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Enterotoxaemia in Iraqi Sheep and Assessment of the Efficacy of the Local Clostridium Vaccine… A Clinical Study

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Abstract

The study aimed to estimate the incidence of enterotoxemia in sheep flocks and to evaluate the efficacy of the local vaccine used by Kindy Company (enterotoxaemia polyvalent Clostridium vaccine: (C. perfringens C, D, C. chauvoei and C. novyi B) known as Co Baghdad in two semigroups of one-year-old Awassy sheep (40 heads). Group 1 was vaccinated, group 2 was not vaccinated and kept as a negative control. All individuals in group 1 were injected with two doses (one month apart). ELISA assessment was used to estimate the efficacy of the vaccine. This group showed a rapid and strong response. After the second dose, the highest antibody levels of antibodies were in the third month after vaccination and gave the maximum rate of antibody titers, which began to decline to the minimum level of protection until the seventh month. Group 2 did not show any significant changes throughout the experiment except two cases died during study time diagnosed with enterotoxaemia clinically and in necropsy findings signs of dehydration, enophthalmos, and skin firmness, within, many multifocal sub-serosal hemorrhages of the rumen. The small intestine was expanded with dark red fluid in the lumen and existing congestion and hemorrhage of the mucosa. In short, this vaccine has protected sheep for a long time against enterotoxaemia and reduced the stress of multiple handling and injections on livestock and the efforts of workers. Therefore, we recommend that breeders must be aware of the necessity of vaccination against enterotoxemia. The breeder must immunize their herds twice a year, including a booster vaccination after a month, as a result, the herd is kept healthy even in harvest, with more eating and dietary changes.

Keywords:

Clostridia spp., polyvalent, vaccine.

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Introduction

Enterotoxaemia in sheep is an important and dangerous disease to animals and usually takes an acute or pre-acute form causing huge economic losses, caused by Clostridium perfringens, a type of anaerobic bacteria (Modhugu, 2023). Spores of Clostridium bacteria are found in soil, freshwater, and marine sediments. These bacteria are also found naturally in the intestines of animals in small amounts and do not cause any harm to animals (Takehara et al., 2020). However, when the immunity of animals is weakened for various reasons such as irregular feeding, lack of sufficient space for feeding, the animal is forced to eat, swallow feed quickly, or young new-born eat large amounts of milk at once, the disease often occurs in the early stages of feeding, in addition to exposed to stress factors such as climate change, these bacteria become active and cause the disease and death the animal in a short period (Alabbody, 2024). The most common serotypes that cause enterotoxaemia in Iraqi sheep are C. perfringens A, B, C, D, and E. D and C are also in many animal species, especially in cattle, goats, and young horses. The presence of Clostridium toxins varies from one type to another, except for the alpha toxin, which is present in all serotypes and plays the main role in the occurrence of acute enterotoxaemia (Hamad et al., 2010).

The symptoms mainly appear in pre-acute form, especially in newborn animals. This form is considered by quick loss that happens within twelve hours after the onset of clinical signs. Symptoms appear as refraining from breastfeeding in new-borns, fever with severe watery diarrhea that may be bloody, loss of appetite depression tending to lie on the ground, severe abdominal pain that can be indicated by the animal kicking its feet in the abdominal area and sitting and standing a lot drooling, tearing and then lying on their sides, and extend their legs, with their head and neck extended back over their withers. This posture is caused by the effects of the toxins on the brain. There is dyspnoea and the animal dies within 12-24 hours after the appearance of signs. Enterotoxaemia may develop so rapidly, that animals may be found dead without any signs of disease (Bryant & Stevens, 2010).

Clostridium species can cause serious health problems for many mammals, birds, and even humans. Clostridium spp. is non-invasive but can cause disease through toxins. Clostridium toxins cause gangrene and foodborne diseases (gastroenteritis) resulting in significant financial losses (Lafta, 2017; Alabbody, 2024). These diseases consist of enterotoxaemia with Clostridium perfringens, lamb dysentery with Clostridium perfringens type B), kidney pulp with Clostridium perfringens type D, blackleg with Clostridium chauvoy, malignant edema with Clostridium septicum, tetanus with Clostridium tetani and black disease with Clostridium edema type B. All these Clostridium diseases can be controlled with different types of vaccines that can be monovalent or polyvalent (Ibrahim et al., 2025).

Vaccinations are vital protection for animals against enterotoxemia (tülay Çağatay et al., 2021). Vaccines are the best item to cut the rate and intensity of the disease (Archana Menon et al., 2024). The effects of vaccination depend on several topics such as species, routes of administration, and site of inoculation, as well as the type of adjuvant and the variety of antigens in the vaccine which may also improve acquired immunity (Modhugu, 2023). Avoidance the Clostridium enterotoxaemia in Iraqi sheep mostly rests on the administration of an active dose of vaccine that consists of toxins of C. perfringens type C and D, C. septicum, C. tetani, and C. novyi and a high cell concentration of formal cultures with great immunogenicity since protection to C. chauvoei which is usually been antibacterial rather than antitoxin (Alabbody, 2024).

The study aimed to evaluate the efficacy of the local enterotoxemia polyvalent Clostridium vaccine: (C. perfringens C, D, C. chauvoei, and C. novyi B) and to estimate the incidence of enterotoximia in sheep flocks in north of Baghdad outskirts.

Material and Methods

Experimental Design

Forty-one-year-old Awassi sheep that appeared in good health were obtained from farmers living on the outskirts of Baghdad. They were used to evaluate the inactivated polyvalent Clostridium vaccine. The vaccine named (Co- Baghdad) contains C. perfringens types C & D, C. chauvoei, and C. novyi were kindly cooled from Al-Kindi Company, a mixed sector company for the production of vaccines and veterinary medicines in Baghdad. Iraq. The trial animals were distributed into two collections, 20 sheep each. Group 1 (vaccinated group): The sheep were vaccinated subcutaneously with two doses (with a pause of 4 weeks between each dose) of the vaccine (3 ml/dose). Group 2 was kept as a negative control group. Blood samples were collected earlier than the first dose, 2 weeks after the first dose, 1 month afterward the second dose, and then once a month until the 7th month from both groups. The collected serum samples were used to evaluate the immunity after vaccination in comparison with the other control group.

Evaluation of the generated antibodies against Clostridia in sheep by Indirect ELISA test was done according to (Buys et al., 2020) using the Co-Baghdad vaccine was purified by ammonium sulfate precipitation and dialysis, with coating solution and then 100 µl of the dilute cells were located in each well. The antigen-coated plates were incubated at 4 °C overnight. The plates were washed three times with a washing solution. The plates were then blocked by adding 100 µl of blocking solution and incubated for 1 h at room temperature. The plates were washed three times and then incubated with 100 µl of the diluted serum samples per well for 1 hour at room temperature. The plates were washed three times. The plates were incubated with 100 µl per well of horseradish peroxidase (HRPO)-conjugated sheep anti-IgG diluted 1:7000 in washing solution for 30 min at room temperature. The plates were washed three times before. Plates were incubated with 100 µl per well of substrate solution (phosphate citrate buffer) for 10 min at room temperature in the dark. Color development was terminated by adding 50 µl per well of 12.5% H2SO4 solution. The optical density at 492 nm (OD 492) of each well was measured using an ELISA reader. Serum samples were proven positive when absorbance values were equal to or higher than the cut-off value (cut-off value = twice the mean OD. of negative sera). Immunoreactivity was resolute where the latest dilution of serum gave a positive result. (Table 1)

Table 1. The antigens used for ELISA test

Clostridium spp. (Antigens)	**Source**
C. perfringens type C, D (Bacterin Toxoid) C. chauvoei C. novyi type B (Bacterin Toxoid)	Kindy Company (enterotoxaemia polyvalent Clostridium vaccine)

Results

The current study did not show any side effects or pathological signs in all vaccinated animals, and all animals were free of any specific antibodies before vaccination. The results showed that the sheep's response against Clostridium suppled to an antibody response two weeks after vaccination. After the second dose of the vaccine (booster dose) a month later, a rapid and strong response occurred, raising the level of antibodies to Clostridium spp. The data revealed that the vaccine gave the maximum rate of antibody titers in the third month, which began to decline to the minimum level of protection for the polyclonal Clostridium vaccine strains until the seventh month. That is, it gave high antibody titers for a long time (figure 1).

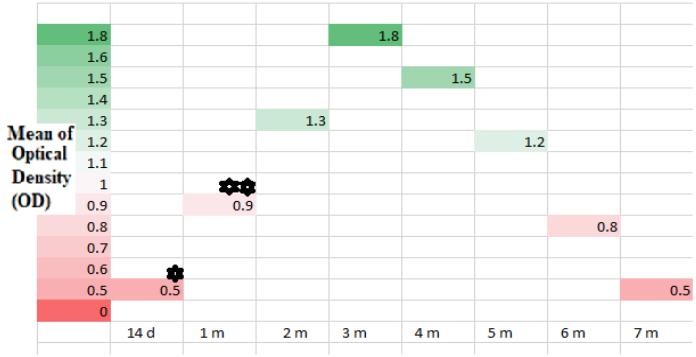

Figure 1. Immune responses of group 1 of sheep before and after the first and booster doses of vaccination: * After the first dose of vaccine, ** after the second dose of vaccine, d: day, m: month

Figure 1 shows sheep's response against Clostridium with the ELISA test used to evaluate the efficacy of the vaccine. Vaccine led to an antibody response after two weeks of the first dose This group showed a rapid and strong response occurred, after the second dose (booster dose) after one month, the highest antibody levels of antibodies was in the third month, and gave the maximum rate of antibody titers, which began to decline to the minimum level of protection until the seventh month. As for the 20 cases that were not vaccinated, there aren't any significant changes in antibody titers. In two cases they were exposed to sudden death, one died in the harvest season in July and another in the winter in January. Both cases were diagnosed as enterotoxaemia as a result of risk factors such as overfeeding or climate changes, a post-mortem was performed on the two cases, which were characterized by: evidence of dehydration, including sunken eyes and loss of skin elasticity. Internally, numerous focal sub-serosal hemorrhages of the rumen. The small intestine was expanded with dim red water in the lumen with existing hemorrhage of the mucosa (Figure. 1). An adequate bloody fluid was contented in the colon and cecum. A fresh segment of the small intestine was transmural with black to red watery content or expanded and full with yellow melted contented (figure 2).

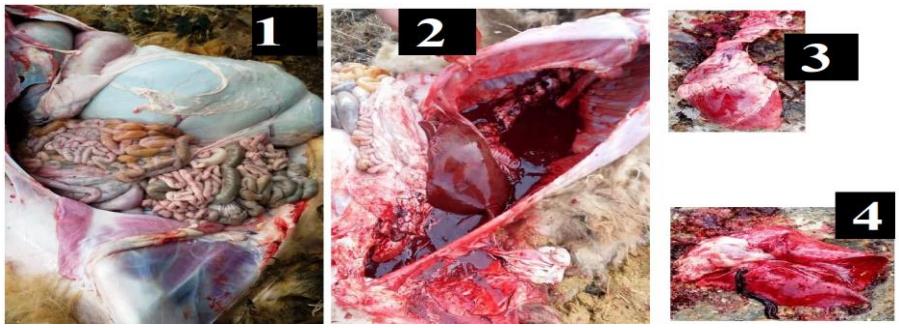

Figure 2. shows the Necropsy findings of an ewe from group 2 with a sudden death.

- A hyperaemic parts on the intestine, petechial and ecchymosis of the belly muscles, and serosa of the bowl,The rumen and abomasum comprise profusion of food, and undigested feed frequently is set up in the ileum
- Chest and abdominal cavities filled with bloody fluid congestion in the liver and other organs of the body
- A fluid-filled pericardial sac., hemorrhagic areas on the myocardium,
- Bilateral pulmonary petechiae, ecchymosis, edema, and congestion.

Discussion

The study showed that vaccination leads to maintaining the herd and enhancing production because enterotoxaemia is a serious disease that causes huge losses whenever the breeder ignores the importance of vaccination. As we note in this study, two cases from group 2 were infected and then died suddenly and were diagnosed with enterotoxaemia during the necropsy examination of the dead animal. Vaccination against enterotoxaemia has great benefits in obtaining protection (Uzal et al., 2008). Clostridium perfringens bacteria cause intestinal diseases, commonly identified as enterotoxaemia, in ruminants. These bacteria can be normal residents of the intestines of the furthermost animal kind, as well as humans, but when the intestinal environs alterations due to rapid changes in diet or other factors, Clostridium perfringens increase and produce strong toxins that act closely or are absorbed into the systemic circulation with usually shocking properties on the host. Case history, clinical signs, and postmortem results are beneficial apparatuses for starting a probable conclusion of enterotoxigenic Clostridium perfringens in sheep and goats. Definitive diagnosis requires laboratory confirmation. Isolation of some Clostridium perfringens types (such as B and C) can be of diagnostic value, but other types (such as A) are so common in the intestines of normal animals that their isolation becomes meaningless from a diagnostic point of view. In this study, ELISA tests were used to evaluate the immunity against Clostridium toxoid antigens in the vaccine The most widely accepted criterion for establishing a definitive diagnosis of enterotoxemia is the detection of Clostridium perfringens toxins in the intestinal fillings. Histological examination of the brain is also valuable in the identification of type D disease, as brain lesions caused by epsilon toxins in ruminants are distinguished as type D enterotoxemia. Additional tests, such as calculating urine glucose or detecting Gram-stained smears of the intestinal mucosa, may be used. Yet, although such tests have probable diagnostic value when positive, they cannot be used to ignore the diagnosis of enterotoxemia when negative. (Radostits et al., 2007).

Enterotoxemia can be confirmed by examining the dead animal's body and observing clinical signs, medical history, and sudden death. It can also be confirmed by culturing aerobic bacteria in the laboratory. Clostridium perfringens can be identified from stool or bowel substances from clinical or necropsy samples of infected animals. The existence of hyperglycemia and sugar in the urine can powerfully suggest enterotoxaemia in live or dead animals. Necropsy information is significant for the judgment (Ivanov & Dragunsky, 2005). So, dead animals or a whole set of necropsy tissues, feces, etc. should be sent to the analytic laboratory to confirm the clinical diagnosis. Postmortem investigation of the large and small intestines can recognize the watery matter, blood, fibrin coagulates, and small ulcers on the mucosa. On external examination, the kidneys may have a soft pulpy consistency, and intracerebral encephalopathy may occur (usually seen only in sheep). On histological inspection, there may be enhanced autolysis or separate necrosis of the proximal tubules in the kidneys. Tiny ulcers and superficial mucosal necrosis with numerous associated clostridial bacteria and mild purulent irritation may be current in intestinal specimens (Hamad et al., 2009). The lumen regularly contains rich clostridial bacteria, indicating enterotoxaemia. Progressive autolysis later in death often avoids a final diagnosis of enterotoxaemia at autopsy due to the overgrowth of clostridial bacteria. Specific nucleic acid (PCR) tests for Clostridium perfringens may be useful to approve the identification, and ELISA may be a means of detecting C. perfringens toxins from intestinal contents (Miserez et al., 1998). In our study, the level of antibodies generated by two doses of polyvalent enterotoxaemia vaccine was evaluated. ELISA has great advantages

ELISA (enzyme-linked immunosorbent assay) is a common laboratory easy that identifies and quantifies proteins, antigens, antibodies, and hormones in body fluid samples like blood, plasma, urine, saliva (spit), and cerebrospinal fluid (CSF). (Uzal et al., 2010).

The results were valuable and the immune response was high in the polyvalent vaccine and remained above the minimum protection against Clostridium species and subspecies for seven months, which is in agreement with other researchers. (Khamas & Nour, 2004). The efficiency of the local vaccine (Co-Baghdad) we agree with the breeders in preferring it over imported vaccines, even its cost seems more expensive than imported vaccines, despite the imported item being more diverse in terms of antigens, this is an indication that the local vaccine for enterotoxemia is highly efficient, perhaps this is due to it is prepared locally from bacterial strains present in the country, so it gives the best results, as previous studies have shown (Khamas & Nour, 2004; Al-Shuwaili & Tarsh, 2022) The second booster dose of the vaccine is always necessary, but some reviewers prefer to give one dose to reduce the financial cost, and it is not enough for the animal to enjoy good health and be safe from the risk of enterotoxemia, as we noticed in this study a sudden and almost non-decreasing rise in the level of antibody titers. Not giving the booster dose is almost like not vaccinating and thus being exposed to losses in the herd (Al-Shuwaili & Tarsh, 2022). The vaccine gave the highest immunity and antibody titers that remained above the minimum level of protection against Clostridium C and D toxins for seven months, which adds another efficiency to the vaccine, as there is a positive relationship that can be benefited from and developed in the future from this immune response (Robi et al., 2023).

The absence of side effects of the vaccine on the antigenic component makes it safe, and thus we agree with previous studies (Gomez et al., 2012), but some injection mechanisms must be taken cautiously, such as avoiding injection directly under the armpit because it causes lameness during the first days due to the local immune reaction where movement and walking occur, and here it causes some discomfort to the animals and owner who believe that the vaccination process has failed, so it is desirable to inject subcutaneous in the front of the chest.

Conclusions

The conclusion is that the local enterotoxemia polyvalent vaccine provided good protective immunity against the toxins of the C.perfringens used did not show any side effects on the animal, the vaccine and can be safely used to protect sheep from clostridial diseases. In addition, it is necessary to diversify the sheep's diet and not limit its food to concentrated feed and high-grade grains, but it must include green and dry herbs. In addition to concentrated feed, when fattening lambs, it is recommended to gradually give concentrated feed and grains and to work on rationing the concentrated feed provided, as some sheep may eat more than they need, which exposes them to several health problems. It must be taken into consideration that the areas allocated for feed are sufficient for all animals so that the food is taken by everyone equally.

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Conflicts of interest

There are no conflicts to declare. The author declared no competing interests.

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