RESEARCH ARTICLE

Pathogen isolation, identification, and histopathological analysis of *Escherichia coli* in lamb diarrhea

Ke Li[1,†], Yanan Guo[2,†,*], Lixia Lei[2,†], Jiandong Wang[2], Beibei Yan[3], Jingsong Wang[1,2], Shenghu He[1,*]

[1]School of Animal Science and Technology, Ningxia University, Yinchuan, Ningxia, China. [2]Animal Science Institute, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, Ningxia, China. [3]Agricultural Comprehensive Training Service Center, Guyuan, Ningxia, China

Received: June 15, 2023; **accepted:** July 15, 2023.

Lamb diarrhea is a common disease in sheep farming and is usually prevalent in lactating lambs. In severe cases, the disease can cause herd morbidity and lead to individual mortality. *Escherichia coli* frequently appears as a pathogen in cases of diarrhea in lambs. This study reported a case of acute diarrhea in a lamb 8 days after birth. The diarrhea of sick lamb was initially diagnosed by the observation of clinical symptoms, and then, confirmed by autopsy performed by a certified veterinary doctor. The isolation of bacterial pathogens was carried out by using 5% sheep blood agar plates, Eosin methylene blue agar plates, and *Clostridium perfringens* identification medium. The genomic DNAs of the isolates were extracted and 16S rRNA genes were amplificated and sequenced. After bacterial isolation and identification and 16S rRNA sequencing analysis, the lamb acute diarrhea caused by *Escherichia coli* was determined. Pathological histology results showed fatty degeneration of hepatocytes, glomerular epithelial hyperplasia in the kidneys, necrosis of the small intestinal villi, congestion and infiltration of lymphocytes, exfoliation and necrosis of the mucosal layer of the large intestine, dilated intestinal glands and lymphocytic infiltration, and a remarkably increase in lymphocytes in the intestinal lymph nodes of the affected lamb. Analysis of the morphological and molecular biology of the pathogen suggested that this case of lamb diarrhea was caused by the infection of *E. coli*. The results of this report could provide reliable data reference for accurate diagnosis and even prevention and control of *E. coli* diarrhea in lambs in sheep farms.

Keywords: lambs; *Escherichia coli*; isolation and identification; molecular biology; pathological histology.

[*]**Corresponding authors:** Yanan Guo, Animal Science Institute, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, Ningxia 750021, China. Email: gyn330@126.com. Shenghu He, School of Animal Science and Technology, Ningxia University, Yinchuan, Ningxia 750021, China. Email: heshenghu308@163.com.

†These authors contributed equally.

Introduction

Lamb diarrhea is a relatively common disease in sheep farming with a variety of causes and closely related to feeding management, climate change, and pathogenic attack, which can develop throughout the year [1]. The onset of the disease usually results in stunting and even death of the lambs. After cured, diarrheic lambs have stunted growth compared to healthy lambs of the same age, which seriously affects the farm's breeding efficiency as well as the healthy and sustainable development of the sheep industry [2]. The clinical symptoms of lamb diarrhea are mainly elevated body temperature, change in fecal traits, and later, lamb weakness, mucus or even blood in feces, and death of some lambs in exhaustion. Pathohistological changes are mainly

necrosis and detachment of intestinal epithelial cells, loss of intestinal epithelial mucosal layer, and inflammatory reaction including mixed infiltration of lymphocytes, plasma cells, and neutrophils [3]. The main pathogens causing diarrhea in lambs are *Bovine Viral Diarrhea Virus* (BVDV) [4], *Rotavirus* [5], *Escherichia coli* [6], *Salmonella* [7], *Shigella* [8], *Staphylococcus aureus* [9], *Clostridium perfringens* [10], *Sphaerozoum fuscum* [11], *Cryptosporidium* [12] and other microorganisms as single infection or mixed infections with multiple pathogens [13]. As a common and frequent epidemic disease in sheep farming, accurate identification of the pathogen and timely symptomatic treatment of lamb diarrhea can reduce farming losses and is of great significance for the healthy development of sheep farming.

E. coli is an important zoonotic pathogen, often present in the intestinal tract of many warm-blooded animals. Most *E. coli* bacteria are normal in the intestinal tract, but some serotype *E. coli* are pathogenic [14]. After invading the intestinal tract of lambs, pathogenic *E. coli* colonizes in the intestinal tract through the bacterial hairs, and then secretes enterotoxins causing electrolyte imbalance in the intestinal epithelium, which eventually leads to clinical disease represented by diarrhea. Its toxin-induced intestinal infections in lambs as well as its high outbreak and high morbidity and mortality rates are of concern and are seriously endanger the healthy development of the sheep farming industry [15]. Although the disease is found in all age groups of sheep, it is most common in lactating and weaning lambs and is also known as lamb dysentery. In newborn lambs, *E. coli* as a pathogenic bacterium can easily invade the body due to imperfect fluid regulation and neuromodulation, poor body resistance, and disorganized flora in the gastrointestinal tract, thus causing diarrhea and a series of symptoms in lambs [16].

Sheep farming is an important industrial pillar of animal husbandry, so the healthy development of sheep farming is very important. However, lamb diarrhea seriously affects farming efficiency and the sustainable development of the sheep farming industry. Although *E. coli* can cause diarrhea in lambs, it is not the only pathogen. The clinical signs and pathological changes in lambs infected with different pathogens that can cause diarrhea are often non-specific. With the continuous development of animal medicine, lamb diarrhea has been controlled to a certain extent. However, there is geographical variability in the pathogens causing lamb diarrhea, which makes it difficult to accurately identify the pathogens by conventional methods [1]. Currently, the diagnosis of diarrhea in lambs is based on the speed of diarrhea onset, clinical characteristics such as the color and smell of feces, and the morphological characteristics of the isolated pathogen. However, the diagnostic accuracy is difficult to guarantee. This study combined pathogen isolation, identification, and sequencing with traditional diagnostic methods to improve the accuracy of diagnosis. In addition, this study analyzed the diarrheic lamb's intestinal pathohistological changes caused by *E. coli* infection to provide a scientific basis for accurate diagnosis and timely prevention and control of this disease.

Materials and Methods

Sample collection

In October 2021, eighteen newborn lambs in a large-scale Hu sheep farm (Lvsheng Agricultural and Animal Husbandry Comprehensive Development Co Ltd, Lingwu City, Ningxia, China) were developed acute diarrhea successively with elevated body temperature and mental distress. The body temperature of the affected lambs rose significantly reaching around 40℃ with depression, loss of appetite, prolonged lying, grey-yellow porridge-like feces, some lambs showing blood-like loose feces, and limping limbs. Six lambs were died. 12 tissue samples of suspicious infected material were aseptically collected from the liver, kidney, duodenum, jejunum, ileum, cecum, colon, rectum, and intestinal lymph nodes from dead lambs within

30 mins of death. The samples were stored in sealed sterile containers and transported in sampling boxes to the Clinical Veterinary Laboratory at Ningxia Academy of Agriculture and Forestry Sciences (Yinchuan, Ningxia, China) for processing, histopathologic diagnosis, and pathohistological examination.

Bacteria isolation, culture, and amplification

The aseptically collected organ tissues, intestinal tissues, and mesenteric lymph nodes were surface flame sterilized. The deep tissues of approximately 2 × 2 × 2 cm^3 in size were cut and inoculated on Eosin methylene blue agar plates (Haibo, Qingdao, Shandong, China), 5% sheep blood agar plates (Lab-Lemco powder 10.0 g/L, peptone neutralized 10.0 g/L, sodium chloride 5.0 g/L, agar 15.0 g/L, and defibrinated sheep blood) (Oxoid, Basingstoke, Hampshire, UK), and *Clostridium perfringens* identification medium (CHROMagar, Paris, France) under aseptic conditions with 2 pieces of each sample in each medium, respectively. The samples were cultured in BSD-YX3200 constant temperature incubator (Boxun, Shanghai, China) at 37°C for 24 hours. The colonies were observed for growth, colony morphology, color, and the presence of miscellaneous bacteria. The suspicious single colony isolate was selected and purified on a new plate by using the three-zone delineation method incubating at 37℃ for 24 h. After the new colonies grew on the new plate, the single colony was picked up for amplification in the Tryptic Soy Broth (tryptone 17.0 g/L, soy peptone 3.0 g/L, sodium chloride 5.0 g/L, K_2HPO_4 2.5 g/L, glucose 2.5 g/L) (Haibo, Qingdao, Shandong, China) according to the colony morphology and was cultured in QYC-200 bacterial culture oscillator (Yiheng, Shanghai, China) at 37℃, 220 rpm for 12 hours before performing Gram staining and observation under the ML41 microscope (Mingmei, Guangzhou, Guangdong, China).

DNA extraction and polymerase chain reaction (PCR)

Two milliliters of bacterial strain culture (> 1 × 10^8 colony forming unit (CFU)/mL) were centrifuged by using Eppendorf 5418R centrifuge (Eppendorf, Hamburg, German) at 12,000 rpm for 2 mins to obtain the precipitation of the bacterial strain. The bacterial isolate genomic DNA was extracted by using TIANGEN TIANamp Bacteria DNA Kit (Tiangen, Beijing, China). 16S rRNA gene was then amplificated by using Sangon 16S rDNA Bacterial Identification PCR kit (Sangon, Shanghai, China) with the forward primer of 5'-AGA GTT TGA TCC TGG CTC AG-3' and reverse primer of 5'- GGT TAC CTT GTT ACG ACT T-3'. The PCR reaction mixture was 50 µL with 2 µL of template DNA, 1 µL each of forward and reverse primes, 25 µL of 2× Taq PCR Mix, 21 µL of ddH_2O. The reaction was performed by using BIO-RAD S1000 thermal cycler (Bio-Rad, Hercules, California, USA) with the program of 95℃ for 5 mins, followed by 30 cycles of 94℃ for 50 s, 57℃ for 1 min, and 72℃ for 1.5 mins, then a final incubation at 72℃ for 10 mins. The PCR products were analyzed by electrophoresis on 1% agarose gel and then purified by using OMEGA.D2500-02 Gel DNA Extraction Kit (Omega, Feiyang, Guangzhou, Guangdong, China). The purified PCR products were sequenced by Shenggong Bioengineering Co., Ltd, Shanghai, China.

Phylogenetic analysis

The sequencing results of the isolates were compared with the standard *E. coli* strains in GenBank (https://www.ncbi.nlm.nih.gov/) by using BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). Homologous species with high similarity were determined and further analyzed by using the neighbor-joining (NJ) method in the MEGA 11.0 software (DNAStar, Madison, Wisconsin, USA). Bootstrap confidence values (1,000 replications) were identified at the respective nodes.

Pathohistological examination

Tissues of kidney, liver, duodenum, jejunum, ileum, cecum, colon, rectum, and intestinal lymph nodes collected aseptically were immersed in 10% neutral formaldehyde solution and fixed for 7 days. The fixed tissues were then dehydrated in JT-12S automatic dehydrator

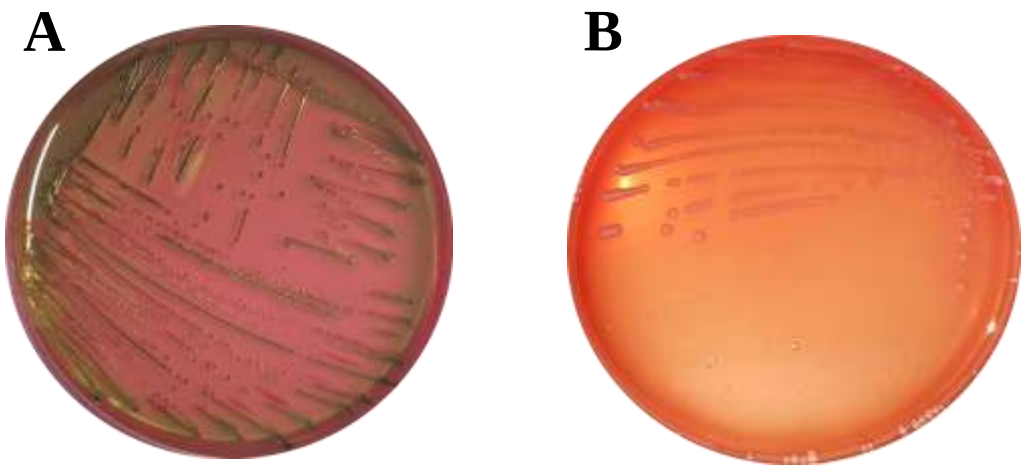

Figure 1. Morphology of *E. coli* isolates on culture media. **A.** Eosin methylene blue agar plates. **B.** 5% sheep blood agar plates.

(Junjie, Wuhan, Hubei, China) by the sequence of 75% ethanol for 4 hours, 85% ethanol for 2 hours, 95% ethanol for 1 h, 4 times of 100% ethanol for 0.5 h, two times of xylene for 10 mins, paraffin wax for 1 h, paraffin wax for 2 hours, paraffin wax for 3 hours. The BMJ-A embedding machine (Zhongwei, Changzhou, Jiangsu, China) was used to embed tissues. Leica-2016 rotary microtome (Leica Microsystems, Wetzlar, Germany) was employed for tissue sectioning and RS36 automatic staining machine (Paisijie, Changzhou, Jiangsu, China) was used for staining of tissues section following the procedures of dewaxing, hematoxylin staining for 10 - 20 mins, rinsing with water for 1 - 3 mins, hydrochloric acid alcohol differentiation for 5 - 10 s, rinsing with water for 1 – 3 mins, immersing in warm water at 50℃ or weakly alkaline aqueous solution until blue color appearing, rinse with water for 1 – 3 mins, adding 85% ethanol for 3 - 5 mins, eosin staining for 3 - 5 mins, washing with water for 3 - 5 s, gradient ethanol dehydration, xylene transparent, and neutral gum sealing. A digital slice scanner (3D HISTECH, Budapest, Hungary) was used to collect images of the slices.

Results

Observation of collected samples

The kidneys demonstrated hemorrhagic spots, while the liver was enlarged and a pseudo-membrane appeared on the surface, forming a vacuolated cavity that could be easily peeled off. There was a large amount of gas in the intestinal lumen. The mesenteric lymph nodes were also enlarged. The intestinal wall was edematous and thickened. The feces were thin, and the intestinal mucosa was detached.

Bacterial colony morphology

After the bacteria plates were incubated in an inverted incubator at 37°C for 24 h, a single black colony with a metallic luster was observed on Eosin methylene blue agar plates (Figure 1A), while a single grayish-white round colony with a medium size and moist surface was generated on 5% sheep blood agar plates (Figure 1B). No colony grew in the *Clostridium perfringens* identification medium. The colonies on Eosin methylene blue agar plates and 5% sheep blood agar plates were picked for Gram staining and observation. The red Gram-negative short rods with bluntly rounded ends were observed under the light microscope (Figure 2). One bacterial strain was isolated from the liver and one from the intestinal lymph nodes, while nine strains were isolated from the intestine. The isolated bacterial strains were named NXGY001 to NXGY011, respectively, following the above order.

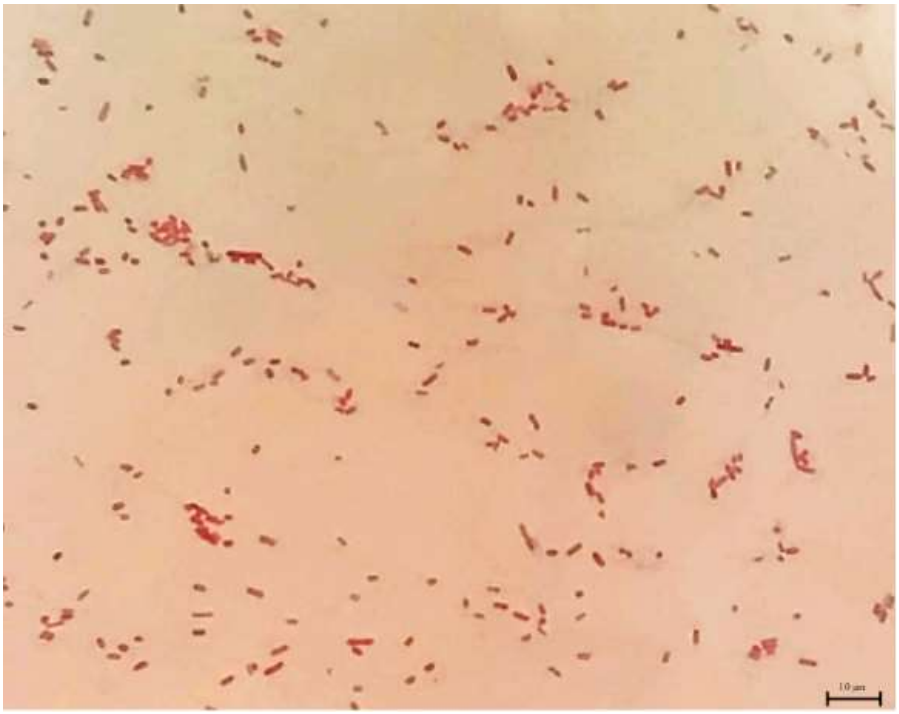

Figure 2. Gram stain negative, microscopic examination of short red rods with blunt rounded ends (1,000×).

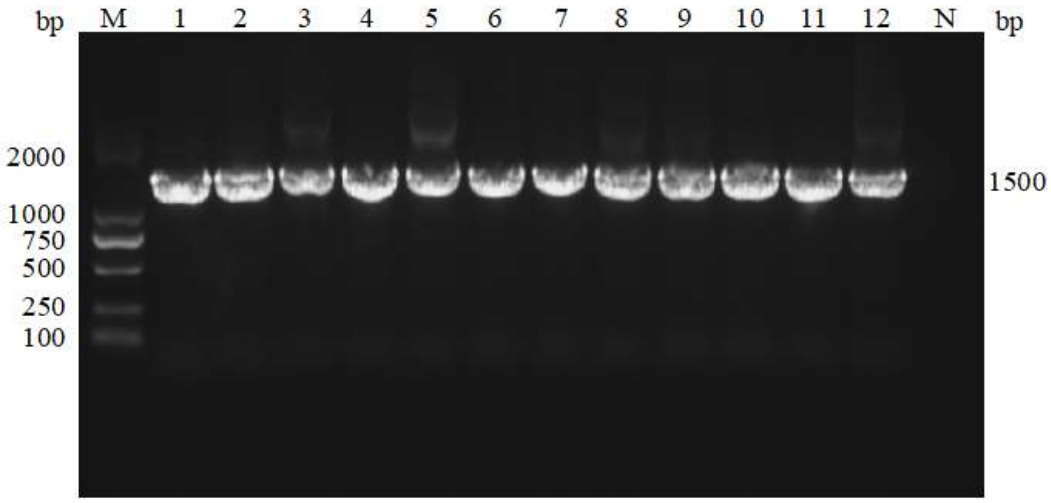

Figure 3. PCR amplification of 16S rRNA. **Line M:** DNA marker DL 2000. **Lines 1 to 11:** PCR products of 1 to 11. **Line 12:** *Escherichia coli* ATCC 25922 (positive control). **Line N:** negative control.

PCR identification and phylogenetic Analysis

Gel electrophoresis analysis of the 16S rRNA amplification products of the 11 isolated strains of pathogenic bacteria revealed that a bright band was observed at approximately 1,500 bp position (Figure 3), which was consistent with the expected result (1,465 bp). After sequencing, the 16S rRNA sequence size was confirmed as 1,465 bp. The sequencing results were compared with the known sequences in GenBank database by using BLAST. The highest similarity of more than 99% was found matching the sequence of *E. coli*,

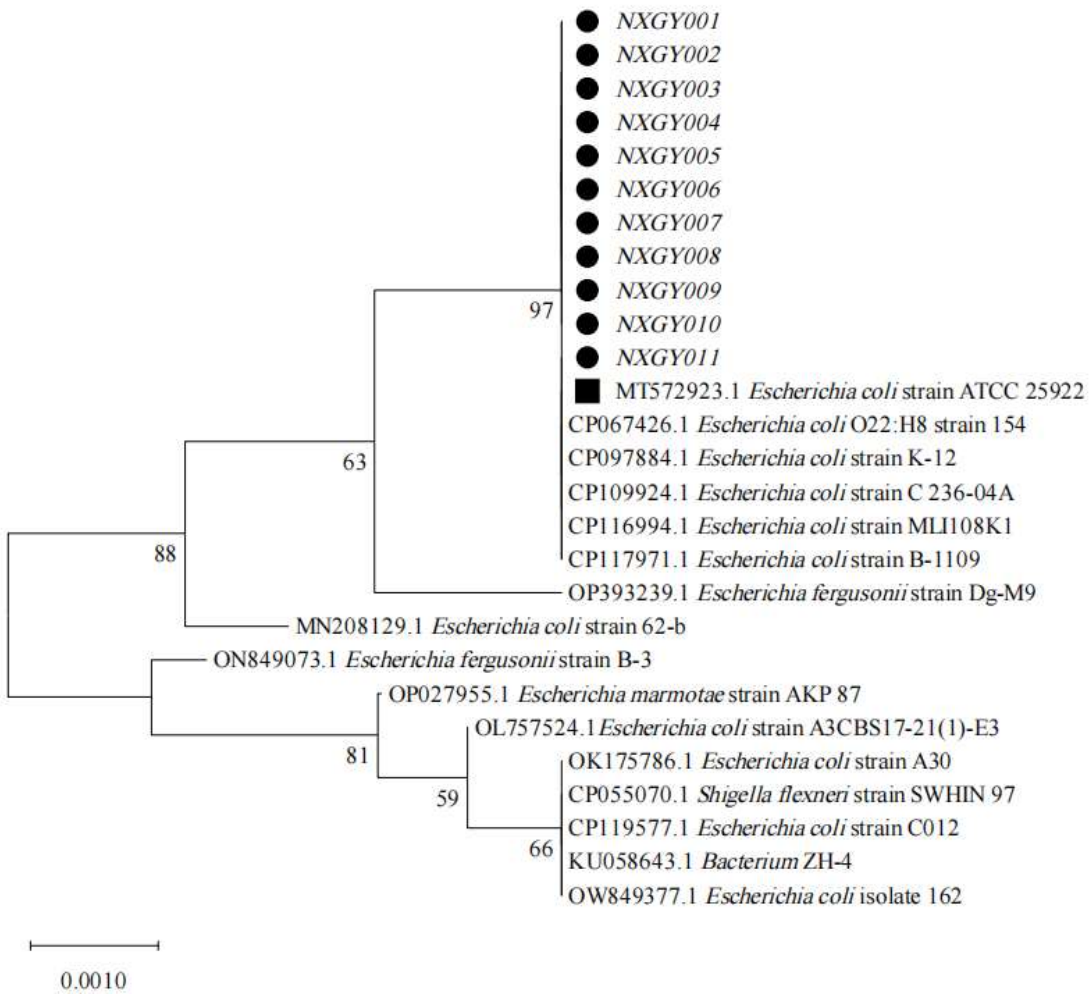

Figure 4. Phylogenetic tree of isolates constructed from 16S rRNA gene sequences.

and therefore, the isolated bacteria were identified. The results of the constructed phylogenetic tree showed that NXGY001 to NXGY011 were on the same branch as *E. coli* ATCC25922 (GenBank ID: MT572923.1), *E. coli* O22:H8 strain 154 (GenBank ID: CP067426.1), *E. coli* K-12 (GenBank ID: CP097884.1), *E. coli* C236-04A (GenBank ID: CP109924.1), *E. coli* MLI108K1 (GenBank ID: CP116994.1), and *E. coli* B-1109 (GenBank ID: CP117971.1), with 97% support (Figure 4).

Pathohistological observation

The liver tissue of diseased lambs showed steatosis of the hepatocytes and contained variable numbers of lipid droplets in the cytoplasm (Figure 5). Localized areas of renal tissue showed proliferation of individual glomerular Bowman's capsule epithelial cells, and homogeneously red-stained glass vesicles were seen inside of some glomerular capsule (Figure 6). On the slices of intestinal sections, extensive necrosis of the intestinal villi in the mucosal layer of the small intestinal tissue, detachment of the mucosal epithelium, blurring of the cellular structure between the glands of the small intestine, dilatation of the capillaries in the mucosal layer were observed accompanied by massive infiltration of inflammatory cells (Figure 7). The mucosal epithelial structure of the colonic tissue was lost, while necrosis of the intestinal glands and a large infiltration of

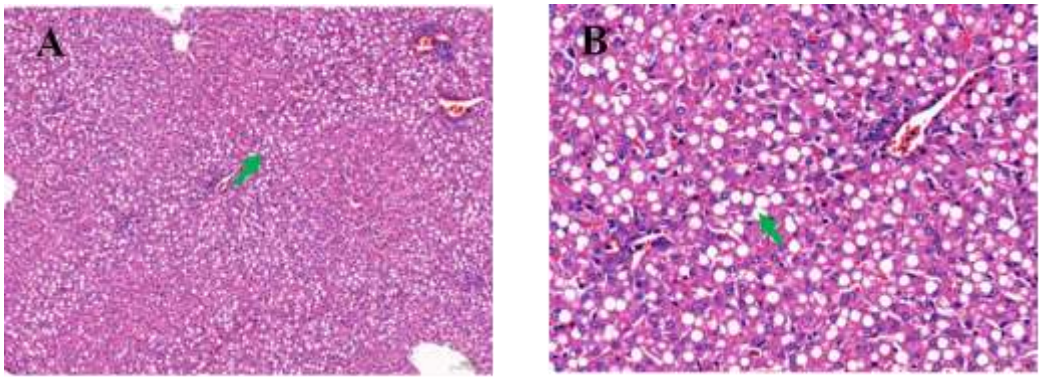

Figure 5. Pathohistological sections of the liver (H.E.). **A.** hepatocellular steatosis (arrow) (100×). **B.** lipid drops (arrow) (400×).

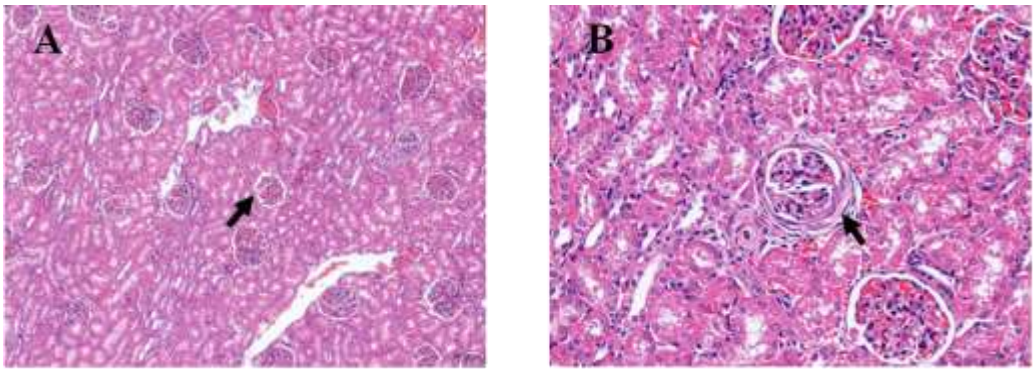

Figure 6. Pathohistological sections of the kidney (H.E.). **A.** glass vesicles (arrow) (100×). **B.** Epithelial hyperplasia of the renal capsule (arrow) (400×).

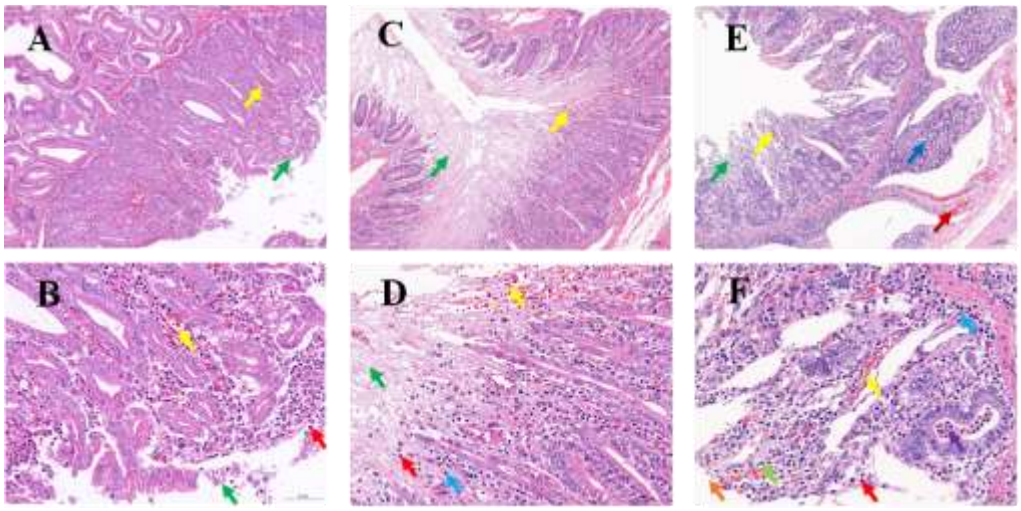

Figure 7. Pathohistological sections of the small intestine (H.E.). **A.** duodenum intestinal villi necrosis (green arrow), capillary congestion in the mucosal layer (yellow arrow) (100×). **B.** duodenum mucosal epithelial detachment (green arrow), capillary congestion in the mucosal layer (yellow arrow), neutrophil infiltration (red arrow) (400×). **C.** jejunum intestinal villi necrosis (green arrow), bleeding in the mucosal layer (yellow arrow) (100×). **D.** jejunum superficial mucosal necrosis, cell disintegration (green arrow), bleeding in the mucosal layer (yellow arrow), neutrophil infiltration (red arrow), lymphocytic infiltration (blue arrow) (400×). **E.** ileum intestinal villi necrosis (green arrow), capillary congestion in the mucosal layer (yellow arrow), lymphopenia of lymphoid follicles (blue arrow), vascular congestion in the submucosa (red arrow) (100×). **F.** ileum plasma cells (light green arrow), capillary congestion in the mucosal layer (yellow arrow), neutrophil infiltration (red arrow), lymphocytic infiltration (blue arrow), loss of mucosal epithelium (orange arrow) (400×).

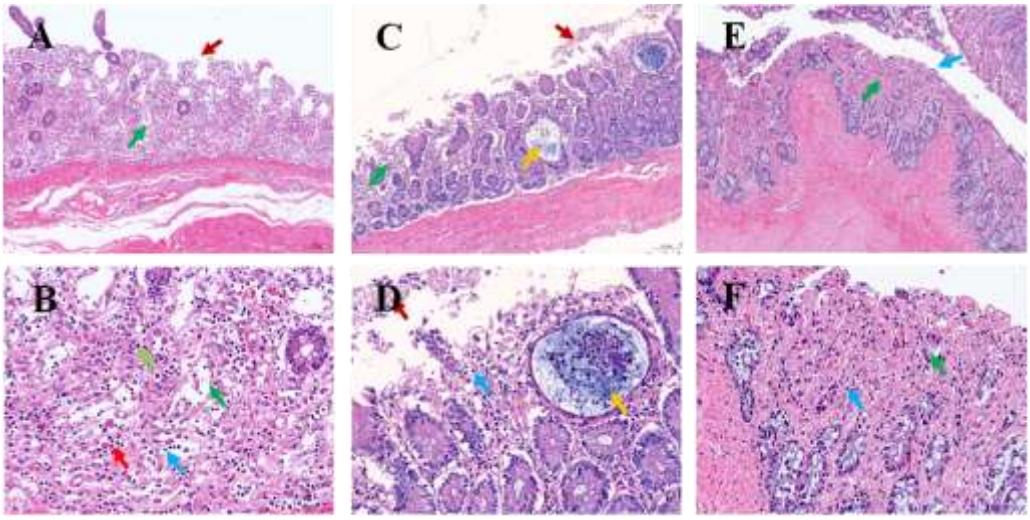

Figure 8. Pathohistological sections of the large intestine (H.E.). **A.** cecum necrosis of the mucosal layer, loss of intestinal glands (green arrow), loss of mucosal epithelial structure (red arrow) (100×). **B.** cecum plasma cells (light green arrow), necrosis of the mucosal layer, loss of intestinal glands (green arrow), neutrophil infiltration (red arrow), lymphocytic infiltration (blue arrow) (400×). **C.** colon necrosis of the mucosal layer (green arrow), mucosal epithelial detachment (red arrow), dilated intestinal glands (orange arrow) (100×). **D.** colon dilated intestinal glands (orange arrow), mucosal epithelial detachment (red arrow), lymphocytic infiltration (blue arrow), cellular debris in the lumen of the intestinal glands (purple arrow) (400×). **E.** rectal necrosis of the mucosal layer, loss of intestinal glands (green arrow), loss of mucosal epithelial structure (blue arrow) (100×). **F.** rectal necrosis of the mucosal layer, loss of intestinal glands (green arrow), lymphocytic infiltration (blue arrow) (400×).

inflammatory cells including lymphocytes and neutrophils in the intestinal tissue were observed (Figure 8). Unclear structure in the cortical area of intestinal lymph node with a remarkable increase in the number of lymphocytes and macrophages was also noticed (Figure 9).

Discussion

Escherichia coli is a common conditional pathogen that can cause infections in humans and many animals under certain conditions [17]. In this study, the observed pathological changes of the organs of dead lambs included hemorrhagic spots in the kidneys, enlarged liver with pseudomembrane formation on the surface, large amount of gas in the intestinal lumen, enlarged mesenteric lymph nodes, edema of the intestinal wall, detachment of intestinal mucosa, and unformed feces. These pathohistological characteristics were consistent with the report of Tzipori, *et al*. [18], which were common in histopathology caused by diarrhea. During the pathogenic isolation of the samples, the isolates produced black round colonies with a metallic luster on Erythromax blue agar medium and opaque single colonies with an off-white color on blood agar medium with Gram stain negative, red color, and short rod with blunt rounded ends on microscopic examination, which were consistent with the results of Morin, *et al*. [19]. The isolated bacteria were initially identified as *E. coli*. In the molecular biology analysis, the PCR amplification product was 1,465 bp with the sequence similarity more than 99% similar to the standard strains of *E. coli* and the homology support rate in the constructed evolutionary tree as high as 97%. Therefore, the obtained bacteria were confirmed as *E. coli* by the colony morphology of the isolated strains, Gram stain microscopic observation, and 16S rRNA sequencing results. The 16S rRNA sequence analysis has become an internationally accepted identification technique for the identification of bacteria with high rRNA conservation [20]. The results of this study showed that the identified *E. coli* isolates with the highest homology support rate were also reported in France, Argentina, Australia, Denmark, Mali, and the United States, indicating a worldwide trend of prevalence of this type of strain.

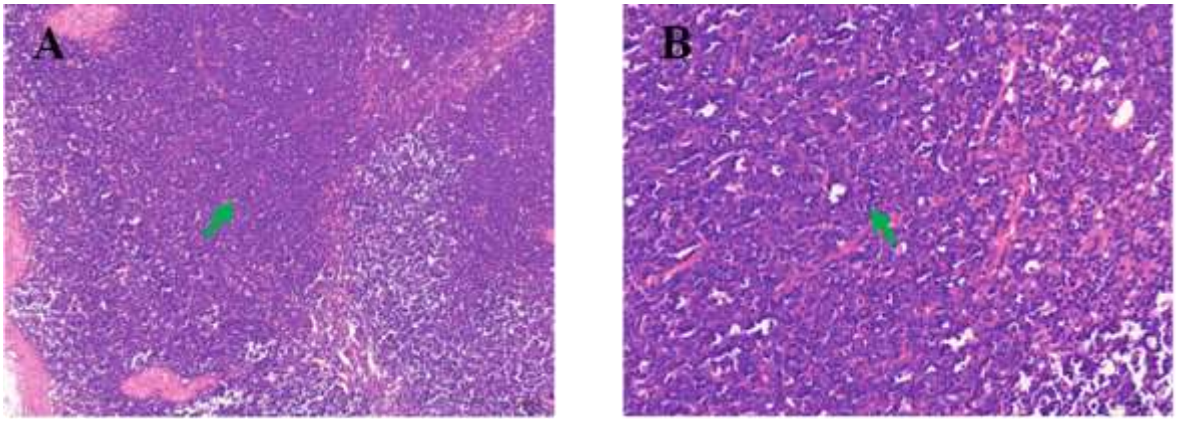

Figure 9. Pathohistological section of intestinal lymph nodes (H.E.). **A.** lymphocytosis (green arrow) (100×). **B.** lymphocytosis (green arrow) (400×).

In pathohistological observations, it was found that *E. coli* diarrhea in lambs caused different degrees of damage to the liver, kidney, and intestinal tissues, mainly in the form of fatty degeneration of hepatocytes, proliferation of kidney cells, necrosis and lymphocytic infiltration with congestion in the small intestine villi, peeling and necrosis of the mucosal layer of the large intestine, dilated intestinal glands, lymphocytic infiltration, and a significant increase in lymphocytes in the intestinal lymph nodes. These damages and inflammations had similarities to previously reported studies of *E. coli* infection or other *E. coli* toxins affecting intestinal morphology [21]. When the organism is exposed to *E. coli* infection, a large number of inflammatory cells accumulate at the site of *E. coli* invasion to clear the pathogen and form lymphocyte infiltration, among other phenomena. However, the inflammatory mediators and enzymes with lytic activity released by the cells damage normal cells and tissues, thus, causing degeneration and necrosis of organ cells [22]. According to the report of Moon, after infection with the enterotoxin-producing *Escherichia coli* (ETEC), the *E. coli* layer adhered to the epithelium of the intestinal villi and usually did not cause significant structural damage to the mucosa. However, the enteropathogenic *E. coli* (EPEC) infection, in which *E. coli* adhered tightly to the surface membrane of the epithelial cells, caused the loss of microvilli at the brush border. The infection with enterohemorrhagic *E. coli* (EHEC) caused the destruction of the epithelial brush border [23]. The pathological effects of EPEC on the intestinal tissues of animals had the common features of the pathohistological phenomena in this study, and therefore, the pathogenic bacteria causing *E. coli* diarrhea in this lamb herd was probably EPEC. The causes of colibacillosis diarrhea in lambs are usually the lambs contacting diseased sheep or the environment contaminated by bacteria, and intaking of bacteria contaminated milk [24]. Rocha-Melogno suggested that, in poorly sanitized premises, enteric pathogens could be transmitted through the air. The data obtained in Bolivia showed that airborne bacterial flux was the main factor influencing the risk of bacterial disease transmission. Among those pathogens*, E. coli* infections were the more serious ones [25]. *E. coli* is a conditionally pathogenic bacterium. So that, it is important to strengthen feeding management and to strictly disinfect the environment and enclosures during breeding, especially during delivery of ewes. Attention should be paid to maintaining environmental hygiene. The feeding of pregnant sheep is also crucial and should be ensured that their diets contain sufficient protein and carotene to synthesize immunoglobulins and to meet the growth needs of the fetus. In addition, the ewes should be vaccinated in time so that the offspring can obtain sufficient maternal antibodies by taking colostrum [26].

Acknowledgement

This study was funded by Key R&D project of Ningxia Hui Autonomous Region, China (2021BEF02026).

References

1. Holland RE. 1990. Some infectious causes of diarrhea in young farm animals. Clin Microbiol Rev. 3(4):345-375.
2. Jacobson C, Larsen JW, Besier RB, Lloyd JB, Kahn LP. 2020. Diarrhea associated with gastrointestinal parasites in grazing sheep. Vet Parasitol. 282:109139.
3. Tsalie E, Kouzi K, Poutahidis T, Abas Z, Sarris K, Iliadis N, *et al*. 2006. Effect of vitamin E nutritional supplementation on the pathological changes induced in the ileum of rabbits by experimental infection with enteropathogenic *Escherichia coli*. J Comp Pathol. 134(4):308-319.
4. Evans CA, Woolford L, Hemmatzadeh F, Reichel MP, Cockcroft PD. 2021. Pathological lesions of lambs infected in utero with bovine viral diarrhea virus type 1c (BVDV-1c). Vet Rec. 188(3):e6.
5. Shabana II, Bouqellah NA, Zaraket H. 2017. Investigation of viral and bacterial enteropathogens of diarrheic sheep and goats in Medina, Saudi Arabia. Trop Biomed. 34(4):944-955.
6. Acosta-Dibarrat J, Enriquez-Gómez E, Talavera-Rojas M, Soriano-Vargas E, Navarro A, Morales-Espinosa R. 2021. Characterization of commensal *Escherichia coli* isolates from slaughtered sheep in Mexico. J Infect Dev Ctries. 15(11):1755-1760.
7. Weber M, Zanolari P, Ardüser F, Stucki D, Akarsu H, Overesch G. 2022. Prevalence and antimicrobial resistance of *Salmonella enterica* subsp. *diarizonae serovar* 61:k:1,5,(7) in Swiss sheep flocks. Prev Vet Med. 206:105697.
8. Omer MK, Hauge SJ, Østensvik Ø, Moen B, Alvseike O, Røtterud OJ, *et al*. 2015. Effects of hygienic treatments during slaughtering on microbial dynamics and contamination of sheep meat. Int J Food Microbiol. 194:7-14.
9. Dinges MM, Orwin PM, Schlievert PM. 2000. Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev. 13(1):16-34.
10. Esteban-Blanco C, Gutiérrez-Gil B, Puente-Sánchez F, Marina H, Tamames J, Acedo A, *et al*. 2020. Microbiota characterization of sheep milk and its association with somatic cell count using 16s rRNA gene sequencing. J Anim Breed Genet. 137(1):73-83.
11. Lasecka L, Baron MD. 2014. The nairovirus nairobi sheep disease virus/ganjam virus induces the translocation of protein disulphide isomerase-like oxidoreductases from the endoplasmic reticulum to the cell surface and the extracellular space. PLoS One. 9(4):e94656.
12. Baillou A, Kasal-Hoc N, Barc C, Cognié J, Pinard A, Pezant J, *et al*. 2021. Establishment of a newborn lamb gut-loop model to evaluate new methods of enteric disease control and reduce experimental animal use. Vet Sci. 8(9):170.
13. Moon HW, McClurkin AW, Isaacson RE, Pohlenz J, Skartvedt SM, Gillette KG, *et al*. 1978. Pathogenic relationships of rotavirus, *Escherichia coli*, and other agents in mixed infections in calves. J Am Vet Med Assoc. 173(5 Pt 2):577-583.
14. Kaper JB, Nataro JP, Mobley HL. 2004. Pathogenic *Escherichia coli*. Nat Rev Microbiol. 2(2):123-140.
15. Kopic S, Geibel JP. 2010. Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, *V. cholerae* and rotavirus infection. Toxins (Basel). 2(8):2132-2157.
16. Lee JB, Kim SK, Yoon JW. 2022. Pathophysiology of enteropathogenic *Escherichia coli* during a host infection. J Vet Sci. 23(2):e28.
17. Ponce-Gordo F, García-Rodríguez JJ. 2021. *Balantioides coli*. Res Vet Sci. 135:424-431.
18. Tzipori S, Robins-Browne RM, Gonis G, Hayes J, Withers M, McCartney E. 1985. Enteropathogenic *Escherichia coli* enteritis: evaluation of the gnotobiotic piglet as a model of human infection. Gut. 26(6):570-578.
19. Morin M, Larivière S, Lallier R. 1976. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can J Comp Med. 40(3):228-240.
20. Sontakke S, Cadenas MB, Maggi RG, Diniz PP, Breitschwerdt EB. 2009. Use of broad range16S rDNA PCR in clinical microbiology. J Microbiol Methods. 76(3):217-225.
21. Touchette KJ, Carroll JA, Allee GL, Matteri RL, Dyer CJ, Beausang LA, *et al*. 2002. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. J Anim Sci. 80(2):494-501.
22. Hart CA, Batt RM, Saunders JR. 1993. Diarrhea caused by *Escherichia coli*. Ann Trop Paediatr. 13(2):121-131.
23. Moon HW. 1997. Comparative histopathology of intestinal infections. Adv Exp Med Biol. 412:1-19.
24. Madzingira O. 2016. Shiga toxin-producing *E. coli* isolated from sheep in Namibia. J Infect Dev Ctries. 10(4):400-403.
25. Rocha-Melogno L, Crank KC, Ginn O, Bergin MH, Brown J, Gray GC, *et al*. 2022. Quantitative microbial risk assessment of outdoor aerosolized pathogens in cities with poor sanitation. Sci Total Environ. 827:154233.
26. Sawyer M, Willadsen CH, Osburn BI, McGuire TC. 1977. Passive transfer of colostral immunoglobulins from ewe to lamb and its influence on neonatal lamb mortality. J Am Vet Med Assoc. 171(12):1255-1259.