assay (29). In contrast, in a report on healthy lambs in Turkey, 6.3% of 63 faecal isolates of *E. coli* produced heat-labile and heat-stable enterotoxins (30).

In the present investigation, the failure to isolate *Salmonellae* from calves indicates that the incidence of *Salmonellae*-induced diarrhoea in calves may be very rare in this region. This agrees with other reports from India and Mozambique (22, 31). In contrast, three isolates of *Salmonellae*, which have a broad host range, were obtained from the diarrhoeic lambs, apparently the first finding in lambs with diarrhoea in India.

The absence of EAEC and DAEC in lambs and calves in the present study is in agreement with reports from Great Britain (32) and Germany (33), where no EAEC or DAEC was detected in animals. These findings suggest that calves and lambs do not serve as reservoirs of these two pathotypes of *E. coli*. In addition, no EAEC or DAEC have been isolated from chickens or pigeons in Kashmir (unpublished data).

The multidrug resistance of the isolates in the present study was alarming and could be the consequence of indiscriminate use of antibacterials in clinical practice. Resistance against frequently used antibacterials, such as fluoroquinolones (norfloxacin, ciprofloxacin,

enrofloxacin), oxytetracycline and streptomycin, was evident, although fluoroquinolone resistance was low compared with resistance to oxytetracycline and streptomycin, perhaps reflecting their recent introduction into animal practice. The resistance of most of the calf and lamb isolates to ampicillin, co-amoxiclav, co-trimoxazole and the cefalosporins may be due to enterobacterial beta-lactamases. All the isolates were found to be sensitive to amikacin, which is seldom used in bovine and ovine practice in Kashmir.

Conclusion

Diarrhoeic calves and lambs in Kashmir carry multidrug-resistant ETEC strains, mostly harbouring the genes encoding LT-I and STa. These animals do not serve as reservoirs for EAEC and DAEC. The study underlines the importance of emerging multidrug-resistant strains. There is considerable need for the judicious use of antimicrobials to effectively control diarrhoea in calves and lambs.

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References

1. Nataro J.P. & Kaper J.B. (1998). – Diarrhoeagenic *Escherichia coli*. *Clin. Microbiol. Rev.*, **11** (1), 142–201.

2. Ansari M.M., Renshaw H.W. & Gates N.L. (1978). – Colibacillosis in neonatal lambs: onset of diarrhoeal disease and isolation and characterization of enterotoxigenic *Escherichia coli* from enteric and septicaemic forms of the disease. *Am. J. vet. Res.*, **39** (3), 11–14.

3. Franck S.M., Bosworth B.T. & Moon H.W. (1998). – Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. *J. clin. Microbiol.*, **36** (6), 1795–1797.

4. Qadri F., Svennerholm A.M., Faruque A.S. & Sack R.B. (2005). – Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment and prevention. *Clin. Microbiol. Rev.*, **18** (3), 465–483.

5. Blanco J., Blanco M., Garabal J.I. & González E.A. (1991). – Enterotoxins, colonization factors and serotypes of enterotoxigenic *Escherichia coli* from humans and animals. *Microbiologia*, **7** (2), 57–73.

6. Huang D.B., Mohanty A., DuPont H.L., Okhuysen P.C. & Chiang T. (2006). – A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. *J. med. Microbiol.*, **55** (10), 1303–1311.

7. Schmidt H., Knop C., Franke S., Aleksic S., Heesemann J. & Karch H. (1995). – Development of PCR for screening of enteroaggregative *Escherichia coli*. *J. clin. Microbiol.*, **33** (3), 701–705.

8. Le Bouguenec C., Lalioui L., Du Merle L., Louve M., Courcoux P., Bouzari S., Selvarangan R., Nowicki B.J., Germani Y., Andremont A., Gounon P. & Garcia M.I. (2001). – Characterization of *AfaE* adhesions produced by extraintestinal and intestinal human *Escherichia coli* isolates: PCR assay for detection of *afaI* adhesions that do or do not recognize Dr blood group antigens. *J. clin. Microbiol.*, **39** (5), 1738–1745.

9. Galan J.E. & Curtiss R. (1989). – Cloning and molecular characterization of genes whose products allow *S.* Typhimurium to penetrate tissue culture cells. *Proc. natl Acad. Sci. USA*, **86**, 6383–6387.

10. Skyberg J.A., Logue C.M. & Nolan L.K. (2006). – Virulence genotyping of *Salmonella* spp. with multiplex PCR. *Avian Dis.*, **50** (1), 77–81.

11. Wani S.A., Bhat M.A., Samanta I., Nishikawa Y. & Buchh A.S. (2003). – Isolation and characterization of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) from calves and lambs with diarrhoea in India. *Lett. appl. Microbiol.*, **37** (2), 121–126.

12. Hussain I., Wani S.A., Nagamani K., Fayaz I., Mir M.A., Nabi A., Qureshi K., Khan M.A. & Nishikawa Y. (2010). – Isolation and characterization of enteroaggregative, enterotoxigenic and diffusely adherent *Escherichia coli* and *Salmonella* Worthington from human diarrhoeic faecal samples in Kashmir and Secunderabad, India. *World J. Microbiol. Biotechnol.*, **26** (10), 1883–1889.

13. Itoh F., Ogino T., Itoh F. & Watanabe H. (1992). – Differentiation and detection of pathogenic determinants among diarrheagenic *Escherichia coli* by polymerase chain reaction using mixed primers [in Japanese]. *Jpn J. clin. Med.* (*Nihon Rinsho*), **50**, 343–347.

14. Lin C.K. & Tsen H.Y. (1996). – Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. *J. appl. Bacteriol.*, **80** (6), 659–666.

15. Alveraz J., Sota M., Vivanco A.B., Perale I., Cisterna R., Rementeria A. & Garaizar J. (2004). – Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. *J. clin. Microbiol.*, **42** (4), 1734–1738.

16. Cortez A.L., Carvalho A.C., Ikuno A.A., Bürger K.P. & Vidal-Martins A.M. (2006). – Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR. *Res. vet. Sci.*, **81** (3), 340–344.

17. Rahman H. (1999). – Prevalence of enterotoxin gene (*stn*) among different serovars of *Salmonella*. *Indian J. med. Res.*, **110** (1), 43–46.

18. Bauer A.W., Kirby W.M., Sherris J.C. & Turck M. (1966). – Antibiotic susceptibility testing by a standardized single disk method. *Am. J. clin. Pathol.*, **45** (4), 493–496.

19. Nagy B. & Fekete P.Z. (1999). – Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. *Vet. Res.*, **30** (2–3), 259–284.

20. Salvadori M.R., Valadares G.F., Leite D.S., Blanco J. & Yano T. (2003). – Virulence factors of *Escherichia coli* isolated from calves with diarrhea in Brazil. *Braz. J. Microbiol.*, **34** (3), 230–235.

21. Guler I., Gunduz K. & Ok U. (2008). – Virulence factors and antimicrobial susceptibility of *Escherichia coli* isolated from calves in Turkey. *Zoonoses public Hlth*, **55** (5), 249–257.

22. Hussain I. & Saikia G.K. (2000). – Isolation and characterization of bacteria from diarrhoeic calves. *Indian J. comp. Microbiol. Immunol. infect. Dis.*, **21** (1), 125–127.

23. Rajkhowa S., Hussain I. & Rajkhowa C. (2008). – Detection of heat-stable and heat-labile enterotoxin genes of *Escherichia coli* in diarrhoeic faecal samples of mithun (*Bos frontalis*) calves by polymerase chain reaction. *J. appl. Microbiol.*, **106** (2), 455–458.

24. Vagh A.A. & Jani R.G. (2010). – Prevalence and comparative studies of some major serotypes of *E. coli* from cattle and buffalo calf scour. *Vet. World*, **3** (10), 458–459.

25. Acres S.D. (1985). – Enterotoxigenic *Escherichia coli* infection in newborn calves: a review. *J. Dairy Sci.*, **68** (1), 229–256.

26. Bandyopadhyay S., Mahanti A., Samanta I., Dutta T.K., Ghosh M.K., Bera A.K., Bandyopadhyay S. & Bhattacharya D. (2011). – Virulence repertoire of Shiga toxin-producing *Escherichia coli* (STEC) and enterotoxigenic *Escherichia coli* (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India. *Trop. anim. Hlth Prod*., **43** (3), 705–710.

27. Blanco J., Cid D., Blanco J.E., Blanco M., Quiteira J.R.S. & de la Fuente R. (1996). – Serogroups, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrhoeic lambs in Spain. *Vet. Microbiol*., **49** (3–4), 209–217.

28. Blanco J., Blanco M., González E.A., Blanco J.E., Alonso M.P., Garabal J.I. & Jansen W.H. (1993). – Serotypes and colonization factors of enterotoxigenic *Escherichia coli* isolated in various countries. *Eur. J. Epidemiol*., **9** (5), 489–496.

29. Osek J. & Winiarczyk S. (2001). – Prevalence of eae and Shiga toxin genes among *Escherichia coli* strains isolated from healthy calves. *J. vet. Med., B.*, **48** (1), 67–72.

30. Gulhan T., Ilhan Z., Aksakal A. & Solmaz H. (2009). – Detection of heat-stable and heat-labile enterotoxins of *Escherichia coli* strains isolated from healthy animals. *J. Anim. vet. Adv*., **8** (10), 2058–2062.

31. Achá S.J., Kuhn I., Jonsson P., Mbazima G., Katouli M. & Mollby R.S. (2004). – Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens. *Acta vet. scand*., **45** (1–2), 27–36.

32. Cassar C.A., Ottaway M., Paiba G.A., Futter R., Newbould S. & Woodward M.J. (2004). – Absence of enteroaggregative *Escherichia coli* in farmed animals in Great Britain. *Vet. Rec*., **154** (8), 237–239.

33. Wieler L.H., Sobjinski G., Schlapp T., Failing K., Weiss R., Menge C. & Baljer G. (2007). – Longitudinal prevalence study of diarrheagenic *Escherichia coli* in dairy calves. *Berl. Münch. tierärztl. Wochenschr*., **120** (7–8), 296–306.

Table I
Primers used to identify faecal isolates from diarrhoeic calves and lambs in Kashmir, India

Primer	Primer sequence (5'→ 3')	Target	Primer concentration (μM)	Product size (base pair)	Reference
LT-f	AGCAGGTTTCCCACCGGATCACCA	*elt* (LT-I)	0.5 each	132	(13)
LT-r	GTGCTCAGATTCTGGGTCTC				
ST-f	TTTATTTCTGTATTGTCTTT	*est* (STa)	1.0 each	171	(13)
ST-r	ATTACAACACAGTTCACAG				
EAEC-f	CTGGCGAAAGACTGTATCAT	*pCVD432*	0.125 each	630	(7)
EAEC-r	CAATGTATAGAAATCCGCTGTT				
afa1	GCTGGGCAGCAAACTGATAACTCTC	*afaBC*	0.5 each	750	(8)
afa2	CATCAAGCTGTTTGTTCGTCCGCCG				
Sal-f	TGTTGTGGTTAATAACCGCA	16s rRNA	1.0 each	574	(14)
Sal-r	CACAAATCCATCTCTGGA				
Ent-f	TGTGTTTTATCTGATGCAAGAGG	*S*. Enteritidis	0.075	304	(15)
Ent-r	TGAACTACGTTCGTTCTTCTGG		0.1		
Typh-f	TTGTTCACTTTTTACCCCTGAA	*S*. Typhimurium	0.1 each	401	(15)
Typh-r	CCCTGACAGCCGTTAGATATT				
Inv-f	TTGTTACGGCTATTTTGACCA	*invA*	0.2 each	521	(16)
Inv-r	CTGACTGCTACCTTGCTGATG				
Sef-f	GCAGCGGTTACTATTGCAGC	*sefA*	0.2 each	330	(16)
Sef-r	TGTGACAGGGACATTTAGCG				
Stn-f	TTGTGTCGCTATCACTGGCAACC	*stn*	1.0 each	617	(17)
Stn –r	ATT CGT AAC CCG CTC TCG TCC				

Table II
Virulence gene profile of enterotoxigenic *Escherichia coli* isolates from diarrhoeic calves and lambs in Kashmir, India

Serogroup	Number of isolates		Virulence genes	
	Calves	Lambs	*elt*	*est*
O1	1	0	–	+
O5	1	0	+	–
O8	3	0	+	–
O8	1	0	–	+
O9	1	0	+	–
O15	0	4	–	+
O15	0	1	+	+
O18	1	0	+	–
O20	1	0	+	+
O21	1	0	+	–
O25	1	0	+	–
O26	1	0	+	–
O33	0	1	–	+
O49	0	1	+	–
O53	1	0	+	–
O55	1	0	+	–
O76	1	0	+	–
O78	0	3	–	+
O79	1	0	+	–
O101	1	0	+	+
O117	1	0	+	–
O119	2	0	–	+
Rough	2	0	+	–
UT	1	0	+	–
UT	0	1	+	–
UT	0	1	–	+
Total	**23**	**12**	**22**	**16**

+: positive

–: negative

UT: untypeable

Table III

Antibacterial sensitivity patterns of enterotoxigenic *Escherichia coli* isolates from diarrhoeic calves and lambs in Kashmir, India

Antibacterial	No. (%) of sensitive calf isolates	No. (%) of sensitive lamb isolates
Ampicillin	5 (22)	2 (17)
Co-amoxiclav	6 (26)	4 (33)
Cefalexin	6 (26)	2 (17)
Ceftriaxone	13 (56)	8(67)
Cefotaxime	13 (56)	6 (50)
Chloramphenicol	13 (57)	7 (58)
Ciprofloxacin	17 (74)	9 (75)
Enrofloxacin	17 (74)	9 (75)
Norfloxacin	16 (70)	8 (67)
Amikacin	23 (100)	12 (100)
Gentamicin	19 (83)	10(83)
Streptomycin	14 (61)	7 (58)
Oxytetracycline	13 (57)	6 (50)
Co-trimoxazole	2 (9)	3 (25)