

Effect of a combined clostridial vaccine on cytokine and immunoglobulin levels in lambs

Efecto de una vacuna clostridial combinada sobre los niveles de citocinas e inmunoglobulinas en corderos

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ABSTRACT

Infections caused by clostridial agents are difficult to treat and have a high mortality rate. This study aimed to determine the effects of the recommended dose of a commercial clostridial vaccine on serum levels of TNF- α , IL-1 β , IL-6, and IL-10, and IgG and IgM in lambs. Methods: A single commercial clostridial vaccine, containing *Clostridium perfringens* types A, B, C, and D, *Clostridium septicum*, *Clostridium novyi* type B, *Clostridium tetani*, and *Clostridium chauvoei*, was administered subcutaneously (2 mL per dose) to 15 female lambs, with two doses given at a four-week interval. Blood samples were taken at 0, 2, 4, 8, 12, and 24 hours after the first dose, and species-specific serum TNF- α , IL-1 β , IL-6, and IL-10 levels were measured using commercial ELISA kits. Serum IgG and IgM levels were determined using an ELISA reader from blood samples taken on days 0, 7, and 21 after each of the first and second vaccinations. While no statistical changes were observed in IL-1 β and IL-10 levels ($P>0.05$), TNF- α peaked at the 2nd hour ($P<0.05$), and IL-6 levels reached the highest levels at the 8th and 12th h after vaccination ($P<0.05$). While no change was detected in IgM levels in the study ($P>0.05$), IgG levels were significantly elevated in the final two samples ($P<0.05$). In conclusion, clostridial vaccine application in lambs stimulates cytokine synthesis and provokes an IgG response responsible for immunity, which is observed after the second application.

Key words: Lambs; clostridial vaccine; cytokines; immunoglobulins; immunity

RESUMEN

Las infecciones causadas por agentes clostridiales son difíciles de tratar y tienen una alta tasa de mortalidad. Este estudio tuvo como objetivo determinar los efectos de la dosis recomendada de una vacuna clostridial comercial en los niveles séricos del TNF- α , IL-1 β , IL-6 e IL-10, e inmunoglobulinas IgG e IgM en corderos. Una sola vacuna clostridial comercial, que contenía *Clostridium perfringens* tipos A, B, C y D, *Clostridium septicum*, *Clostridium tetani* y *Clostridium chauvoei*, se administró por vía subcutánea (2 mL por dosis) a 15 corderas (de 2 a 3 meses de edad), con dos dosis administradas en un intervalo de cuatro semanas. Se tomaron muestras de sangre a las 0, 2, 4, 8, 12 y 24 horas después de la primera dosis, y se midieron los niveles séricos específicos de especie de TNF- α , IL-1 β , IL-6 e IL-10 utilizando kits ELISA comerciales. Los niveles séricos de IgG e IgM se determinaron utilizando un lector ELISA a partir de muestras de sangre tomadas los días 0, 7 y 21 después de cada una de la primera y segunda vacunación. Si bien no se observaron cambios estadísticos en los niveles de IL-1 β e IL-10 ($P>0,05$), el TNF- α alcanzó su punto máximo en la segunda hora ($P<0,05$) y los niveles de IL-6 alcanzaron los niveles más altos en la octava y duodécima hora después de la vacunación ($P<0,05$). Aun cuando no se detectaron cambios en los niveles de IgM en el estudio ($P>0,05$), los niveles de IgG se elevaron significativamente a los 7 y 21 días posteriores a la revacunación ($P<0,05$). En conclusión, la aplicación de la vacuna clostridial en corderos estimula la síntesis de varias citoquinas y eleva la respuesta IgG responsable de la inmunidad después de la segunda aplicación.

Palabras clave: Corderos; vacuna clostridial; citosinas; inmunoglobulinas; inmunidad

INTRODUCTION

Clostridia are anaerobic, sporiferous, rod-shaped bacteria. The length of the bacteria varies between 3 and 10 μm and their width varies between 0.5 and 1.5 μm . While usually staining Gram-positive, older cultures have been reported to stain Gram-negative. Some clostridial agents can both produce toxins and be invasive. Variation in toxin production ability can also be observed among strains of the same clostridial species. Under certain conditions, certain clostridial strains are unable to produce toxins, while other strains can produce high amounts of lethal toxins. This variability can make diagnosis difficult. Gross post-mortem lesions and detection of the relevant toxin are key to successful diagnosis [1]. *Clostridium* spp. can survive in environmental conditions for several years. Clostridial agents *C. perfringens*, *C. botulinum*, *C. chauvoei*, *C. septicum*, *C. novyi*, *C. tetani*, *C. haemolyticum*, and *C. difficile* can cause serious and potentially fatal infections [2, 3].

Several types of *C. perfringens* are toxin-producing, with B, C, and D being the most pathogenic. Enterotoxemia caused by *C. perfringens* is the most common clostridial disease in sheep farming countries. Type B of *C. perfringens* causes lamb dysentery, while type D causes fibrous kidney disease. Although type A has recently been shown to cause death in sheep (*Ovis aries*), it is generally considered a commensal in most sheep, typically not producing toxins [1]. *C. septicum* is considered the most important cause of gas gangrene (malignant edema), leading to high mortality rates, especially in sheep and cattle (*Bos taurus*). *C. chauvoei*, *C. perfringens* type A, *C. novyi* type A, and *C. sordellii* can also cause gas gangrene [4]. *C. tetani* is identified as the causative agent of fatal neuromuscular disease. Clinical symptoms include a decreased sucking reflex, jaw failure, convulsions, seizures, fever, and respiratory failure [5]. *C. novyi* type A causes gas gangrene [6], while type B causes infectious necrotic hepatitis in sheep, an acute infection with a high mortality rate [7]. *C. chauvoei* causes blackleg [6].

Vaccination is considered as the best effective methods of preventing clostridial infections, with effective vaccines available for domestic animals such as cattle, sheep, and goats (*Capra hircus*). Clostridial vaccines have been available since the 1950s. Clostridial vaccines include various combinations of 2–8 agents. Clostridial diseases are quite common and vaccination is often recommended because they usually cause sudden death in a short time. Since most clostridial vaccines are inactive, two doses (3–4 weeks apart) must be given in the first application. Sufficient protection is provided 1–2 weeks after the second dose [2, 8]. Vaccines against clostridial infections for ruminants are licensed for sale [9, 10].

Cytokines are polypeptide substances produced and secreted by many types of cells (lymphocytes, monocytes, macrophages, some somatic cells). Cytokines can cause the differentiation of lymphoid system cells in the living, antimicrobial effect, activation of inflammatory cells and their migration to the inflammatory site, wound healing, hematopoiesis, fever, shock and death in high concentrations. Cytokines are classified as pro-inflammatory (inflammation stimulating) or anti-inflammatory (inhibitory) cytokines according to their role in inflammation. Interleukin (IL) 1- β , IL-6 and Tumor necrosis factor (TNF)- α are defined as pro-inflammatory cytokines. TNF- α is stated as the most important mediator of acute inflammation. Together with IL-1 and IL-6, it is responsible for the disseminated intravascular coagulation

and shock that develop in infection. IL-1 is reported to cause the secretion of acute phase proteins and fever in the living. Another pro-inflammatory cytokine, IL-6, is responsible for B lymphocyte proliferation and differentiation, synthesis of acute phase proteins, and production of immunoglobulins (Ig). IL-10 inhibits cytokine synthesis and suppresses inflammation [11]. It has been reported that serum cytokine levels increase after plague (*Peste des petits ruminants*) vaccination in sheep [12] and that *Corynebacterium cutis* lysate administered to sheep causes statistical fluctuations in IL-1 β , IL-6, IL-10 and TNF- α levels [13].

Ig are synthesized by B lymphocyte cells in living organisms [14]. It is stated that the primary function of Ig in living beings is to bind to antigens and form antigen-antibody compounds, facilitate their removal by phagocytosis and render them ineffective. They also play a role in mucosal immunity, antiviral effect, activation of the complement system, prevention of microorganisms adhering to mucosa and prevention of absorption of certain substances from the intestine. It has been determined that there are five different types of immunoglobulins immunologically. These are called IgG, IgM, IgE, IgA and IgD. While IgG, IgM, IgE and IgA are determined to be found in all mammals, IgD is stated to be found only in monkeys (Cercopithecidae), bovine (Bovinae), humans, dogs (*Canis lupus familiaris*) and rats (*Rattus norvegicus*). It has been reported that IgG constitutes the majority of the total amount of immunoglobulin (75%) and is the most important immunoglobulin for living beings [15, 16].

IgM is the first immunoglobulin produced in response to an antigen [14]. Increases are observed in the acute phase of infections in the blood IgM level. However, IgM is a short-lived immunoglobulin. When the level of IgM begins to decrease, the long-lived IgG begins to increase. Determining the level of IgM in the blood higher than the level of IgG is considered an indicator of the presence of an acute infection [15]. It has been stated that there are increases in serum antibody levels after the plague (*Peste des petits ruminants*) vaccine administered to sheep [12].

Considering that changes have been observed in blood cytokine [12, 17] and Ig [12, 18, 19, 20]. Levels after some vaccinations, we hypothesized that there may be increases in serum cytokine and Ig levels after clostridial vaccination in lambs. The purpose of this research was to determine the effects of a combined clostridial vaccine containing *C. perfringens* types A, B, C, and D, *C. novyi* type B, *C. tetani*, *C. septicum*, and *C. chauvoei* on serum cytokine (IL-1 β , IL-6, IL-10 and TNF- α), IgG and IgM levels in healthy lambs.

MATERIALS AND METHODS

Animals

The Selcuk University Faculty of Veterinary Medicine Ethics Committee (SUVDA MEK 2023/049) approved this study protocol. In the study, 15 female Merino lambs (2–3 months, 17.17 $\pm$ 0.44 kg) (Immax, Imamoglutarti, Istanbul, Turkiye) were administered a commercial clostridial vaccine (Cogvalax Polyvalent Inactivated Vaccine, Ceva Animal Health, Inc., Budapest, Hungary) containing *C. perfringens* types (A, B, C, and D), *C. novyi* (type B), *C. tetani*, *C. chauvoei* and *C. septicum*. The vaccine was injected subcutaneously in two doses of 2 mL, four weeks apart. Blood samples for cytokine measurements were collected at 0, 2, 4,

8, 12 and 24 hours (h) following the first vaccine injection. For measurements of Ig values, blood samples were taken on days (d) 0, 7 and 21 after the first vaccine injection and on days 0, 7 and 21 after the second vaccine injection (FIG. 1).

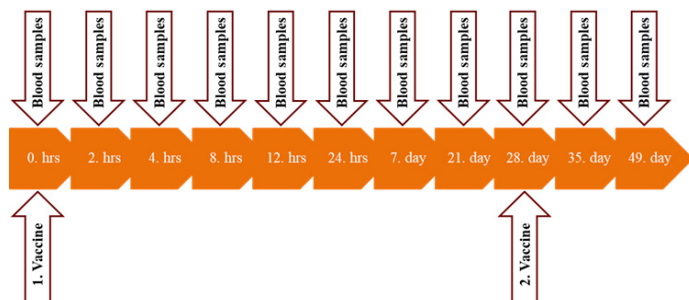


FIGURE 1. Vaccination and blood sampling times of lambs

ELISA measurements

In the study, ELISA kits of a commercial company (BT LAB, Shanghai, China) were used and all kits were selected to be specific to the sheep species. TNF- α , IL-1 β , IL-6, IL-10, IgG and IgM serum levels were measured from the serum extracted from the blood samples using commercial kits in an ELISA reader (MWGt Lambda Scan 200, Bio-Tec Instruments, Winooski, VT, USA).

Statistical analysis

The study data is presented as mean $\pm$ standard error (SE). The study results were evaluated with ANOVA and Tukey tests (SPSS 29.0). Results of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Serum IL-1 β , IL-6, IL-10 and TNF- α levels obtained from lambs after vaccination are presented in FIG. 2. No statistically significant changes were determined in IL-1 β and IL-10 levels in the study ($P > 0.05$). IL-10, however, shows a slight increase at 2 hours, increasing in a non-significant manner at 4 and 8 hours and then falling until 24 hours post vaccination. A peak increase in TNF- α was observed at h 2 post-vaccination ($P < 0.05$), before continually decreasing to baseline levels across the further sampling intervals. IL-6 levels at 8 and 12 h were statistically higher than at 24 h ($P < 0.05$). Post-vaccination serum IgG and IgM levels are presented in FIG. 3. While no statistical change was observed in the IgM level across the study ($P > 0.05$), IgG levels peaked at the final sampling point at 49 d after the start of vaccination ($P < 0.05$).

In Veterinary Medicine, clostridial agents [2, 3] are known to cause difficult-to-treat infections with high mortality rates, such as blackleg, gas gangrene (malignant edema), tetanus, botulism, infectious necrotic hepatitis, and enterotoxemia. As infections are often observed in flocks, treatment can be costly and time-consuming. For this reason, clostridial vaccination is recommended for ruminants as opposed to treatment after infection [10, 21, 22]. In this study, it was determined that clostridial vaccination of lambs had no statistically significant effect on serum IL-1 β and IL-10 levels

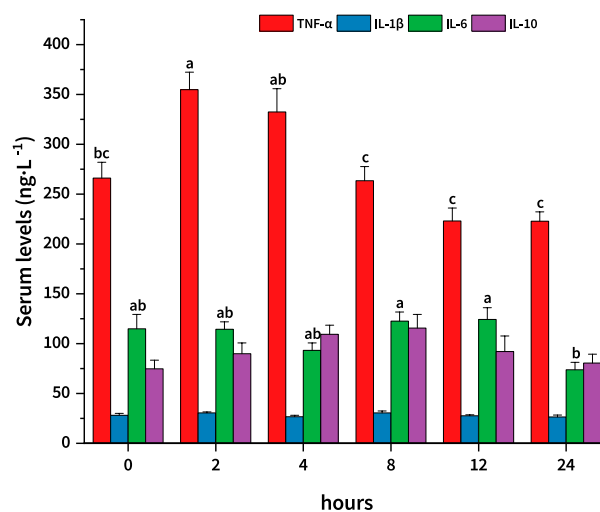


FIGURE 2. Effect of clostridial vaccine administration to lambs on serum TNF- α , IL-1 β , IL-6 and IL-10 concentrations (mean $\pm$ SE)

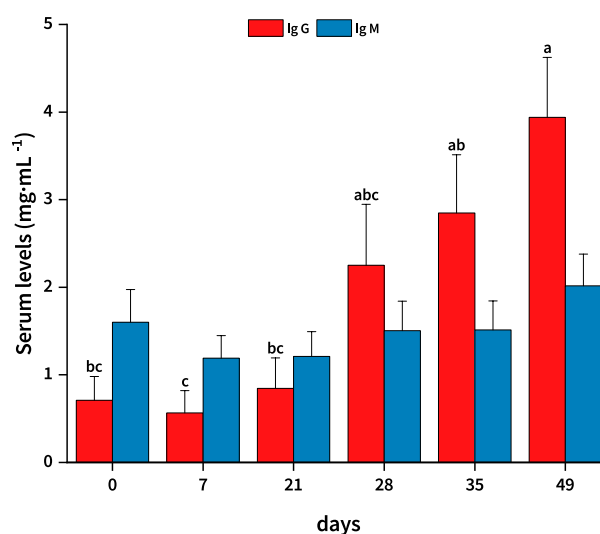


FIGURE 3. Effect of clostridial vaccination on serum IgG and IgM concentrations in lambs (mean $\pm$ SE)

($P > 0.05$), while TNF- α concentration increased and peaked at 2 h post-administration ($P < 0.05$). Fluctuations in IL-6 levels were also observed, with the level of IL-6 at 8 and 12 h being higher than at 24 h ($P < 0.05$; FIG. 2). No studies have reported previously on the effect on cytokines of clostridial vaccines administered to sheep. However, it has been reported that the *Peste des petits ruminants* vaccine administered to sheep increases IL-6 and IL-10 levels [12], foot-and-mouth disease type O vaccine administered to guinea pigs (*Sus domesticus*) increases TNF- α and IL-10 gene expression [23], and a canine coronavirus vaccine administered to mice (*Mus musculus*) increased TNF- α and IL-1 β gene expression [24]. Meanwhile, serum IL-1 β , IL-6, IL-10 and TNF- α levels were higher in humans infected with *C. difficile* [25]. It has been reported

that COVID-19 vaccines may induce a cytokine storm [17], while the Bacille Calmette–Guerin (BCG) vaccine has no effect on TNF- α and IL-1 β in humans, but causes increases in IL-6 and IL-10 levels [26]. It has been reported that clostridial vaccination could initiate immunity by stimulating the synthesis of TNF- α , the first and most important mediator of the acute response in lambs, and to support IL-6 immunity. Although no statistical difference has been observed, fluctuations in other cytokines suggest that the immune system is stimulated within the cytokine storm. Cytokine monitoring in lambs after clostridial vaccination can provide information about whether the immune system has been stimulated.

While no statistical changes were determined in serum IgM levels at the sampling times ($P>0.05$), IgG levels started to increase 7 d after the second vaccination (experimental d 28) and reached the highest level at the final sampling point (experimental d 49) ($P<0.05$; FIG. 3). Previous reports have shown that antibodies against *C. perfringens* reached their highest level on the 45th d after the administration of a clostridial enterotoxemia vaccine in sheep [27]. However, another report observed no changes in IgG and IgM levels on the 35th d in sheep vaccinated with an enterotoxemia vaccine containing *C. perfringens* types C and D, *C. chauvoei*, *C. septicum*, *C. haemolyticum*, *C. novyi*, and *C. sordellii* [28].

Antibody levels have also been shown to increase after the administration of vaccines for *C. perfringens* to sheep, goats, and rabbits (*Oryctolagus cuniculus*) [29], *C. perfringens* and *C. oedematiens* to rabbits [30], and *Peste des petits ruminants* to sheep [12]. It has been reported that, in human studies, serum anti-toxin A IgG levels increase after *C. difficile* toxoid vaccine administration [31, 32]. Shigella conjugate [18], and SARS-CoV-2 [33] vaccines increase serum IgG levels in humans. However, decreases in total IgG and IgM levels have also been reported after the administration of the measles vaccine to children [34]. Most clostridial vaccines used in the Veterinary field are inactivated and are recommended to be administered twice, with an interval of 21–28 d; sufficient immunity develops 7–14 d after the second dose [2]. In the current study, the highest IgG levels were observed 21 d after the second application (d 49 overall). It is important to emphasize that the purpose of measuring post-vaccination cytokines is to determine, within a few hours, whether an innate response indicative of an effective specific response has begun. As previously indicated, the specific immune response requires 21 to 28 d and a booster vaccination to demonstrate vaccine titers. Although total IgM and IgG levels can provide some indication of an immune response, the ideal approach would be to measure a specific response using a Clostridial antigen ELISA.

CONCLUSIONS

Two vaccinations against clostridial agents in lambs (*Ovis aries*), administered 21 days apart, increased the levels of TNF- α , the primary mediator of the acute immune response, and IgG, which provides long-term humoral immunity. However, although the increase in total IgG appears to enhance immunity, specific anticlostridial antibodies should also be measured. A significant increase in IL-6 and TNF- α levels after vaccination can be used as an early biomarker of the effectiveness of anticlostridial vaccination in lambs.

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Conflict of interest

The authors stated that they have no conflict of interest.

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