Pakistan J. Zool., pp 1-8, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231005073531

Molecular Epidemiology of Enteric Pathogens Associated with Diarrheal Disease in Goat Kids

Maria Azmat, Jawaria Ali Khan*, Muhammad Ijaz, Kamran Ashraf and Omer Naseer

Department of Clinical Medicine, University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT

Neonatal diarrhea is one of the leading cause of mortalities in goats leading to economic losses. The current research was conducted to study the prevalence of *Salmonella* and *Escherichia coli* in diarrheic goat kids. Fecal samples of 200 diarrheic goats up to two months of age were collected from different government and private sector veterinary hospitals, livestock farms and small households in and around district Bahawalpur between year 2021 and 2022 on seasonal basis. Microbiological samples underwent processing to isolate *E. coli* and *Salmonella*, and the identification of these isolates was achieved through a combination of cultural, morphological, and biochemical characteristics, complemented by molecular characterization. PCR results showed amplification of 521 bp segment of the *invA* gene and 314bp of *K99* gene in *Salmonella* and *E. coli,* respectively. The results revealed that bacterial prevalence was 43.5%, among which *E. coli* prevalence was 23.5% and *Salmonella* prevalence was 17.5%. Moreover, in male goat kids, both *Salmonella* and *E. coli* infection rate was higher (65.71% and 31.25% respectively) as compared to females (7.27% and 13.64% respectively). Concerning the local breeds, Desi breed has the highest prevalence of *Salmonella* (21.21%) and *E. coli* (29.41%). Seasonal variations were found to be statistically significant in *Salmonella* with high prevalence in winter (41.18%) but non- significant in *E. coli* with high prevalence in Autumn (29.41%). Furthermore, varying factors impact infection rates such as, age, weather conditions, management practices and hygiene measures.

Article Information
Received 05 October 2023
Revised 08 March 2024
Accepted 26 March 2024
Available online 29 May 2024
(early access)

Authors' Contribution
MA and JAK conceptualized the study. MA collected the samples and analyzed them. MI, KA and ON validate the data and helped in investigation. MA and JAK drafted the manuscript. MI, ON and KA reviewed and improved the manuscript.

Key words
Neonatal goats, Diarrhea, *Salmonella, E. coli*, Prevalence, PCR

INTRODUCTION

Diarrhea represents the primary cause of both high morbidity and mortality among young animals, resulting in substantial economic losses in the global livestock industry (Yadegari *et al*., 2019; Shrivastava *et al*., 2022). Diarrhea leads to deficit of health and drop in production potential of animals (Koirala and Bhandari, 2019). According to the National Animal Health Monitoring System, approximately 57% of calf deaths at the weaning stage can be attributed to diarrhea (Cho and Yoon, 2014). Small ruminants play a vital role in the agricultural systems of developing countries, both economically and ecologically (Devendra, 2005). The mortality rate for lambs typically falls within the range of 10 to 25%, while estimates for goat kid mortality range from 11.5 to 37% (Thiruvenkadan and Karunanithi, 2007).

The causes of diarrhea in sheep and goats encompass both non-infectious and infectious factors (Abdou *et al*., 2021). Among bacterial pathogens, enterotoxigenic *E. coli* (ETEC) and *Salmonella* hold significant economic importance (Achá *et al*., 2004). Although the presumed cause is typically attributed to specific bacteria like *E. coli*, investigations into diarrhea among both young kids and adult goats have consistently been unable to identify specific causes in the majority of instances (Gavin *et al*., 2018). In both cattle and dairy goats, research has demonstrated variations in the expression patterns of different virulence genes in *E. coli*, depending on the host's age, season and geographic location (Dwell *et al*., 2008; Fernández *et al*., 2009). *E. coli* typically induces watery diarrhea and weakness in newborn calves aged 1–4 days. Usually, death often ensues within 24 h as a result of severe dehydration (Cho *et al*., 2010). Additionally, stress has been identified as a factor that can lead to an elevated prevalence of specific virulent strains (Bach *et al*., 2004; Ndegwa *et al*., 2020). *Salmonella* infection stands as a prevalent cause of diarrheal illness in both sheep and goat flocks (Shabana *et al*., 2017). The extent of *Salmonella* infection's impact varies, contingent on bacterial factors such as serovar, virulence, and antimicrobial susceptibility. It's worth noting that the global count of identified

* Corresponding author: jawaria.khan@uvas.edu.pk
0030-9923/2024/0001-0001 $ 9.00/0

Salmonella serovars exceeds 2600, with new serovars emerging annually (Jajere, 2019).

Understanding the epidemiology, prevalence, and virulence factors of these pathogens in specific regions is crucial for effective disease management and prevention strategies. The current research was conducted to study the prevalence of *E. coli* and *Salmonella* in diarrheic goats in Bahawalpur, Pakistan (Fig. 1).

MATERIALS AND METHODS

A total of 200 goats suffering from clinical signs of diarrhea (irrespective of breed and sex) up to two months of age were included in this study. The fecal samples were collected directly from the diarrheic goats and the data was recorded using a pre-tested form termed as Data Capture form (DCF). The parameters in DCF included age, breed, sex, duration of diarrhea, fecal scoring, season, stagnant water, colostrum feeding, environmental condition, and body condition.

Each animal's rectum was used to collect approximately 5 g of feces. DNA from colonies of positive samples were extracted using bacterial DNA extraction kit (WizPrepTM gDNA cell/tissue kit, Korea) and confirmed through PCR (Haq *et al*., 2017) by using new primers.

Isolation and purification of E. coli *and* Salmonella

Bacterial pathogens like *E. coli* and *Salmonella* were identified and isolated by culturing (Kar *et al*., 2017; Haq *et al*., 2017). The samples were mixed with 5mL phosphate buffer saline solution in test tubes, followed by inoculation on Nutrient Agar were further streaked on MaConkey agar and EMB agar and incubated further at 37°C aerobically for 24 h. Only pink colonies from MaConkey agar, and green metallic sheen colonies from EMB agar were chosen for *E. coli.* These colonies were further purified by multiple streaking methods on the MacConkey agar and EMB agar and further processed for identification and confirmation of *E. coli*.

For isolation and purification of *Salmonella,* bacterial cultures were streaked on to nutrient agar, yielding translucent circular *Salmonella* colonies after 24 h and then streaked onto *Salmonella-shigella* agar (SSA), incubated at 37°C for 24 h, and processed to purify the bacteria by multiple streaking method.

Pure colonies were proceeded for identification of *E. coli* by observing macroscopic, microscopic and biochemical characters. It was followed by motility test using hanging drop method (Cheesbrough, 2009).

The isolates that were Gram negative after the gram staining were then subjected for the biochemical tests which include different tests, MR test, VP test, indole test, catalase test, oxidase test, citrate utilization test and urease test and H_2S production tests (Reiner, 2010; Koneman, 2016).

Molecular diagnosis of bacteria

For molecular diagnosis of bacteria positive fecal samples were processed for detection of *E. coli* and *Salmonella* DNA, using primer sequence specific for *inaA* gene and *F5* gene (Tables I and II).

Table I. Primer sets used for characterization of *E. coli* and *Salmonella.*

Gene	Primer sequence 5' →3'	Length of PCR product (bp)
invA	F TTGTTACGGCTATTTTGACCA	521
	R CTGACTGCTACCTTGCTGATG	
F5	F TATTATCTTAGGTGGTATGG	314
	R GGTATCCTTTAGCAGCAGTATTTC	

Table II. Biochemical characteristics of *Salmonella* and *E. coli.*

Biochemical test	*Salmonella*	*E. coli*
Gram staining	Negative	Negative
Motility	Positive	Positive
Catalase test	Positive	Positive
Oxidase test	Negative	Negative
Indole test	Negative	Positive
Methyl red test	Positive	Positive
Citrate utilization test	Positive	Negative
VogesProskauer test	Negative	Negative

For genomic DNA extraction of Gram-negative bacteria fresh culture of isolates was taken from MaConkey agar plates and transferred into micro centrifuge tube (1.5ml) containing normal saline from fecal samples using genomic DNA extraction Kit (GeneAll®, Exgene™ Catalogue No. 105-101).

PCR recipe was arranged in an absolute volume of 20µl containing of 10µl of TOPreal™ qPCR 2x PreMIX, 2µl of DNA sample and 1µmol of separately primer. Reaction was cycled 35 times afterwards first denaturation at 95°C for 5 min with denaturation at 95°C, annealing at 60°C and extension phase at 72°C, each phase was given 45 seconds, last elongation at 72°C for 10 min was done. A negative control was involved in each PCR run. The PCR yields were detected on agarose gel (2%)for positive bands against a 100bp molecular ladder. Amplified products were visualized using UV Transilluminator.

Statistical analysis

The data were statistically analyzed using SPSS 25.0

version. Analysis of variance (ANOVA) was used in case of more than two variables within group while Student's t-test was employed for the two groups.

RESULTS

Prevalance of E. coli *and* Salmonella *in diarrhoeic goats*

Out of 200 samples 47 goats were positive for *E. coli* and 35 goats were positive for *Salmonella* infection after initial identification on basis of microscopic examination and biochemical profile. The PCR analysis confirmed the presence of *Salmonella* by 521bp amplified band of *invA* gene and *E. coli* by amplified band of *K99* gene.

The results revealed that bacterial prevalence was 43.5% (97/200). Among which *E. coli* prevalence was 23.5% and *Salmonella* prevalence was 17.5% in Bahawalpur district, Punjab, Pakistan.

Age

The goats were examined across three age groups: G1 (0-15 days), G2 (16-30 days), and G3 (>30 days). Among these groups, 18.60% goats in G1, 22.22% goats G2, and 13.83% goats in G3 were tested positive for *Salmonella* (Table III). The statistical analysis indicated that age was a significant factor (P>0.05) in relation to *Salmonella* infection. In case of *E. coli* infection, maximum positive cases were recorded in G1 (34.88%) while in G2 and G3 the positive cases were 20.63% and 20.21%, respectively (Table III). In the present study, age factor was significant (P<0.05): *Salmonella* infection was found to be most infected G2 while G1 was found to be most infected age group with *E. coli*.

Breed

The results showed that Desi breed has the maximum rate (21.21%) of infection followed by Beetal (19.35%), Teddy (17.78) and Rajanpuri breeds (13.33) (Table III). The results were non-significant (P>0.05) for *Salmonella* infection causing diarrhea.

In case of *E. coli*, the results showed that Desi breed showed maximum (29.41%) positive cases followed by Teddy (26.09%) and Beetal (24.59%). The Rajanpuri breed had the least infected cases in terms of percentage (16.95%). The variations on the basis of breed were found to be significant (p<0.05). In both cases, Desi breed was most infected.

Gender/Sex

Regarding sex, male goats showed more prevalence in both infections: *Salmonella* 65.71% and *E. coli* 31.25% (Table III). Infections in female were 7.27% in the case of *Salmonella* while 13.64% in the case of *E. coli.* The results were significant (P<0.05) in both infections.

Table III. Prevalence of *Salmonella* and *Escherichia coli* infection in diarrheic goats kids.

Parameters	*Salmonella*		*E. coli*	
	Positive/ total (%)	P value	Positive	P value
Age				
0-15 days	8/43 (18.60 %)	0.038	15/43 (34.88 %)	0.013
16-30days	14/63 (22.22%)		13/63 (20.63 %)	
>30days	13/63 (13.83%)		19/94 (20.21 %)	
Breed				
Teddy	8/45 (17.78 %)	0.756	12/46 (26.09 %)	0.001
Beetal	12/62 (19.35%)		15/61 (24.59 %)	
Rajanpuri	8/60 (13.33 %)		10/59 (16.95 %)	
Desi	7/33 (21.21 %)		10/34 (29.41 %)	
Sex				
Male	23/35 (65.71%)	0.001	35/112 (31.25%)	0.004
Female	12/165 (7.27%)		12/88 (13.64 %)	
Season				
Winter	7/17 (41.18 %)	0.073	12/46 (26.09 %)	0.001
Spring	6/46 (13.04 %)		10/59 (16.95 %)	
Summer	13/68 (19.12%)		15/61 (24.59 %)	
Autum	9/69 (13.04 %)		10/34 (29.41 %)	
Colostrum feeding				
Yes	26/128(20.3%)	0.016	19/102 (18.63%)	0.097
No	9/72(12.50 %)		28/98 (28.57 %)	
Duration of diarrhea				
1 Day	8/57 (14.04 %)	0.066	22/84 (26.19 %)	0.063
2 Day	13/64 (20.31%)		14/71 (19.72 %)	
3 or >3 days	14/79 (17.72%)		11/45 (24.44 %)	
Body condition				
Fatty	4/51 (7.84 %)	0.579	8/43 (18.60 %)	0.027
Emaciated	20/82 (24.39%)		30/94 (31.91%)	
Good	11/67 (16.42%)		9/63 (14.29 %)	
Environmental condition				
Good	8/57 (14.04 %)	0.069	9/71 (12.68 %)	0.037
Fair	11/61 (18.03%)		22/47 (46.81%)	
Poor	16/82 (19.51%)		16/82 (19.51%)	
Stagnant water				
Yes	23/118 (19.4%)	0.037	34/108 (31.48%)	0.004
No	12/82 (14.63%)		13/91 (14.13 %)	
Fecal scoring				
1 (Normal feces)	4/21 (19.05%)	0.047	4/18 (22.22 %)	0.003
2 (Semi solid feces)	12/73 (16.44%)		10/46 (21.74%)	
3(Dysentery)	6/49 (12.24 %)		3/35 (8.57 %)	
4(Very liquid)	8/36 (22.22 %)		19/58 (32.76%)	
5 (Watery)	5/21 (23.81 %)		11/43 (25.58%)	

Fig. 1. Bahawalpur district location map, Pakistan.

Seasonal analysis

The seasonal analysis revealed that *Salmonella* infection was highest (41.18%) during winter season followed by summer (19.12%). The percentage of infection was same in spring and autumn (13.04%). The seasonal variations were found to be non-significant ($p<0.05$).

The prevalence of *E. coli* infection was highest in Autumn (29.41%) followed by winter (26.09%) and summer (24.59%). The infection rate was minimum in spring (16.95%) (Table III). The seasonal variations were found to be significant statistically ($p<0.05$).

Colostrum feeding

A total of 128 goats were given colostrum's feeding among which 26 (20.31%) showed *Salmonella* cases (Table III). The results were found to be significant ($P<0.05$).

In the case of *E. coli*, 102 goats were given colostrum feeding among which 19 (18.63%) showed infection (Table III). Overall, the results were non-significant ($P>0.05$).

Diarrhea Duration

In the context of diarrhea duration among goats, the data indicated that goats with a one-day duration of diarrhea had a lower *Salmonella* (14.04%) rate while higher (26.19%) *E. coli* infection, whereas those with a two-day duration showed a higher rate of infection in *Salmonella* (20.31%) and lower rate in *E. coli* (19.72%) infection as compared to one-day duration. Furthermore, goats experiencing diarrhea for three or more days exhibited an infection rate of 17.72% in *Salmonella* and 24.44% in case of *E. coli* (Table III). The study revealed that there were no statistically significant associations between diarrhea duration and *Salmonella* infection ($p>0.05$) as well as between *E. coli* infection and diarrhea duration ($p<0.05$).

Body condition

Regarding body conditions, the results stated that emaciated goats showed maximum (24.39%) positive cases while good healthy goats showed 11% positive cases of *Salmonella* followed by lowest infection rate in fatty goats 7.84% (Table III). The results were non-significant ($P>0.05$). In case of *E. coli* infection, emaciated goats had maximum (31.91%) prevalence followed by fatty condition (18.60%) and healthy goats in good condition (14.29%) (Table III). The results were statistically significant ($P<0.05$). In both infections, emaciated condition had maximum prevalence.

Environmental condition

Regarding environmental condition, the data revealed that good environmental condition showed 14.04% *Salmonella* infection, fair environmental condition showed 18.03% positive cases while poor condition showed 19.51% infection (Table III). In this study, the results were non-significant ($P>0.05$). In the case of *E. coli*, the results revealed that fair environmental condition showed maximum (46.81%) positive cases of infection (Table III) followed by poor (19.51%) and good environmental condition (12.68%). The results were statistically significant ($P<0.05$).

Stagnant water

The presence of stagnant water showed 19.49% positive cases of *Salmonella* as compared to its absence (14.63%) (Table III). *E. coli* infection was also more prevalent (31.48%) in the presence of stagnant water as compared to its absence (14.13%) (Table III). The findings were significant ($P<0.05$) in both cases of infections.

Fecal scoring

The maximum cases (23.81%) of infection were recorded in watery feces (score 5) followed by liquid feces (score 4) and normal feces (score 1) with 22.22 and 19.05% respectively. Infection rate in semi solid feces (score 2) was 16.44%. Least number of cases for positive infection were recorded in score 3 (dysentery) with 12.24% infection rate (Table III). In case of *E. coli* infection, maximum positive cases were recorded in fecal score 4 (32.76%) followed by score 5 (25.58%) and score 1 (22.22%) (Table III). The results were found to be significant ($P<0.05$) in both infections.

DISCUSSION

Neonatal diarrhea is one of the leading causes of calf

death in animal industry (Urie *et al*. 2018). Diarrhea and health issues in young goats pose significant challenges to the well-being of these animals, impacting the overall profitability of the goat industry (Cheng *et al*., 2021). It is essential to gain a deeper insight into potential biomarkers that can serve as indicators for mortality and morbidity in neonatal goats suffering from diarrhea (Dwyer *et al*., 2015). The current study revealed that bacterial prevalence was 43.5% in goat kids.

E. coli stands as the primary and most influential factor responsible for neonatal diarrhea in ruminants (Brunauer *et al*., 2021). The *E. coli* prevalence in this study was much lower (23.5%) than prevalence reported by previous studies in diarrheic goats; such as, 57.8% (Shabana and Enazi, 2020), 48.7% (Abd El-Tawab *et al*., 2020), 31.7% (Kiziltepe and Ayvazoğlu, 2022) and 27.3% (Osman *et al*., 2013). Mishra *et al*. (2018) identified 193 isolates out of 300 (64.3%). The differences in *E. coli* prevalence may be linked to variations in geographic distribution, calf age, weather conditions, management practices, and hygiene measures (Alam *et al*., 2009; Vanselow *et al*., 2017; Abd El-Tawab *et al*., 2020).

The *Salmonella* prevalence was 17.5% in the current study which was lower than *E. coli* infection. Lower rate (7%) of *Salmonella* infection in goats was also reported by Hawwas *et al*. (2022), 3.86% by Farouk *et al*. (2021) and 3.49% by Abd El-Twab *et al*. (2016). Another study reported 75.6% *E. coli* and 18.1% *Salmonella* infection in diarrheic calves (El-Seedy *et al*., 2016). Diagnosing salmonellosis in sheep and goats poses challenges because the clinical symptoms are not specific (OIE, 2000). Contaminated water can be considered as one of the leading causes of *Salmonella* infection in goat kids (Ghimire and Bhattarai, 2019).

Young goats are vulnerable to diarrhea during the initial week of their lives (Caffarena *et al*., 2021). In case of *Salmonella* infection G2 was found to be most infected. The results were in accordance with Grünberg (2020) and Duffy *et al*. (2010). In case of *E. coli* G1 was most infected age group. Moreover, the *Salmonella* isolation rate was highest (13.5%) between one and two years of age (Hawwas *et al*., 2022). *E. coli* K99 was found to be most common factor of diarrhea in kids in Kuwait (Majeed *et al*., 2018; Abdou *et al*., 2021) and Saudi Arabia (Shabana *et al*., 2017).

Male goats showed more prevalence in both infections, *Salmonella* (65.71%) and *E. coli* (31.25%). In Ethiopia, a hundred percent higher isolation rate of *Salmonella* was recorded in males as compared to females (0%). Saha *et al*. (2014) also reported higher (15.78%) prevalence in male goats as compared to females (10.71%).

The seasonal analysis revealed that *Salmonella* infection was highest (41.18%) during winter season followed by summer (19.12%). The percentage of infection was same in spring and autumn (13.04%). In case of *E. coli* infection, the prevalence was highest in Autumn (29.41%) followed by winter (26.09%) and summer (24.59%). Abdou *et al*. (2021) recorded more cases of *E. coli* diarrhea in dry season as compared to wet season.

CONCLUSIONS

Neonatal diarrhea poses a significant threat to goat mortality, leading to economic losses. A year-long research project examined *Salmonella* and *Escherichia coli* prevalence in diarrheic goat kids. Fecal samples from 200 goats under two months old were collected from various sources in the Bahawalpur district. Microbiological analysis identified bacterial presence, with higher rates in male goat kids compared to females. Local Desi breed goats had the highest prevalence. Seasonal variations were significant for *Salmonella*, favoring winter, while *E. coli* showed no significant seasonal variation.

DECLARATIONS

Funding

The study was not funded by any organization.

IRB approval

The study was approved by the IRB of University of Veterinary and Animal Sciences, No/624/DVM, dated: (16/02/2022).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Twab, A.A., El-Hofy, I.F. and Amira, M.R., 2016. Molecular characterization of Quinolones and β-lactams resistant *Salmonella* Serovars determinants in diarrheic calves, lambs and goats-kids in the Middle of Nile Delta, Egypt. *Benha Vet. med. J.,* **30**: 171-182. https://doi.org/10.21608/bvmj.2016.31361

Abd El-Tawab, A.A., Maarouf, A.A., El-Hofy-Fatma, I. and Nesma, M.G.A., 2020. Phenotypic characterization of some food poisoning bacteria isolated from meat and meat products in Kaliobia, Egypt. *Benha Vet. med. J.,* **38**: 146-151. https://doi.org/10.21608/bvmj.2020.25678.1186

Abdou, N.E., Majeed, Q.A.H., El-Azazy, O.M.E., Tahrani, L.M.A., AlAzemi, M.S. and Alajmi, A.,

2021. Risk factors of diarrhea in small ruminants in Kuwait. *Iran. J. Vet. Res.*, **22**: 146.

Achá, S.J., Kuhn, I., Jonsson, P., Mbazima, G., Katouli, M. and Mollby, R.S., 2004. Studies on calf diarrhoea in Mozambique: Prevalence of bacterial pathogens. *Acta Vet. Scand.,* **45**: 27–36. https://doi.org/10.1186/1751-0147-45-27

Acha, S.J., Kühn, I., Jonsson, P., Mbazima, G., Katouli, M. and Möllby, R., 2004. Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens. *Acta Vet. Scand.*, **45**: 1-10. https://doi.org/10.1186/1751-0147-45-27

Alam, M.J., Renter, D.G., Ives, S.E., Thomson, U.D., Sanderson, W.M., Hollis, C.L. and Nagaraja, G.T., 2009. Potential associations between fecal shedding of *Salmonella* in feedlot cattle treated for apparent respiratory disease and subsequent adverse health outcomes. *J. Vet. Res.,* **40**: 1-13. https://doi.org/10.1051/vetres:2008040

Bach, S.J., McAllister, T.A., Mears, G.J. and Schwartzkopf-Genswein, K.S., 2004. Long-haul transport and lack of preconditioning increases fecal shedding of *Escherichia coli* and *Escherichia coli* O157: H7 by calves. *J. Fd. Prot.,* **67**: 672–678. https://doi.org/10.4315/0362-028X-67.4.672

Brunauer, M., Roch, F.F. and Conrady, B., 2021. Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with bovine coronavirus, *Escherichia coli* K99 and *Cryptosporidium* spp.: A meta-analysis. *Animals,* **11**: 1014. https://doi.org/10.3390/ani11041014

Caffarena, R.D., Casaux, M.L., Schild, C.O., Fraga, M., Castells, M., Colina, R., Maya, L., Corbellini, L.G., Riet-Correa, F. and Giannitti, F., 2021. Causes of neonatal calf diarrhea and mortality in pasture-based dairy herds in Uruguay: A farm-matched case-control study. *Braz. J. Microbiol.,* **52**: 977-988. https://doi.org/10.1007/s42770-021-00440-3

Cheng, Y., Yang, C., Tan, Z. and He, Z., 2021. Changes of intestinal oxidative stress, inflammation, and gene expression in neonatal diarrhoea kids. *Front. Vet. Sci.*, **8**: 598691. https://doi.org/10.3389/fvets.2021.598691

Cheesbrough, M., 2009. *District laboratory practice in tropical countries.* 2nd ed. Cambridge University Press, Cambridge, UK. 62-70: 178-180.

Cho, Y., Kim, W., Liu, S., Kinyon, J.M. and Yoon, K.J., 2010. Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J. Vet. Diagn. Invest.,* **22**: 509–517. https://doi.org/10.1177/104063871002200403

Cho, Y.I. and Yoon, J., 2014. An overview of calf diarrhea infectious etiology, diagnosis, and intervention. *J. Vet. Sci.,* **15**: 1–17. https://doi.org/10.4142/jvs.2014.15.1.1

Devendra C., 2005. Small ruminants in Asia; contribution to food security, poverty alleviation and opportunities for productivity enhancement. In: *Proceeding of international workshop on small ruminant production and development in South East Asia.* MEKARN, Nong Lam, HCMC, Vietnam, 19-32.

Dewell, G.A., Simpson, C.A., Dewell, R.D., Hyatt, D.R., Belk, K.E., Scanga, J.A., Morley, P.S., Grandin, T., Smith, G.C., Dargatz, D.A. and Wagner, B.A., 2008. Impact of transportation and lairage on hide contamination with *Escherichia coli* O157 in finished beef cattle. *J. Fd. Prot.,* **71**: 1114–1118. https://doi.org/10.4315/0362-028X-71.6.1114

Duffy, L.L., Small, A. and Fegan, N., 2010. Concentration and prevalence of *Escherichia coli* O157 and Salmonella serotypes in sheep during slaughter at two Australian abattoirs. *Aust. Vet. J.,* **88**: 399–404. https://doi.org/10.1111/j.1751-0813.2010.00623.x

Dwyer, C.M., Conington, J., Corbiere, F., Holmøy, I.H., Muri, K., Nowak, R., Rooke, J., Vipond, J. and Gautier, J.M., 2016. Invited review: Improving neonatal survival in small ruminants: Science into practice. *Animal*, **10**: 449-459. https://doi.org/10.1017/S1751731115001974

El-Seedy, F.R., Abed, A.H., Yanni, H.A. and Abd El-Rahman, S.A.A., 2016. Prevalence of *Salmonella* and *E. coli* in neonatal diarrheic calves. *Beni-Suef Univ. J. Basic appl. Sci.*, **5**: 45-51. https://doi.org/10.1016/j.bjbas.2015.11.010

Farouk, M.M., El-Molla, A., Salib, F.A. and Soliman, Y.A., 2021. Epidemiology of *Salmonella* species in diarrheic sheep and goats. *Pakistan J Zool.*, **54**: 1-9. https://doi.org/10.17582/journal.pjz/20201012161016

Fernández, D., Rodriguez, E.M., Arroyo, G.H., Padola, N.L. and Parma, A.E., 2009. Seasonal variation of Shiga toxin-encoding genes (stx) and detection of *E. coli* O157 in dairy cattle from Argentina. *J. appl. Microbiol.,* **106**: 1260–1267. https://doi.org/10.1111/j.1365-2672.2008.04088.x

Gavin, W.G., Porter, C.A., Hawkins, N., Schofield, M.J. and Pollock, J.M., 2018. Johne's disease: A successful eradication programme in a dairy goat herd. *Vet. Rec.,* **182**: 483-483. https://doi.org/10.1136/vr.104507

Grünberg, W., 2020. *Salmonellosis in animals*. The Merck veterinary manual.

Ghimire, T.R. and Bhattarai, N., 2019. A survey of gastrointestinal parasites of goats in a goat market in Kathmandu, Nepal. Journal of parasitic diseases: Official organ of the Indian Society. *Parasitology,* **43**: 686–695. https://doi.org/10.1007/s12639-019-01148-w

Haq, I., Durrani, A.Z., Khan, M.S., Mushtaq, M.H. and Ahmad, I., 2017. A study on causes of pathogenic diarrhea in foals in Punjab, Pakistan. *J. Equine Vet. Sci.,* **56**: 88-92. https://doi.org/10.1016/j.jevs.2017.05.010

Hawwas, H.A.E.H., Aboueisha, A.K.M., Fadel, H.M. and El-Mahallawy, H.S., 2022. *Salmonella* serovars in sheep and goats and their probable zoonotic potential to humans in Suez Canal Area, Egypt. *Acta Vet. Scand.*, **64**: 17. https://doi.org/10.1186/s13028-022-00637-y

Jajere, S.M., 2019. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet. World,* **12**: 504–521. https://doi.org/10.14202/vetworld.2019.504-521

Kar, J., Barman, T.R., Sen, A. and Nath, S.K., 2017. Isolation and identification of *Escherichia coli* and *Salmonella* sp. from apparently healthy Turkey. *Int. J. Adv. Res. Biol. Sci.,* **4**: 72-78.

Kiziltepe, Ş. and Ayvazoğlu, C., 2022. Iğdır Yöresindeki Neonatal Kuzularda İshal Etkenlerinin Araştırılması. *ISPEC J. agric. Sci.*, **6**: 189-194. https://doi.org/10.46291/ISPECJASvol6iss1pp189-194

Koirala, A. and Bhandari, P., 2019. Seroprevalence of paratuberculosis in goat of Lumbini zone, Nepal. *Nepal J. agric. Sci.,* **18**: 115-121.

Koneman, E.W., 2016. *Color atlas and textbook of diagnostic microbiology*. J.B. Lippincott Company, Philadelphia, PA.

Majeed, Q.A., El-Azazy, O.M., Abdou, N.E.M., Al-Aal, Z.A., El-Kabbany, A.I., Tahrani, L.M., AlAzemi, M.S., Wang, Y., Feng, Y. and Xiao, L., 2018. Epidemiological observations on cryptosporidiosis and molecular characterization of *Cryptosporidium* spp. in sheep and goats in Kuwait. *Parasitol. Res.,* **117**: 1631–1636. https://doi.org/10.1007/s00436-018-5847-1

Mishra, A.K., Singh, D.D., Gururaj, K., Kumar, A., Dixit, A., Shrama, N., Gupta, G. and Yadav, S., 2019. Molecular characterization of diarrhoegenic *Escherichia coli* isolated from neonatal goat-kids. *J. Anim. Res.*, **9**: 51-59. https://doi.org/10.30954/2277-940X.01.2019.7

Ndegwa, E., Alahmde, A., Kim, C., Kaseloo, P. and O'Brien, D., 2020. Age related differences in phylogenetic diversity, prevalence of Shiga toxins, Intimin, Hemolysin genes and select serogroups of *Escherichia coli* from pastured meat goats detected in a longitudinal cohort study. *BMC Vet. Res.*, **16**: 1-15. https://doi.org/10.1186/s12917-020-02479-0

Office International des Épizooties (OIÉ). 2000. *Salmonellosis. Manual of standards for diagnostic tests and vaccines*. OIÉ. Paris, pp. 832-842.

Osman, K.M., Mustafa, A.M., Elhariri, M. and Abdelhamed, G.S., 2013. The distribution of *Escherichia coli* serovars, virulence genes, gene association and combinations and virulence genes encoding serotypes in pathogenic E. coli recovered from diarrhoeic calves, sheep and goat. *Transbound. Emerg. Dis.,* **60**: 69-78. https://doi.org/10.1111/j.1865-1682.2012.01319.x

Reiner, K., 2010. Catalase test protocol. *Am. Soc. Microbiol.,* **1**:1-9.

Saha, G.K., Paul, A.K., Samad, M.A., Islam, M.A. and Khan, M.S.R., 2014. Prevalence of *Salmonella* associated with goats in Bangladesh. *Suranaree J. Sci. Technol.*, **21**: 193-199.

Shabana, I.I., Bouqellah, N.A. and Zaraket, H., 2017. Investigation of viral and bacterial enteropathogens of diarrheic sheep and goats in Medina, Saudi Arabia. *Trop. Biomed.,* **34**: 944-955.

Shabana, I.I. and Al-Enazi, A.T., 2020. Investigation of plasmid-mediated resistance in E. coli isolated from healthy and diarrheic sheep and goats. *Saudi J. biol. Sci.*, **27**: 788-796. https://doi.org/10.1016/j.sjbs.2020.01.009

Shrivastava, S., Mishra, K.K., Shrivastava, N., Walwadkar, K.K., Singh, A.K. and Risheen, G.D., 2022. Prevalence of caprine diarrhea due to *Escherichia coli* in the Vindhya region (Rewa) of Madhya Pradesh. *Gene*, **10**: 13-14.

Thiruvenkadan, A.K. and Karunanithi, K., 2007. Mortality and replacement rate of Tellicherry and its crossbred goats in Tamil Nadu. *Indian J. Anim. Sci.,* **77**: 590–594.

Urie, N.J., Lombard, J.E., Shivley, C.B., Kopral, C.A., Adams, A.E., Earleywine, T.J., Olson, J.D. and Garry, F.B., 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *J. Dairy Sci.,* **101**: 9229-9244. https://

doi.org/10.3168/jds.2017-14019

Vanselow, B.A., Hornitzky, M.A., Walker, K.H., Eamens, G.J., Bailey, G.D., Gill, P.A., Coates, K., Corney, B., Cronin, J.P. and Renilson, S., 2017. *Salmonella* and on farm risk factors in healthy slaughter-age cattle and sheep in Eastern Australia. *Aust. Vet. J.*, **85**: 498-502. https://doi.org/10.1111/j.1751-0813.2007.00233.x

Yadegari, Z., Brujeni, G.N., Ghorbanpour, R., Moosakhani, F. and Lotfollahzadeh, S., 2019. Molecular characterization of enterotoxigenic *Escherichia coli* isolated from neonatal calves diarrhea. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. *Vet. Res. Forum,* **10**: 73-78.