Characterization of shiga-toxin producing *E. coli* (STEC) and Enteropathogenic *E. coli* (EPEC) from domestic animals for virulence and colonisation factors and their antimicrobial resistance

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Abstract

Aim: To determine the occurrence and molecular characterization of Shiga toxin-producing *E. coli* (STEC) and Enteropathogenic *E. coli* (EPEC) from different domestic animals for serogrouping, virulence factors and their antibiotic resistance from Jammu region.

Methodology: Faecal samples were collected from 200 different animals including calves (39), rabbits (24), dogs (38), slaughtered pigs (22), poultry (41), sheep and goat (36). All the 200 strains were screened for the presence of stx1, stx2 and eae genes by m-PCR and screening of confirmed EPEC isolates with respect to their serogroup, virulence factors (bfpA, astA and ecpA genes) by PCR and antibiotic resistance.

Results: Of total 200 *E. coli* isolates, 38 (19.00%) were detected as EPEC and 28 (14.00%) as STEC. 5 (12.82%), 17 (43.58%) isolates from calves and 12 (33.33%), 11 (30.55%) isolates from sheep were found as EPEC and STEC, respectively. In rabbits, pigs and dogs, 6 (25.0%), 10 (45.45%) and 5 (13.15%) isolates were detected as EPEC, respectively. The most predominant EPEC serogroups were O88 (36.84%), O118 (18.42%). All 38 EPEC (100%) isolates carried ecpA gene and 18 (47.36%) isolates carried astA gene. Of 38 EPEC, only 5 (13.15%) isolates from dog carried bfpA gene, therefore considered as typical EPEC and 33 (86.84%) isolates were designated as atypical EPEC. Antimicrobial sensitivity test (AST) of 38 EPEC isolates showed resistance to nalidixic acid (55.26%), kanamycin (42.10%) followed by streptomycin (42.10%), doxycycline hydrochloride (28.94%), ciprofloxacin (13.15%), tetracycline (13.15%). Surprisingly, all the 38 isolates were sensitive to ampicillin.

Keywords: AST, EPEC, STEC, bfpA, astA, ecpA

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) are commonly recovered from the faeces of food-producing animals and pose threats to health of humans and livestock [29]. Healthy cattle, sheep, and pigs appear to be important reservoirs of EPEC but they have also been implicated as causative agent of diarrhoea in number of animal species, including rabbits, pigs, cattle and dogs [23]. STEC strains carry genes encoding Shiga toxins and may possess other virulence genes for intimin and enterohemolysin [40]. EPEC isolates are defined as intimin-containing diarrhoeagenic *E. coli* isolates that possess the ability to form attaching and effacing (AE) lesions on intestinal cells and do not possess genes coding for Shiga toxin. This protein called ‘intimin’ is encoded by eae gene which is used for the molecular diagnosis of EPEC *E. coli* strains. Multiple steps are involved in producing the characteristic A/E histopathology first of which is localized adherence, which is dependent on the presence of a plasmid designated the EPEC adherence factor (EAF) plasmid. This plasmid encodes a type IV pilus called the ‘bundle-forming pilus (BFP)’ whose structural gene is referred as bfpA. All those EPEC which carry EPEC adherence factor (EAF) are called typical EPEC, whereas, atypical EPEC contain the locus of enterocyte effacement (LEE), but do not contain the EAF plasmid. There are additional potential virulence factors present in EPEC. Some EPEC strains produce a low-molecular-weight Enteroaggregative heat-Stable Toxin 1 (EAST1) encoded by astA gene [38]. Similarly, there are reports of additional fimbrial structures produced by EPEC, one among them is *E. coli* common pilus (ECP), which is
Materials and methods
Sample collection
Faecal samples were collected from 200 different animals including calves [39], rabbits [24], dogs [38], slaughtered pigs [22], poultry [24], sheep and goat [36] from Jammu region during the period from March 2016 to April 2017. The samples included rectal swab from live animals and intestinal swabs from slaughtered animals. Theses samples were transported to the laboratory on ice, where experimental works were carried out. The samples were processed immediately or stored at 4°C to be processed later on.

Isolation of E. coli
All the samples were inoculated on MacConkey’s agar plates (HiMedia, India) and incubated for 24 hrs at 37°C. At least 3 well isolated pink colonies were randomly picked from each faecal sample showing the greening of EPEC for virulence factors; their serogroup, accessory virulence attributes and antibiotic resistance.

Antimicrobial susceptibility testing
Central Research Institute, Kasauli (H.P) and their virulence factors and colonization factors is lacking. So it becomes essential to characterize the EPEC isolates with respect to their serogroup, accessory virulence attributes like bfpA, astA and ecpA genes and antibiotic resistance.

Table 1: List of primer sequences and predicted amplicon length

| Primer | Sequence (5’-3’) | Target gene | Amplicon size (bp) | Reference |
|--------|------------------|-------------|--------------------|-----------|
| Stx1 F | ATAAATCGCCATTCTTGACTACAGAACGCCACCGACTAC | stx1 | 180 | (30) |
| Stx1 R | ATACAGGCTGCTTGCTGC | | | |
| Stx2 F | GGCACCTGCTGAACTGCTCCTGGCAATTCGACTTCTTG | stx2 | 255 | (30) |
| Stx2 R | AGAACGCCCACTGAGATCATC | | | |
| eae F | GACCCCGCAACAGCAATAGG | eae | 384 | (30) |
| eae R | GGCACCTGCAAGCAACAGGAG | | | |
| EAST11a | CCAATCAACAGATATCCGAAGTGCGGAGTCGTTG | astA | 111 | (42) |
| EAST11b | CCAATCAACAGATATCCGAAGTGCGGAGTCGTTG | | | |
| ecPA F | GCAACAGCACAAAAAGACACC | ecPA | 477 | (20) |
| ecPA R | CCAACGGTCGGCGTGAAC | | | |
| EP1 | AATGGTGCTGTGCGTCTGC | bfpA | 326 | (18) |
| EP2 | CGCGCTTATCCACCTGTGA | | | |

Molecular screening of EPEC for virulence factors bfpA, astA and ecpA
All the E. coli isolates carrying eae gene and devoid of stx1 and stx2 were declared as EPEC and screened for bfpA as per Gunzburg et al. (1955). ecpA as per Hernandes et al. (2011) and astA is as per Yamanoto and Echeverria (1996) using specific primers (Table 1). The amplified product of bfpA and ecpA was electrophoresed in a 1% (w/v) agarose gel and astA ampiclons in a 2% (w/v) agarose gel for 1 hr at 5 V/cm with a Standard molecular weight marker.

Serogrouping of E. coli Isolates
All the E. coli isolates carrying one or more of the virulence genes screened were serogrouped on the basis of their O antigen by the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (H.P) - 173204 (India).

Antimicrobial susceptibility testing
The EPEC isolates were tested for antimicrobial susceptibility against 12 antibiotics using disc diffusion technique [5] following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). Antimicrobial discs (HiMedia Pvt. Ltd., India) used were ampicillin (Amp 10 μg); cefixime (Cfm 5 μg); chloramphenicol (C 30 μg); ciprofloxacin (Cip 5 μg); doxycycline hydrochloride (Do 30 μg); kanamycin (K 30 μg); minocycline (MI 30 μg); nalidixic acid (NA 30 μg); norfloxacin (NX 10 μg); streptomycin (S 10 μg); tetracycline (TE 30 μg) and trimethoprim (TR 5 μg).

Results and Discussion
Isolation of presumptive E. coli
A total of 560 presumptive E. coli isolates were obtained from 200 faecal samples (Plate 1). Out of these 560 bacterial isolates, only one isolates from each sample showing the biochemical characteristics of E. coli on HiMotility E. coli test kit were selected for further processing. The identity of the selected isolates were further confirmed by +ve, +ve, -ve and -ve IMViC pattern as depicted in plate-2.
Plate 1: Isolation of ESBL producing *E. coli* on MLA and EMB agar.

Plate 2: IMViC pattern of *E. coli* isolates from faecal samples. The isolates show positive test results for indole production and methyl red test while negative results for Voges-Proskauer and citrate utilization test.

Detection of EPEC and STEC

All the 200 *E. coli* isolates, one each from each faecal sample, were subjected to molecular screening for *stx*1, *stx*2 and *eae* genes by multiplex PCR. Isolates carrying *stx*1 gene produced an amplicon of 180 bp, those carrying *stx*2 showed an amplicon of 255 bp and the presence of *eae* gene was detected by the amplification of 384 bp product. The species wise relative distribution of these genes is depicted in table 2 and the representative gene profiles are depicted in plate-3.

Table 2: Virulence gene profiles of *E. coli* isolates from different animal species

| S. No. | Species | No. of Isolates | *stx*1 | *stx*2 | *eae* |
|--------|---------|----------------|--------|--------|-------|
| 1      | Rabbit  | 6              | -      | -      | +     |
| 2      | Pig     | 10             | -      | -      | +     |
| 3      | Calf    | 5              | -      | -      | +     |
| 4      | Calf    | 8              | +      | +      | -     |
| 5      | Calf    | 2              | -      | +      | -     |
| 6      | Calf    | 6              | +      | -      | +     |
| 7      | Calf    | 1              | +      | -      | -     |
| 8      | Dog     | 5              | -      | -      | +     |
| 9      | Sheep   | 7              | +      | +      | -     |
| 10     | Sheep   | 1              | -      | +      | -     |
| 11     | Sheep   | 1              | +      | +      | +     |
| 12     | Sheep   | 2              | +      | -      | -     |
| 13     | Sheep   | 12             | -      | -      | +     |
| Total  |         | 66             | 25     | 19     | 45    |
In the present study, only 5 (12.82%) isolates out of 39 *E. coli* isolates from calves carried *eae* gene alone and thus the carriage of 12.82% EPEC is comparable to the 10.4% in Germany but higher than 2.7% in Sao Paulo, Brazil [1] and 5.83% reported in Dublin, Ireland [11]. However, higher percentage (41%) was reported by Waltner-Toews et al. (1986) from Ontario. In case of sheep 12 (33.33%) isolates out of 36 *E. coli* isolates carried *eae* gene alone. The previous studies have shown that a high percentages of sheep were colonized with EPEC [13, 21]. Aktan et al. (2004) from England detected EPEC in less than 17.7% of the sheep studied, while in present study 33.33% of sheep harboured EPEC. However, higher percentage (55%) was reported by Frohlicher et al. (2008).

Out of 24 *E. coli* isolates from rabbits, 6 (25.0%) isolates were detected as EPEC. However their carriage rate in healthy animals is lacking. In our study 25% of rabbits carried EPEC, which is comparatively less than that of 74% and 83% as reported by Blanco et al. (1997) from Spain and Swennes et al. (2012) from USA, in diarrhoeic rabbits, respectively. Similarly in pigs, 10 (45.45%) isolates out of 22 turned out to be EPEC, Porcine EPEC are associated with post weaning diarrhoea in pigs (44). However in Hungary, Malik et al. (2006) found no difference in the frequency of occurrence of *eae*+ *E. coli* in diarrhoeic and non-diarrhoeic pigs. In the present study, EPEC was detected in 45.45% of healthy pigs. There are few reports on pigs as a reservoir of EPEC and in one study by Frohlicher et al. (2008) the proportion of *eae*+ *E. coli* samples in pigs was 89%. This is quite high when compared with the proportion of 45.45% in our study. But De la Fuente et al. (2002) reported less than 20%: Aktan et al., 2004 from England and Wales reported 0.75%, Krause et al., 2005 from Germany reported 17.6%, which were lower than our study. On the same pattern, out of 38 *E. coli* isolates from dogs, 5 (13.15%) carried *eae* gene alone. EPEC strains with virulence properties similar to human strains are frequently isolated from diarrhoeic and healthy dogs [16]. The shedding rate of 13.15% of EPEC in dogs in the present study is, comparable to that of 12.6% reported from Brazil [26], but higher than 7.2%, reported from Germany [23] and 5.5% reported by Mainil et al. (1998). From poultry, 41 isolates were screened for these three genes and none of them carried any gene.

In the present study, out of all the domestic animals studied, STEC could be isolated from only 17 (43.58%) isolates of calves and 11 (30.55%) isolates of sheep. The carriage of 43.58% STEC in calves supported the findings of other workers, who showed that calves are a possible reservoir of STEC in their gastrointestinal tract [17]. The frequency, detected in the present study is comparable to the 44% in Brazil [27], 46% in Japan [28] reported for calves. These variations are likely due to geographical differences. It has been shown that the environment may have an influence on the shedding of STEC in calves [37]. In the present study the carriage of STEC in sheep is 30.55% which is higher than 12.32% reported for lambs in Jammu and Kashmir state [7], but lower than the 68% and 88% found in lambs in Germany and Spain (6,10), respectively.

**Plate 3**: Representative *stx*, *stx* and *eae* genes profile of *Escherichia coli* isolates using multiplex polymerase chain reaction (m-PCR). Lane M-100bp DNA ladder. Lane 1, positive control. Lane 2, negative control. Lane 3, *stx* positive. Lane 4, *stx* positive. Lane 5, *stx1*, *stx2* positive. Lane 6, *eae* positive. Lane 7, *stx1*, *eae* positive. Lane 8, *stx1*, *stx2*, *eae* positive. Lane 9, *stx2*, *eae* positive.

**Serogrouping and Virulence gene profile of EPEC**

All the 38 EPEC isolates were serogrouped for somatic ‘O’ antigen at National Saloonella and Escherichia Centre, Central Research Institute, Kasauli, H.P. The most predominant serogroups were O88 (36.84%) and O118 (18.42%) followed by O149 (7.89%), O22 (5.26%) and O11 (5.26%). The different serogroups found in the study are depicted in table 3. In contrast to our study, the serogroup O88 was also isolated by Rehman et al. (2014) from Cattle in Jammu and by Frohlicher et al. (2008) from Pig in Switzerland. Where as the serogroups found in sheep and rabbit have not been reported yet. Nakazato et al. (2004) also reported the isolation of *eae*+ *E. coli* belonging to serogroup O11 from dogs in Brazil. All the EPEC isolates were screened for the presence of *bfpA*, *ecpA* and *astA* genes by PCR as depicted in plate 3 and their virulence profile is detailed in table 3. In dog all the isolated EPEC in the present study harboured *bfpA* gene which matches to the study reported by Goffaux et al., 2000 and Nakazato et al., 2004. A study by Salehi et al. (2010) from Iran indicated that not only diarrhoeic dogs but also healthy dogs act as potential reservoirs of EPEC and should be considered as a potential risk factor in transmission of diarrhoea to humans. The *ecpA* gene was reported from all the EPEC isolates in contrast to
Hernandes et al., 2011. Several reports have previously shown that this gene is highly conserved among the different intestinal and extraintestinal E. coli pathotypes, including commensal strains [31,34,35,36]. A recent gene search study with Brazilian aEPEC strains showed that ecpA was present in all strains displaying an LAL pattern on HEp-2 cells by Scaletsky et al., 2010. In the present work, the distribution of the astA genes in a large collection of EPEC strains isolated from cattle, sheep, pig, dog and rabbit in Jammu was examined. In cattle astA gene was present in 80% of atypical EPEC isolates, which is comparable with 87% reported by De Sousa and Dubreuil (2001) from Canada. In sheep, astA gene was present in 16.6% of atypical EPEC isolates, this occurrence is much higher than 1.6% found by Yuste et al. (2006) in Spain, but lower (26.4%) as reported by Frohlicher et al. (2008) from Switzerland. Similarly, 80% (Eight out of Ten) astA gene was present in pig atypical isolates, which is 8% reported by Frohlicher et al. (2008) from Switzerland. In dogs astA gene was present in 80% (Four out of Five) EPEC isolates. Probably this is the first study of this kind as we do not have the reference to compare our results.

Table 3: Virulence gene profile and serogroup of EPEC from animals

| Species | Serogroup | No. of Strains | Virulence genes |
|---------|-----------|----------------|-----------------|
|         |           |                | bfpA | ecpA | astA |
| Calves  | O22       | 2              | -    | -    | +    |
|         | O88       | 1              | -    | +    | +    |
|         | O88       | 1              | -    | +    | -    |
|         | O149      | 1              | -    | +    | +    |
| Sheep   | O88       | 4              | -    | -    | +    |
|         | O88       | 2              | -    | -    | +    |
|         | O118      | 3              | -    | -    | -    |
|         | UT        | 3              | -    | -    | -    |
| Pig     | O88       | 4              | -    | +    | +    |
|         | O118      | 4              | -    | +    | +    |
|         | UT        | 2              | -    | -    | -    |
| Dog     | O11       | 2              | +    | +    | -    |
|         | UT        | 1              | +    | +    | -    |
|         | UT        | 2              | +    | +    | +    |
| Rabbit  | O88       | 2              | -    | +    | -    |
|         | O149      | 2              | -    | +    | -    |
|         | UT        | 2              | -    | -    | -    |
| Total   | 38        | 5              | 38   | 18   |

*‘+’ Positive; ‘-’, negative.

Plate 4: Representative bfpA, ecpA and astA genes profile of Escherichia coli isolates using multiplex polymerase chain reaction (m-PCR). Lane M 100bp DNA ladder. Lane 1, positive control. Lane 2, negative control. Lane 3, bfpA positive. Lane 4, astA positive. Lane 5, ecpA positive.

Antimicrobial susceptibility testing

The prevalence of antimicrobial resistance among the EPEC strains isolated from animals is shown in Table 4 and depicted in plate 5 and 6. Overall, 26 (68.42%) of the isolates were resistant to at least one of the 12 antibiotics tested, 12 (31.57%) had an intermediate susceptibility, and no isolate was pan-sensitive. In this study, the prevalence of tetracycline resistance in EPEC isolates was low. This is contrary to the results of Meyer et al. (2008), who reported higher rate of tetracycline resistance in E. coli isolated from animals. To our surprise all the isolates were sensitive to ampicillin. This is contrary to the findings of Hoyle et al. (2006), who observed wide spread ampicillin resistance among E. coli isolates obtained from animal faeces living in organic farms even when the use antimicrobial agents is restricted. In our study all of the EPEC isolates of calves were sensitive to chloramphenicol, ciprofloxacin and norfloxacin, which is in agreement with Rehman et al. (2014). However, in our study antibiotic kanamycin is resistant to all isolates of calves and dogs, which is comparable to the findings of Bolton et al. (2014) from Dublin, Ireland and Banik et al. (2016) from South Bengal, respectively. The in vitro sensitivity assay carried out to determine the antibiotic resistance profile of ovine E. coli isolates revealed that the isolates were sensitive to a range of antimicrobials like ampicillin, chloramphenicol, ciprofloxacin and nalidixic acid. However, intermediate resistance was found against antibiotics like kanamycin. Similar results were reported by Purkayastha et al. (2010) who reported sheep E. coli isolates to be sensitive to chloramphenicol, ciprofloxacin and nalidixic acid.
### Table 4: Number of susceptible (S), intermediate (I), and resistant (R) strains of enteropathogenic *Escherichia coli* isolated from animals to 12 antimicrobial agents.

| Antimicrobials                      | Calves(5) | Sheep(12) | Pig(10) | Dog(5) | Rabbit(6) |
|-------------------------------------|-----------|-----------|---------|--------|-----------|
|                                     | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| Ampicillin                          | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 30 | 0 | 0 | 6 | 0 | 0 |
| Cefixime                            | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Chloramphenicol                     | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Ciprofloxacin                       | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Doxycycline hydrochloride           | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Kanamycin                           | 0 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Minocycline                         | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Nalidixic acid                      | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Norfloxacin                         | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Streptomycin                        | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Tetracycline                        | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |
| Trimethoprim                        | 5 | 0 | 0 | 12 | 0 | 0 | 10 | 0 | 0 | 5 | 0 | 0 | 6 | 0 | 0 |

Figure in parenthesis indicates number of isolates

### Conclusion

This study concluded the role of dogs as a reservoir of EPEC and can play a crucial role in transmission of certain pathogens to humans and livestock due to faecal contamination in the environment. Meanwhile high proportion of EPEC and STEC in other healthy animal species may sow seed for future infections in humans around the globe. The study also reports, for the first time, the isolation and characterization of *bfpA* and *asta* gene in dog and EPEC serogroups O88, O118 from healthy sheep and rabbit in India.

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### References

1. Aidar-Ugrinovich L, Blanco J, Blanco M, Blanco JE, Leomil L, Dahbi G *et al.* Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in Sao Paulo, Brazil. International Journal of Food Microbiology 2007;115:297-306.
2. Akton I, Spriggins K, La Ragione RM, Faulkner LM, Paiba GA, Woodward MJ. Characterization of attaching-effacing *Escherichia coli* isolated from animals at slaughter in England and Wales. Veterinary Microbiology 2004;102:43-53.
3. Avelino F, Saldana Z, Islam S, Monteiro-Netoc V, DalAgnol M, Eslava CA *et al.* The majority of enterohaemorrhagic *Escherichia coli* strains produce the *E. coli* common pilus when adhering to cultured epithelial cells. International Journal of Medical Microbiology 2010;300:440-448.
4. Banik A, Isore DP, Joardar SN, Batabyal K, Dey S. Characterization and antibiogram of enteropathogenic *Escherichia coli* isolated from diarrhoeic and non-diarrhoeic dogs in South Bengal. Indian Journal of Animal Research 2016;50:773-775.
5. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 1966;45:493-496.
6. Beutin L, Geier D, Zimmermann S, Aleksic S, Gillespie HA, Whittam TS. Epidemiological Relatedness and
Clonal Types of Natural Populations of Escherichia coli Strains Producing Shiga Toxins in Separate Populations of Cattle and Sheep. Applied and Environmental Microbiology 1997;63:2175-2180.

7. Bhat MA, Nishikawa Y, Wani SA. Prevalence and virulence gene profiles of Shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli from diarrhoeic and healthy lambs in India. Small Ruminant Research 2008;75:65-70.

8. Blackburn D, Husband A, Saldana Z, Nada RA, Klena J, Qadri F et al. Distribution of the Escherichia coli common pilus among diverse strains of human enterotoxigenic E. coli. Journal of Clinical Microbiology 2009;47:1781-1784.

9. Blanco JE, Blanco M, Blanco J, Mora A, Balaguer L, Cuervo L et al. Prevalence and characteristics of enteropathogenic Escherichia coli with the eae gene in diarrhoeic rabbits. Microbiology and Immunology 1997;41:77-82.

10. Blanco M, Blanco JE, Mora A, Rey J, Alonso JM, Hermoso M et al. Serotypes, virulence genes, and intimin types of Shiga Toxin (Verotoxin)-producing Escherichia coli isolates from healthy sheep in Spain. Journal of Clinical Microbiology 2003;41:1351-1356.

11. Bolton DJ, Ennis C, McDowell D. Occurrence, virulence genes and antibiotic resistance of enteropathogenic Escherichia coli (EPEC) from twelve bovine farms in the North-East of Ireland. Zoonoses Public Health 2014;61:149-156.

12. Buchanan RE, Gibbon NE. Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore, MD: Williams and Wilkins 1994, 787.

13. De la Fuente R, Garcia S, Orden JA, Ruiz-Santa-Quiteria JA, Diez R, Cid D. Prevalence and characteristics of attaching and effacing strains of Escherichia coli isolated from diarrhetic and healthy sheep and goats. American Journal of Veterinary Research 2002;63:262-266.

14. De Sousa CP, Dubreuil JD. Distribution and expression of the astA gene (EAST1 toxin) in Escherichia coli and Salmonella. International Journal of Medical Microbiology 2001;291:15-20.

15. Frohlicher E, Krause G, Zweifel C, Beutin L, Stephan R. Characterization of attaching and effacing Escherichia coli (AEEC) isolated from pigs and sheep. BMC Microbiology 2008;8:144.

16. Goffaux F, China B, Janssen L, Mainil J. Genotypic characterization of enteropathogenic Escherichia coli (EPEC) isolated in Belgium from dogs and cats. Research in Microbiology 2000;151:865-871.

17. Guler L, Gunduz K, Ok U. Virulence factors and antimicrobial susceptibility of Escherichia coli isolated from calves in Turkey. Zoonoses Public Health 2008;55:249-257.

18. Gunzburg ST, Tornepoth NG, Riley LW. Identification of enteropathogenic Escherichia coli by PCR-based detection of the bundle-forming pilus gene. Journal of Clinical Microbiology 1995;33:1375-1377.

19. Hernandez RT, Elias WP, Vieira MAM, Gomes TAT. An overview of atypical enteropathogenic Escherichia coli. FEMS Microbiology Letters 2009;297:137-149.

20. Hernandez RT, Velsko I, Sampaio SCF, Elias WP, Robins-Browne RM, Gomes TAT et al. Fimbrial adhesins produced by atypical enteropathogenic Escherichia coli strains. Applied and Environmental Microbiology 2011; 77: 8391-8399.

21. Hoyle DV, Davison HC, Knight HI, Yates CM, Dobay O, Gunn GJ et al. Molecular characterisation of bovine faecal Escherichia coli shows persistence of defined ampicillin resistant strains and the presence of class 1 integrons on an organic beef farm. Veterinary Microbiology 2006; 115: 250-257.

22. Kobayashi H, Shimada J, Nakazawa M, Morozumi T, Pohjanvirta T, Pelkonen S et al. Prevalence and characteristics of Shiga toxin-producing Escherichia coli from healthy cattle in Japan. Applied and Environmental Microbiology 2001;67:484-489.

23. Krause G, Zimmermann S, Beutin L. Investigation of domestic animals and pets as a reservoir for intimin-(eae) gene positive Escherichia coli types. Veterinary Microbiology 2005;106:87-95.

24. Mainil JG, Jacquesmin E, Bez S, Pohl P, Kaeckenbeek A. Les souches pathogens d’Escherichia coli chez les chiens et chats detection des souches enterotoxignonnes (ETEC), enteropathogenes (EPEC), verotoxigones (VTEC), enterohemorragiques (EHEC) et ecrotoxigones (NTEC). Annales De Medicine Vetenaire 1998;142:39-46.

25. Malik A, Toth I, Beutin L, Schmidt H, Taminiabia B, Dow MA et al. Serotypes and intimin types of intestinal and faecal strains of eae+ Escherichia coli from weaned pigs. Veterinary Microbiology 2006;114:82-93.

26. Meyer E, Lunte K, Kist M, Schwab F, Frank U. Antimicrobial Resistance in Escherichia coli strains isolated from food, animals and humans in Germany. Infection 2008;36:59-61.

27. Moreira CN, Pereira MA, Brod CS, Rodrigues DP, Carvalhal JB, Aleixo JAG. Shiga toxin-producing Escherichia coli (STEC) isolated from healthy dairy cattle in southern Brazil. Veterinary Microbiology 2006;93:179-183.

28. Nakazato G, Gyles C, Ziebell K, Keller R, Trabulsi LR, Gomes TAT et al. Attaching and effacing Escherichia coli isolated from dogs in Brazil: characteristics and serotypic relationship to human enteropathogenic E. coli (EPEC). Veterinary Microbiology 2004;101:269-277.

29. Nataro JP, Kaper JB. Diarrhoeagenic Escherichia coli. Clinical Microbiology Reviews 1998;11:142-201.

30. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohaemorrhagic E. coli hlyA, rfbO111, and rfbO157. Journal of Clinical Microbiology 1998;36:598-602.

31. Poult R, Westerlund-Wikstrom B, Lang H, Alstki V, Virkola R. Saarella U et al. catB, a common fimbriil gene of Escherichia coli, expressed in a genetically conserved, virulent clonal group, Journal of Bacteriology 2001;183:4727-4736.

32. Purkayastha M, Khan MSR, Alam M, Siddique MP, Begum F, Mondal T et al. Cultural and biochemical characterization of sheep Escherichia coli isolated from in and around Bau campus. Journal of Veterinary Medicine 2010;8:51-55.

33. Rehman MU, Rashid M, Sheikh JA, Bhat MA. Molecular epidemiology and antibiotic resistance pattern of enteropathogenic Escherichia coli isolated from bovines and their handlers in Jammu, India. Journal of Advanced Veterinary and Animal Research 2014;1:177-181.

34. Rendon MA, Saldana Z, Erdem AL, Monteiro-Neto V,
Vazquez A, Kaper JB et al. Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. Proceedings of the National Academy of Sciences of the United States of America 2007;104:10637-10642.

35. Salehi TZ, Badouei MA, Gohari IM. Molecular detection and antibacterial susceptibility of enteropathogenic *Escherichia coli* (EPEC) and Shiga toxigenic *Escherichia coli* (STEC) strains isolated from healthy and diarrhoeic dogs. Comparative Clinical Pathology 2010;19:585-589.

36. Scaletsky ICA, Aranda KRS, Souza TB, Silva NP. Adherence factors in atypical enteropathogenic *Escherichia coli* strains expressing then localized adherence-like pattern in HEp-2 cells. Journal of Clinical Microbiology 2010;48:302-306.

37. Shaw DJ, Jenkins C, Pearce MC, Cheasty T, Gunn GJ, Dougan G et al. Shedding patterns of verocytotoxin producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm. Applied and Environmental Microbiology 2004;70:7456-765.

38. Silva LEP, Souza TB, Silva NP, Scaletsky ICA. Detection and genetic analysis of the enteroaeggregative *Escherichia coli* heat-stable enterotoxin (EAST1) gene in clinical isolates of enteropathogenic *Escherichia coli* (EPEC) strains. BMC Microbiology 2014;14:135.

39. Swennes AG, Buckley EM, Parry NMA, Madden CM, García A, Morgan PB et al. Enzootic enteropathogenic *Escherichia coli* infection in laboratory rabbits. Journal of Clinical Microbiology 2012;50:2353-2358.

40. Tristao LC, Gonzalez AG, Coutinho CA, Cerqueira AM, Gomes MJ, Irino K et al. Virulence markers and genetic relationships of Shiga toxin-producing *Escherichia coli* strains from serogroup O111 isolated from cattle. Veterinary Microbiology 2007;119:358-365.

41. Waltner-Toews D, Martin SW, Meek AH. An epidemiological study of selected calf pathogens on Holstein dairy farms in south western Ontario. Canadian Journal of Veterinary Research 1986;50:307-313.

42. Yamamoto T, Echeverria P. Detection of the enteroaeggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. Infection and Immunity 1996;64:1441-1445.

43. Yuste M, De La Fuente R, Ruiz-santa-quiteria JA, Cid D, Orden JA. Detection of the astA (EAST1) gene in attaching and effacing *Escherichia coli* from ruminants. Journal of Veterinary Medicine 2006;53:75-77.

44. Zhu C, Harel J, Jacques M, Fairbrother JM. Interaction with pig ileal explants of *Escherichia coli* O45 isolates from swine with postweaning diarrhoea. Canadian Journal of Veterinary Research 1995;59:118-123.