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Peracute Enterotoxemia in Saanen and Alpine Goat Herd

Hossein Esmaeili *, *Seyed Mehdi Joghataei*

Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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ABSTRACT

Background: Enterotoxemia" or "overeating disease" is considered a common and often fatal disease affecting the economy of small ruminant breeding systems. This study presents findings on the occurrence of a peracute form of enterotoxemia in a herd of Saanen and Alpine, including an examination of the clinical signs, post-mortem findings, and methods for diagnosis.

Methods: In a herd of goats a distressing situation occurred where the goats displayed signs (Sudden death and high fever) of peracute enterotoxemia (125 kids). It is noteworthy that these goats had previously been vaccinated against enterotoxemia. The onset of the condition seemed to be linked to three instances of interruption and reconnection of concentrate feed. To obtain a definitive diagnosis, the findings from postmortem examinations and ELISA were utilized.

Results: In total, 60 Alpine and 65 Saanen succumbed to this peracute form. These losses occurred over three days but were successfully stopped by re-vaccination after two days. Clinical signs, post-mortem observations, bacterial analysis, and ELISA results all provided confirmation of enterotoxemia. Notable findings included high fever (90%), duodenum hyperemia (89%), pulmonary edema (82%), convulsions (43%), and hemorrhage in the pericardium and endocardium (58%).

Conclusion: Frequent and consecutive changes in goats' diet or discontinuation of concentrate supply by breeders can disrupt the vaccine-induced immune barrier, increasing the likelihood of enterotoxemia, and leading to casualties and economic losses. Consistent and stable nutrition practices are essential for maintaining vaccination efficacy and preventing enterotoxemia in goats, particularly kids.

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*Corresponding Authors: Hossein Esmaeili, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Tel: +98-21-61117051, *E-mail:* hesmaeli@ut.ac.ir

Introduction

Enterotoxaemia, also called pulpy kidney disease or overeating disease, is a substantial bacterial illness observed in sheep and goats worldwide. It is particularly prevalent in well-nourished animals. The etiological agent responsible for enterotoxaemia is a Gram-positive, anaerobic, spore-forming bacterium known as *Clostridium perfringens* type D. This organism is widely distributed and can produce toxins, leading to various clinical manifestations in different animal species. All types of *C. perfringens* produce Alpha toxin (CPA), while Epsilon toxin (ETX) specifically induces enterotoxaemia in sheep and goats (1, 2). This principle applies to Alpine and Saanen goats, which play a substantial role in farm systems by contributing to the economy and ecological balance (3).

Despite initial reports dating back to 1941, caprine enterotoxaemia remains a disease with several unresolved aspects. It continues to be a prominent cause of sudden death in goats across different age groups worldwide. It is crucial to recognize that several predisposing factors commonly observed in sheep may not necessarily apply to goats. Therefore, a comprehensive understanding of the specific risk factors for enterotoxaemia in goats is essential (4, 5). Many sources indicate that a lack of vaccination and increased intake of carbohydrates serves as a predisposing factor for enterotoxemia. Concentrates, a widely used source of nutrients, are often implicated in this regard (5-7). Regular vaccination is widely recognized as effective for controlling clostridial infections worldwide. It plays a crucial role in preventing and mitigating the impact of these infections in various animal populations (8).

Enterotoxaemia has been observed to exhibit various aspects and manifestations across different breeds of goats. This suggests that breed-specific factors may influence the disease's occurrence, severity, and clinical presentation. Understanding these breed-specific variations is essential for effective management and prevention strategies (4). In 2007, Miyashiro et al. reported a case of enterotoxemia in an 18-month-old Boer goat from a commercial herd of 50 goats. The goat developed enterotoxemia despite regular vaccination against *C. perfringens* using commercial vaccines. Necropsy examination revealed remarkable findings in various organs, including extensive necrotic areas in the renal cortex and medulla (pulpy kidney disease), hyperemia and centrilobular necrosis in the liver, necrosis of the small intestine wall, pulmonary edema, and duodenum hyperemia (9). Notably, these goats had received the enterotoxaemia vaccine, indicating that the disease still occurred in this specific breed despite vaccination.

In a 2022 study conducted by Hussain et al. in Pakistan, three herds of two different breeds (Makhi Cheeni and Beetal) and one herd of Teddy breed experienced cases of sudden death attributed to enterotoxemia (102 deaths). The definitive diagnosis was established through necropsy and bacteriological investigations. The affected goats exhibited a range of clinical signs, including high fever, diarrhea, convulsions, anorexia, depression, and dehydration. During the necropsy, specific findings were observed, such as the presence of straw-colored and light pink fluid in the thoracic, peritoneal, and abdominal cavity. The intestines showed severe congestion, hyperemic mucosa, and multifocal hemorrhagic enteritis. This study emphasized the important fact that enterotoxemia can occur in goats regardless of the season. It underlined the significance of giving special attention to vaccination as a preventive measure against enterotoxemia in goats (10).

Preventing *C. perfringens* type D infection is crucial and should involve a comprehensive approach that includes vaccination and gradual dietary adaptation as part of the management strategy. These preventive measures are of utmost importance for controlling the occurrence and impact of enterotoxaemia in goats (11). The objective of the present study is to document the incidence of a peracute form of enterotoxemia in a herd of Alpine and Saanen kids despite regular vaccination against enterotoxemia.

Materials and Methods

Animals and Herd Conditions

The study comprised a herd of 600 goat kids, with 320 Alpine and 280 Saanen (Male and Female) between 6-11 weeks old. The herd was situated in an industrial farm within the Tehran province, known for its excellent health conditions. The animals in this herd had been appropriately vaccinated, including Rev 1, enterotoxemia, PPR, goat-pox, and FMD, following the guidelines set by the Iranian Veterinary Organization.

Upon birth, the kids were separated from their mothers and provided with powder milk and colostrum. They received vaccinations using a tetravalent vaccine against enterotoxemia, which targeted *C. perfringens* types D, C, and B, and *Clostridium* septicum (obtained from Razi Vaccine Research and Serum Institute, Iran). The mothers received subcutaneous injections of the vaccine in the neck region during the 12th and 16th weeks of pregnancy, while the kids received three doses of the vaccine during the second, fourth, and eighth weeks after birth. The age range of the included goats was between four and ten weeks.

The kids' nutrition primarily consisted of alfa alfa and concentrate feed with a protein content of 19%. While alfa alfa was consistently available, there were periods of interruption and reconnection of the concentrate feed, due to issues related to the availability or supply of starter concentrate, certain challenges arose. Initially, the concentrate feed was discontinued for four days. Subsequently, it was reintroduced to the kids for another four days. This cycle of disconnecting and reconnecting the concentrate feed was repeated for the second and third instances. Following each period of concentrate interruption, the concentrate was gradually reintroduced, starting with a small quantity of 30 grams on the first day and increasing by 50 grams each subsequent day until the end of the fourth day.

After the manipulation of the concentrate feed, there was a sudden onset of death among the kids, characterized by clinical signs indicative of peracute enterotoxemia. This mortality continued for three days. Subsequently, the goats were re-vaccinated using the quadruple vaccine mentioned earlier. Following the re-vaccination, the deaths among the goats ceased two days later.

Post-mortem Examination and Sampling

Upon the losses, close monitoring of the kids was immediately initiated. In cases where animals were found dead, post-mortem examinations were performed. For these examinations, samples of the intestinal contents from the small intestine of the deceased animals were collected sterilely. To preserve the samples, 1% chloroform was added as a preservative. The samples were placed in separate and numbered sterile containers and sent to the laboratory on the same day under standard conditions. Upon arrival at the laboratory, the samples were processed immediately. In addition to collecting intestinal samples, the animals were also sampled for bacteriological investigations. During the necropsy of dead goats, intestine samples were collected for further examination and analysis. Samples were kept at −20 °C until used. To assess glucosuria using a strip (Kimia Pojohan, Iran), urine sampling was conducted directly from the bladder of the animals.

To process the intestinal specimens, they were diluted with endotoxin-tested distilled water in a ratio of 1:5. The mixture was then centrifuged at 2000 ×g for 20 minutes at a temperature of 4 °C. After centrifugation, the supernatants were carefully removed and passed through 0.45-µm membrane filters. The filtered samples were stored at a temperature of -70 °C until they were used for further analysis.

The bacteriological assessements

A bacteriological procedure was performed to diagnose enterotoxemia caused by *C. perfringens* type D. Gram-stained direct smears of the intestinal content were examined to detect high-volume gram-positive bacilli. A total of 60

samples were analyzed using this method. However, quantitative anaerobic growth on blood agar is the gold standard for confirming enterotoxemia suspicion due to *C. perfringens* (4, 12). For culture and isolation, four samples were employed in the study. Based on Smith and Holdman's method (13), the following procedures were employed: Inoculation on Robertson's cooked meat medium: Samples were inoculated into sterile cooked meat medium and incubated anaerobically at 37 °C for 24-48 hours. Isolation on selective media: A loopful from the incubated tube was streaked onto sheep blood agar and *Clostridium* agar media. The plates were incubated anaerobically at 37 °C for 24-48 hours. Colonies with a doubled zone of hemolysis were further identified. Nagler's reaction: *C. perfringens* CPA antitoxin was inoculated in one-half of egg yolk agar plates and allowed to dry in the incubator for 30 minutes. The suspected isolated organisms were streaked across the antitoxin-free half of the plate. After incubating anaerobically at 37 °C for 24 hours, the plates were evaluated for the appearance of opalescence and the formation of pearly layers on the half of the plate without antitoxin, following Koneman's procedure. The collected *C. perfringens* isolates were subjected to biochemical analysis (14-16).

ELISA analysis

The detection of enterotoxins produced by *C. perfringens*, including Alpha, Beta, and Epsilon toxins, was carried out using the Bio-X enterotoxemia ELISA kit (Bio-X Diagnostics, Belgium). This commercial kit was utilized to analyze a total of 46 samples. The kit was also used to confirm the presence of the bacteria itself in the collected samples. To perform the ELISA assay, 100 µl of the diluted samples and negative and positive controls were added to the designated wells of 96-well microtitration plates. The plates were then incubated with the lid closed at 21 ± 3°C for one hour. Following the first incubation, the contents of the microplates were emptied, and the plates were washed three times by adding 300 µl of washing solution to each well. Next, 100 µl of conjugate solutions were added to their respective microplate wells. The plates were incubated again with the lid closed at 21 ± 3 °C for one hour. After the secondary incubation and another round of washing, 100 µl of chromogen solution was added to each well. The plates were then incubated for 10 minutes at 21°C ± 3°C, keeping the lid open and away from light. Following the final incubation, 50 µl of stop solution was added to each well to terminate the reaction. Finally, the wells' optical densities were measured using an ELISA reader, allowing for the quantification and analysis of the results.

Results

Occurrence of Disease and Clinical Findings

Even though the kids had been given a booster dose of the enterotoxemia vaccine three weeks before the manipulation of the concentrate feeding occurred, 60 Alpine (18.8%) and 65 Saanen (23.3%) experienced the peracute form of the disease. The presence of clinical signs, necropsy findings, bacteriological analyses, and ELISA results all confirmed the occurrence of this disease. The clinical course of the disease was typically brief, lasting less than one hours, and predominantly exhibited the peracute form of enterotoxemia. The most commonly observed sign in the affected kids was sudden death (73%). Peracute form of enterotoxemia was characterized by various signs that were observed in kids shortly before their death (27%), including diarrhea (40%), anorexia (64%), high fever (41 and 42 °C) (90%), abdominal discomfort (43%), cold extremities, and opisthotonos (76%) and intermittent convulsions (43%). The initial diarrhea was described as yellow-green and pasty in consistency, but it quickly progressed to watery or mucoid with intestinal mucus and blood.

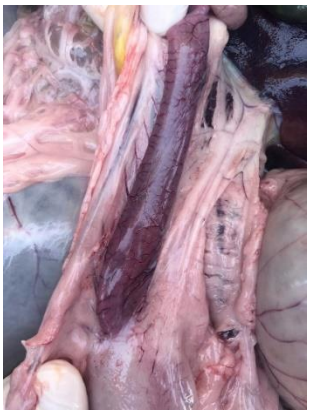

Fig 1. Gross appearance at necropsy of the guts of a kid with hyperemia in the duodenum.

Necropsies findings

In a total of 125 necropsies conducted on kids with enterotoxemia, the most consistent gross lesions were hyperemic areas in the intestines, especially in the duodenum (89%) (Figure 1) and pulmonary edema (82%) (Figure 2). The most pronounced changes were observed in the small and large intestines, which were filled with watery and bloody contents, along with fibrin clots and gelatinous fluid in the abdominal cavity and pericardial sac. Pulpy kidney lesions were observed in 24 cases of enterotoxemia (19%). In 96 affected kids, excess straw-colored pericardial fluid that clots upon exposure to air (77%), subendocardial hemorrhages, epicardial petechiae (58%), congestion, and edema of peripheral and mesenteric lymph nodes were observed. During the post-mortem examination, no external lesions were observed on the bodies of the deceased kids. Glucosuria was identified in 12 Alpine and 16 Saanen.

Bacteriological Findings

Microscopic examination of smears prepared from freshly deceased animals revealed a considerable quantity of *C. perfringens* bacteria in the intestinal contents, particularly in the Ileum. This finding strongly indicates the occurrence of enterotoxemia (4). The biochemical profile of the isolates obtained from the samples was found to be entirely consistent with the specified characteristics described in the sources (14, 15), thus confirming the presence of *C. perfringens* type D bacteria.

The biochemical tests on the isolates revealed negative results for Indole, Methyl Red, Urease, Oxidase, Catalase, and Mannitol fermentation. However, positive results were observed for Voges Proskauer, Citrate Utilization, Glucose and Sucrose fermentation, Gelatin Liquification Test, Stormy Milk Test, Lecithinase Test, and H_2S gas production. The results obtained through bacteriology were consistent with the guidelines and references provided by Markey et al. and Quinn recognized as authoritative sources for bacterial identification (14, 15).

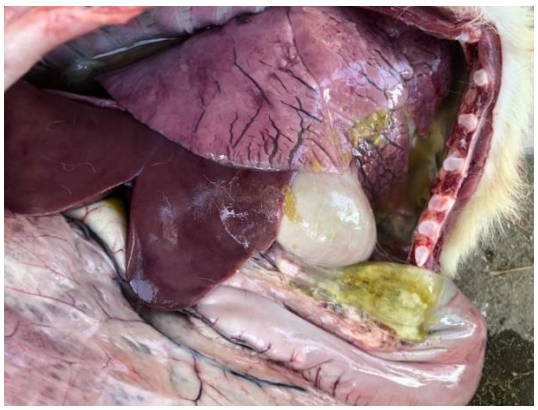

Fig 2. Gross photograph of the pulmonary edema in an Alpine.

Identification of Enterotoxins by ELISA

Based on the results obtained from ELISA testing and the calculations performed following the instructions provided by the kit manufacturer, it was observed that the intestinal contents of All 46 samples contained both CPA and ETX enterotoxins. Additionally, the presence of *C. perfringens* type D was confirmed through ELISA

testing, which supports the findings obtained from the bacteriological procedure.

Discussion

Vaccination against enterotoxemia is a common preventive measure goat farmers use to protect their herds from this disease. However, it is important to note that vaccination does not provide a 100% guarantee immunity against enterotoxemia. The vaccine currently utilized in Iran for immunization against enterotoxemia, known to be effective in conferring immunity in sheep, may not offer an equivalent level of protection in adult goats or goat kids (4, 7). Similar to the findings of the current study, the occurrence of 125 deaths despite vaccination efforts serves as confirmation of the persistence of this issue. Despite vaccination, the occurrence of enterotoxemia cases and the resulting losses suggest that some factors or circumstances can compromise the effectiveness of the vaccine-induced immune response. The study highlights the importance of identifying and addressing these conditions to enhance the protection provided by vaccination against enterotoxemia in goats. Immunity levels due to vaccination proved inadequate in preventing the onset of enterotoxemia when alterations were made in the goats' dietary intake. The vaccination failed to prevent the onset of the peracute form of the disease.

Administration of the polyvalent enterotoxemia vaccine on day three effectively halted the deaths caused by enterotoxemia. According to the claim made, in cases of enterotoxemia, the administration of the vaccine can effectively halt the death rate within two to three days. According to this claim in this situation, the vaccine can effectively prevent the progression of the disease and stop the death rate without the need for the typical two-week immunogenic period (17, 18). The findings of the present study provide evidence that the vaccine can effectively prevent the death rate and control peracute enterotoxemia within a shorter timeframe of two to three days.

The fact that the deaths ceased two days after vaccination, by itself, serves as evidence of the occurrence of enterotoxemia. Moreover, this circumstance highlights the potential limitation of the duration of immunity conferred by goat vaccination, considering that the goats had recently received a booster dose three weeks before the enterotoxemia occurrence. The sources emphasize the importance of promptly ceasing excessive carbohydrate feeding in a herd once a diagnosis is confirmed and administering a booster dose to previously vaccinated animals (4, 19).

Various sources have highlighted the decline in antibody concentrations over 10-12 months following the administration of the enterotoxemia vaccine in sheep. Consequently, it is imperative to administer annual booster vaccinations on sheep farms. Furthermore, the vaccination schedule can be adjusted based on the specific management system employed on each farm. In cases where risk factors are prevalent at different time points during the annual production cycle, shorter intervals between vaccinations may be introduced, especially in countries where livestock feed changes for various reasons, including seasonal changes. However, limited research has been conducted on the vaccination of goats. Additionally, studies have indicated that preventing clostridial diseases in goats necessitates further consideration of species-specific factors (20).

Multiple surveys have consistently demonstrated that the duration of protective antibody titers following clostridial vaccination is shorter in goats than in sheep (4, 21, 22). Additionally, certain researchers argue that toxoid vaccines have limited effectiveness in preventing caprine enterotoxemia. In a study conducted by Green et al. (1987), it was observed that antibody titers in goats were both lower and had a shorter duration when compared to sheep. Similarly, Blackwell et al. (1991) discovered that vaccination-induced antibody titers against ETX toxin protected toxemia but not caprine enterocolitis (4).

In a study conducted by Asadi et al. in 2023, it was observed that the duration of immunogenicity resulting from the enterotoxemia polyvalent vaccine manufactured by Razi Vaccine and Serum Institute was longer in sheep than in goats. Furthermore, the antibody titer in goats decreased significantly earlier than sheep, approaching zero (23). In a separate study by Veschi in 2014, it was concluded that the immunogenicity of the enterotoxemia vaccine was weaker in goats than in sheep (24). In the present study, the examined herd had been following a well-defined vaccination program using the polyvalent vaccine from the Razi Vaccine and Serum Institute for the past three years. During this period, no issues regarding immunity against enterotoxemia disease were observed. However, the current problem arose due to regular and consecutively disconnection and reconnection of the concentrate feed. It is emphasized that excessive and consecutively changes in the diet, even in vaccinated goats, could potentially undermine the immune barrier established by vaccination. Therefore, regularly and consecutively changes in diet should be taken into consideration as a contributing factor to the occurrence of enterotoxemia, despite prior vaccination.

There are three distinct clinical forms of enterotoxemia in goats: peracute, acute, and chronic. The peracute form is more commonly observed in young goats than adults (1). Typically, the peracute form has a clinical course of less than twenty-four hours and may go unnoticed until one or more dead animals are found, serving as the first indication of enterotoxemia in a herd. The larger and more robust individuals that aggressively feed are often affected in milk-fed kids. The present study documented the occurrence of peracute enterotoxemia, confirming the findings related to this severe form of the disease. The recorded observations align with the clinical signs typically associated with peracute enterotoxemia, including sudden loss of appetite, profound depression, marked abdominal discomfort characterized by arching of the back and kicking at the belly, loud and painful screaming, profuse watery diarrhea containing blood and shreds of mucus, and high fevers. The study further highlighted the rapid progression of the condition, with affected goats quickly becoming weak and unable to stand, often leading to coma and death within a few hours. The incidence of peracute enterotoxemia underscores the severity and urgency of this condition (4, 19).

In a study conducted in 2019 by Ortega et al., the researchers examined 44 cases of sudden deaths caused by enterotoxemia. The study focused on detecting CPA and ETX using ELISA in the intestinal contents of the infected goats. These goats exhibited clear necropsy lesions that were consistent with the current study's findings. These lesions included intestinal hyperemia, fluid in various cavities such as hydrothorax, hydro pericardium, and pulmonary congestion and edema (25). It is worth noting that this study's diagnosis method and the necropsy findings closely resembled those of the present study. In the present study, similar to previous studies and existing literature, a high percentage of cases of enterotoxemia in goats exhibited lesions of pulmonary edema. This observation highlights the significance of this manifestation in cases of enterotoxemia in goats. Multiple sources have emphasized that the presence of pulmonary edema lesions is commonly observed in cases of acute enterotoxemia in goats. This finding further contributes to our understanding of the complex nature of enterotoxemia in goats and underscores the importance of considering respiratory complications in its diagnosis and management (4, 19, 26).

In 2017, Karthik et al. conducted a study in Tamil Nadu, India, observing the sudden death of three two-month-old goats out of 19 purchased. Surprisingly, these goats showed no signs before their demise. Upon post-mortem examination, the researchers discovered pulmonary edema, hyperemia in the intestines, and straw-colored pericardial fluid. The acute nature of death, along with the clinical signs and necropsy findings, led to the diagnosis of enterotoxemia. To further confirm their findings, the researchers performed molecular toxinotyping using PCR, which

revealed the presence of CPA and ETX genes, indicating that the isolate was *C. perfringens* type D. The study also highlighted that the newly purchased goats had not been vaccinated on a specific date, were introduced to a new diet, and were well-fed (1). These factors were considered potential causes for enterotoxemia in these goats. Similarities between the present study and the abovementioned study regarding the diagnosis and necropsy findings can be observed.

Conclusion

The present study finds that goats are more susceptible to enterotoxemia than sheep, with shorter vaccine immunity. Disruptions in diet, particularly frequent changes, can weaken vaccine efficacy, increasing the risk of enterotoxemia and economic losses. Kids are especially vulnerable. Maintaining consistent feeding practices is crucial to support vaccination effectiveness and prevent enterotoxemia in goats, especially young ones.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Karthik K, Manimaran K, Bharathi R, et al. Report of enterotoxaemia in goat kids. *Adv Anim Vet Sci*. 2017; **5**(7):289-92.
2. Alsaab F, Wahdan A, Saeed EM. Phenotypic detection and genotyping of *Clostridium perfringens* associated with enterotoxemia in sheep in the Qassim Region of Saudi Arabia. *Vet World* 2021; **14**(3):578.
3. Lobato FC, Lima CG, Assis RA, et al. Potency against enterotoxemia of a recombinant *Clostridium perfringens* type D epsilon toxoid in ruminants. *Vaccine* 2010; **28**(38):6125-7.
4. Sumithra T, Chaturvedi V, Siju S, et al. Enterotoxaemia in goats—A review of current knowledge. *Small Rum Res* 2013; **114**(1):1-9.
5. Saminathan M, Rana R, Ramakrishnan MA, et al. Prevalence, diagnosis, management and control of important diseases of ruminants with special reference to Indian scenario. *J Exp Biol Agric Sci* 2016; **4**(3S):3338-67.
6. Johns J, Heller M. Hematologic conditions of small ruminants. *Veterinary Clinics* 2021; **37**(1):183-97.
7. Esmaeili H, Hamedi M. Color atlas of seep and goat disease of Iran. second ed. Iran: University of Tehran Press; 2017.
8. Abdolmohammadi Khiav L, Zahmatkesh A. Vaccination against pathogenic clostridia in animals: A review. *Trop Anim Health Production* 2021; **53**(2):284.
9. Miyashiro S, Nassar A, Del Fava C, et al. *Clostridium perfringens* types A and D associated with enterotoxemia in an 18-month-old goat. *J Venom Anim Toxins Trop Dis* 2007; **13**:885-93.
10. Hussain R, Guangbin Z, Abbas RZ, et al. *Clostridium perfringens* types A and D involved in peracute deaths in goats kept in Cholistan ecosystem during winter season. *Front Vet Sci* 2022; **9**:849856.
11. Pugh DG, Baird N, Edmondson M, et al. Sheep, Goat, and Cervid Medicine: Elsevier Health Sciences; 2020.
12. Hassanein KMA, Sayed MM, Hassan AM.

Pathological and biochemical studies on enterotoxemia in sheep. *Comparative Clinical Pathology* 2017; **26**(3):513-8.
13. Moustafa S, Zakaria I, Moustafa A, et al. Bacteriological and serological investigation of Clostridium perfringens in lambs. *Scientific Reports* 2022; **12**(1):19715.
14. Markey B, Leonard F, Archambault M, et al. Clinical veterinary microbiology E-Book: Elsevier Health Sciences; 2013.
15. Quinn PJ. Clinical veterinary microbiology: United Kingdom: Wolfe; 1994.
16. Quinn PJ, Markey BK, Leonard FC, et al. Veterinary microbiology and microbial disease: Wiley; 2011.
17. Goossens E, Verherstraeten S, Valgaeren BR, et al. Toxin-neutralizing antibodies protect against *Clostridium perfringens*-induced necrosis in an intestinal loop model for bovine necrohemorrhagic enteritis. *BMC Vet Res* 2016; **12**:1-8.
18. Oliveira DM, Pimentel LA, Pessoa AF, et al. Focal symmetrical encephalomalacia in a goat. *J Vet Diag invest* 2010; **22**(5):793-6.
19. Smith MC, Sherman DM. Goat medicine. Third edition ed: John Wiley & Sons, Inc.; 2023.
20. Lacasta D, Ferrer L, Ramos J, et al. Vaccination schedules in small ruminant farms. *Vet Microbiol* 2015; **181**(1-2):34-46.
21. Khan MA, Khan SB, Ahmad S, et al. Effect of vitamin E supplementation on humoral immunity following the administration of enterotoxaemia vaccine in goats. *Pakistan J Zoology* 2019; **51**(6):1-3.
22. Areekal NN, Thirumoorthy S. Vaccination schedule of goat. *Indian J Livestock Vet Anim Sci* 2023; **3**(1):25-32.
23. Asadi A, Emadi A, Asadi N. Study of immune responses of enterotoxemia vaccine in Kermani sheep and goats. *New Cellular and Molecular Biotechnology Journal* 2023; **13**(50):67-76.
24. Dorca-Arévalo J, Pauillac S, Díaz-Hidalgo L, et al. Correlation between in vitro cytotoxicity and in vivo lethal activity in mice of epsilon toxin mutants from *Clostridium perfringens*. *PloS one* 2014; **9**(7):e102417.
25. Ortega J, Verdes JM, Morrell EL, et al. Intramural vascular edema in the brain of goats with *Clostridium perfringens* type D enterotoxemia. *Vet Path* 2019; **56**(3):452-9.
26. Uzal FA, Songer JG, Prescott JF, et al. Clostridial Diseases of Animals: Wiley; 2016.