

Persistence of Maternal-Derived Antibody against *Clostridium Perfringens* Type D Epsilon Toxin in Goat Kids

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Received: 24.03.2025

Accepted: 02.06.2025

Abstract

Enterotoxemia occurs in goats as a common disease with high mortality. The present study aimed to investigate maternal-derived antibodies against epsilon toxin to improve vaccination strategies for enterotoxemia in goats in Iran. Kids born from 12 vaccinated goats and kids born from 7 unvaccinated goats were sampled at 0, 3, 14, 28, 42, 56, 70, and 86 days of age. A commercial blocking ELISA kite was used for serological analysis of samples. The percentage of inhibition was significantly higher in vaccinated dams compared to unvaccinated ones. In kids, the percentage of inhibition in both groups was statically similar until 42 days of age. After this point, the percentage of inhibition in the control group decreased significantly, becoming notably lower compared to the treatment group at 56 and 70 days old. At 86 days old, the percentage of inhibition in both groups was statically indifferent. In the unvaccinated group, the percentage of inhibition at 56 days of age is significantly lower than the percentage of inhibition at 42 days of age. Moreover, in the vaccinated group, the percentage of inhibition at 70 days of age was significantly lower than at 56 days of age. In conclusion, vaccinating dams during the last month of pregnancy is effective in enhancing maternally derived antibody titers against epsilon toxin in kids. In goat kids born to vaccinated and non-vaccinated dams, 56–70 and 42–56 days of age, respectively, seem to be the proper times for the administration of the first dose of the enterotoxemia vaccine.

Key words: *Clostridium perfringens*, Entrotoxemia, Epsilon toxin, Kids, Vaccine

Introduction

Enterotoxemia occurs in goats as a common disease with high mortality (Rajamohan and Rajasekaran, 2025). Depending on their ability to produce the four typing toxins (α , β , ϵ , ι toxins), *Clostridium perfringens* is classified into

five toxinotypes (A, B, C, D, E) (Sumithra et al, 2013). *Clostridium. perfringens* type D is the principal organism involved in caprine enterotoxemia (Rajamohan and Rajasekaran, 2025; Sumithra et al, 2013). However, types B and C have also been

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mentioned as its etiology (Sumithra et al, 2013). Epsilon toxin is considered to be the most important toxin in *Clostridium perfringens* type B and D enterotoxemia (Sumithra et al, 2013).

Clostridium perfringens, considered part of the normal flora in goats, proliferates rapidly and produces large amounts of toxins when the intestines are altered by sudden changes in diet (Sumithra et al, 2013). Consequently, overfeeding is the main predisposing factor (Rajamohan and Rajasekaran, 2025). Epsilon toxin induces an increase in the permeability of intestinal mucosa, facilitating the passage of toxins into blood, and their spread into various organs is considered the causative virulence factor of all symptoms and lesions caused by *Clostridium perfringens* type D infection (Sumithra et al, 2013).

Enterotoxemia occurs commonly in goat farms in Iran and leads to significant economic losses for farmers (Alimolaei and Ezatkah, 2022; Hayati et al, 2020; Hussain et al, 2022). Peracute enterotoxemia has been reported in two goat herds with high mortality in Iran (Esmaeili and Joghataei, 2024). The report states that the disease has been well controlled by vaccination (Esmaeili and Joghataei, 2024). Many studies have been published on the importance of vaccination in controlling enterotoxemia in goats (Asadi, et al, 2023; Naz et al, 2012; Veschi et al, 2006). However, many aspects of vaccination against enterotoxemia in goats are not fully understood. For example, it is not yet fully clear when maternal derived antibodies against enterotoxemia in kids decay or, more precisely, when kids should be vaccinated against enterotoxemia so that these maternal-derived antibodies are sufficiently reduced and probably do not neutralize the active antibodies induced by vaccination. The present study aims to investigate these issues to improve vaccination strategies against enterotoxemia in goats in Iran.

Material and method

Animals

This study was conducted on a dairy goat farm (housing nearly 5000 Murciana goats). Goats are kept indoors all year round.

A total of 30 maiden goats, approximately 11 months old, and carrying one fetus at 6 weeks before the expected parturition date, were selected for the study. The number of fetuses in each goat and the pregnancy dates were determined based on ultrasound examination findings and mating records. The selected goats were divided into two equals: treatment and control. The treatment group was vaccinated at 6 and 4 weeks before the expected parturition, while the control group was not vaccinated.

Sufficient colostrum (20% of body weight) was administered to the kids via bottle feeding. The first feeding occurred within 30 minutes after birth, followed by three colostrum feedings every four hours. After that, milk feedings were started 3 times a day. Water and starter were made available to the kids at 3 days of age. The kids were weaned at approximately 90 days of age. Milk and colostrum were pasteurized before being given to the kids.

The enterotoxemia vaccine used in this study was Polivac cl 7[®] (Vetal com, Turkey).

Blood sampling

Blood sampling was performed at different times, as shown in Figure 1. Blood was collected from the dams at one week before parturition (3 weeks after the second dose of vaccination). Kids were sampled at 0 (before colostrum intake), 3, 14, 28, 42, 56, 70, and 86 days old.

Exclusion criteria

Dams

Goats affected by any disease (such as pregnancy toxemia) during pregnancy, those with a low body condition score, udder problems, mastitis, with low colostrum production, or those that experienced abortion or stillbirth were excluded from the study.

Kids

Kids that did not receive sufficient colostrum at a proper time, those that died during the study, those with low birth weight (weak kid), those born with dystocia, and those with congenital defects were excluded from the study.

As a result of applying these exclusion criteria, some goats and their kids were removed from the study, leaving samples from 12 goats in the treatment group and 7 goats in the control group for serological analysis.

ELISA testing

Blocking ELISA was used to measure antibody titers against *Clostridium perfringens* epsilon toxin in samples, as previously mentioned (Rousselet et al, 2021) and according to the kit manufacturer's instructions [Monoscreen ELISA Kit (BioX Diagnostics, BIO K 222 - Monoscreen AbELISA *Clostridium perfringens* epsilon toxin / blocking)].

Inhibition percentage was calculated using the following formulas:

$$\% \text{ Inhibition sample} = [(OD_{450 \text{ nm}} \text{ negative sera} - OD \text{ sample}) / OD_{450 \text{ nm}} \text{ negative sera}] \times 100,$$

$$\% \text{ Inhibition positive} = [(OD_{450 \text{ nm}} \text{ negative sera} - OD \text{ positive sera}) / OD_{450 \text{ nm}} \text{ negative sera}] \times 100.$$

The test was validated only if the OD negative – OD positive was >0.7 and inhibition of the positive control was $>30\%$.

Data analysis

The normal distribution of data in each group was checked using the Shapiro–Wilk test. After confirming normal distribution, independent t-test was used to compare the groups. A *P*-value of less than 0.05 was considered statistically significant.

Results

During the study, no clinical disease was observed on the studied farm. The percentage of inhibition was significantly higher in vaccinated dams than in unvaccinated ones; however, unvaccinated dams also exhibited high percentages of inhibition.

In kids, the percentage of inhibition in both groups was statically similar until 42 days of age. After that, percentage of inhibition in the control group decreased significantly, becoming notably lower than that in the treatment group at 56 and 70 days of age. At 86 days old, the percentage of inhibition in both groups was statically comparable.

Additionally, the percentage of inhibition in the unvaccinated group at 56 days of age was significantly lower than at 42 days of age.

In the vaccinated group, the percentage of inhibition at 70 days of age was significantly lower than at 56 days of age.

Table 1: The percentage of inhibition in the control and treatment groups at different times

	BP	BC	3-d	14-d	28-d	42-d	56-d	70-d	86-d
Treatment (mean±sem)	96.2±0.28	13.2±2.4	79.69±5.4	91.3±1.43	92.3±0.61	92.2±1.06	90.8±1.58	78.3±4.63	57.7±5.62
Control (mean±sem)	75.4±7.8	17.6±4.2	86.35±5.1	94.1±1.49	90.12±1.97	85.9±2.8	71.7±4.55	56.4±10.29	52.9±12.53
<i>P</i> Value	0.038*	0.339	0.439	0.223	0.192	0.085	0.005*	0.043*	0.737

**P*<0.05, BP: before parturition, BC: before colostrum intake

Table 2: Comparison between percentage of inhibition in the treatment and control groups in different times after birth

Days		0 , 3	3 , 14	14 , 28	28 , 42	42 , 56	56 , 70	70 , 86
P value	Treatment	0.000*	0.064	0.952	0.881	0.493	0.025*	0.07
	Control	0.000*	0.194	0.134	0.257	0.028*	0.197	0.836

* $P < 0.05$

Discussion

Enterotoxemia associated with *C. perfringens* type D is a disease of ruminant animals, primarily of lambs and kids, and is worldwide in its distribution (Constable et al, 2017). Also, one of the most important diseases in kids is enterotoxemia associated with *C. perfringens* type D and the highest incidence of the disease is in suckling kids between 3 and 10 weeks of age (Constable et al, 2017; Rajamohan and Rajasekaran, 2025); the most important strategy for controlling this disease in goat kids is dam vaccination (Rajamohan and Rajasekaran, 2025). Dam vaccination is an effective way to protect kids from diseases, but the challenge is determining when this immunity ends. Research has been conducted in this field for some diseases like Contagious agalactia (Abdollahi et al, 2022) Pox, (Abdollahi et al, 2024), PPR (Abdollahi et al, 2023) in kids in Iran. However, despite the importance of enterotoxemia in goat kids, few studies have been conducted on maternally acquired immunity against epsilon toxin in kids in Iran and, to the best of our knowledge, this is the first study on this topic.

The roles of β - and ϵ -toxins in dysentery and enterotoxemia in lambs and kids have been well established (Alimolaei and Shamsaddini Bafti, 2023). The β -toxin produced by type B strains causes dysentery in young lambs and kids (<14 days old), while the ϵ -toxin produced by type D strains is a common cause of enterotoxemia in older lambs and kids (Alimolaei and Shamsaddini Bafti, 2023). Despite the recognized importance of type B in postnatal enterotoxemia, a study conducted in Iran found that the second most prevalent *Clostridium perfringens* toxinotype was

type D (Alimolaei and Shamsaddini Bafti, 2023). Additionally, another study in Iran reported that type B isolates were the least frequent among *C. perfringens* strains collected from sheep and goats (Hayati et al, 2020). Therefore, we focused our study on type D. Nevertheless, investigating β -toxins remains a promising direction for future research.

In our study, all kids born to vaccinated dams had a percentage of inhibition above 50% at 70 days of age. At 86 days of age, approximately 60% of kids born to vaccinated dams had a percentage of inhibition above 50%, with the lowest percentage of inhibition recorded at 37%. In contrast, at 70 days of age, approximately 60% of kids born to unvaccinated dams had no titer and at 86 days of age, this proportion decreased to about 30%. Therefore, we can conclude that dam vaccination with the enterotoxemia vaccine can protect kids against enterotoxemia up to 86 days of age. Conversely, kids born to mothers who were not vaccinated in the last months of pregnancy but had previously undergone a regular and repeated vaccination program were protected against enterotoxemia up to 56 days of age. The significantly higher percentage of inhibition at 56 and 70 days of age in kids born to vaccinated dams compared to those born to unvaccinated dams, could be another reason for this conclusion. However, the percentage of inhibition in the vaccinated group begins to decline at 56 days of age, and by 70 days of age, it is significantly lower than at 56 days. Similarly, in the unvaccinated group, the percentage of inhibition begins to decline at 42 days of age, and at 56 days of age, it is significantly lower than at 42 days. It has been mentioned

that vaccination of maiden ewes twice-at an interval of at least 1 month, with the last vaccination approximately 4 weeks before lambing-results in good passive immunity in young lambs, with 97% of lambs having protective antibody levels at 8 weeks of age, with a significant proportion still protected at 12 to 16 weeks of age (Constable et al, 2017). Additionally, one study showed that vaccination of ewes before lambing provides passive protection to lambs up to 84 days of age (de la Rosa et al, 1997). Although these findings are consistent with our study, some research suggests that the antibody titer induced by the enterotoxemia vaccine in goats is lower and has a shorter duration compared to that in sheep (Asadi et al, 2023; Asadi et al, 2023; Biotech et al, 2012). To our knowledge, there are few studies on the persistence of maternal antibodies against epsilon toxin in goat kids. Only a few textbooks and review articles recommend that kids be vaccinated initially at four to six weeks of age (Constable et al, 2017); (Sumithra et al, 2013). Since the maternal-derived antibody against epsilon toxin can be affected by the type of vaccine used in dam, husbandry and management conditions, and the frequency of previous vaccinations administered to the dam, further studies are needed to conclude definitively about maternally derived immunity against epsilon toxin in kids.

A study conducted in Iran on the humoral immune response against *Clostridium perfringens* epsilon toxin in goats and sheep showed that the duration of the humoral immune response in goats upon administration of the clostridial vaccine was relatively brief, requiring multiple booster

injections (Asadi et al, 2023). The goats in our study had received multiple booster injections and they had been vaccinated 4 times (two doses at 45 days old and then two doses at 6 months old) until they reached 11 months (the time selected for the study). This routine vaccination regimen and the multiple booster injections may explain the high percentage of inhibition in the unvaccinated dams, as well as the insignificant differences in percentage of inhibition in both groups until 42 days of age. On the other hand, another study states that natural acquired antibodies against *Clostridium perfringens* epsilon toxin can appear in young goats and increase with age, without any evidence of clinical disease (Veschi et al, 2008). The presence of these naturally acquired antibodies could also explain the high percentage of inhibition in unvaccinated goats. However, the percentage of inhibition was significantly higher in vaccinated dams than in unvaccinated ones, underscoring the importance of vaccination in late pregnancy. Later significant decrease of the percentage of inhibition in goat kids born to vaccinated dams compared to unvaccinated ones also highlights the importance of vaccination in late pregnancy.

In conclusion, vaccinating dams during the last month of pregnancy is effective in enhancing maternally derived antibody titers against epsilon toxin in kids. In goat kids born to vaccinated and non-vaccinated dams, 56–70 and 42–56 days of age, respectively, seem to be the proper times for the administration of the first dose of the enterotoxemia vaccine.

Acknowledgements

The authors wish to thank everyone who contributed their time and experience to this study.

Conflict of Interest

The authors declare no conflict of interest.

Funding

No financial aid was received for this study.

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Received: 24.03.2025

Accepted: 02.06.2025

بررسی روند تداوم پادتن مادری ضد توکسین اپسیلون کستریدیوم پرفرنجنس تیپ D در بزغاله‌ها

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تاریخ پذیرش: ۱۴۰۴/۳/۱۲

تاریخ دریافت: ۱۴۰۴/۱/۴

چکیده

انتروتوکسمی به صورت یک بیماری رایج و با مرگ و میر بالا در بزها رخ می‌دهد. این مطالعه با هدف بررسی پادتن‌های مادری ضد توکسین اپسیلون کستریدیوم پرفرنجنس تیپ D انجام گرفت تا بتواند برنامه واکسیناسیون مقابله با انتروتوکسمی در بزها در شرایط ایران را بهبود بخشد. برای انجام این مطالعه نمونه خون از بزغاله‌های متولد شده از ۱۲ رأس بز که در ۶ و ۴ هفته قبل زایش با واکسن آنتروتوکسمی واکسینه شده بودند و همچنین نمونه خون از بزغاله‌های متولد شده از ۷ رأس بز غیرواکسینه، در زمان‌های ۰، ۳، ۱۴، ۲۸، ۴۲، ۵۶، ۷۰ و ۸۶ روز پس از تولد، گرفته شد. برای ارزیابی سرولوژیکی از الایزای مهارتی استفاده شد. نتایج مطالعه نشان داد که درصد مهار در مادران واکسینه نسبت به مادران غیرواکسینه به صورت قابل توجهی بالاتر است. در بزغاله‌ها، درصد مهار در هر دو گروه تا سن ۴۲ روزگی تفاوت آماری نداشت. پس از این سن، درصد مهار در گروه کنترل به صورت قابل توجهی کاهش یافت و در سن ۵۶ و ۷۰ روزگی به صورت قابل توجهی پایین‌تر بود. در ادامه، از نظر آماری تفاوتی در درصد مهار در سن ۸۶ روزگی بین دو گروه مشاهده نشد. در گروه غیرواکسینه، درصد مهار در سن ۵۶ روزگی نسبت به درصد مهار در سن ۴۲ روزگی به صورت قابل توجهی پایین‌تر بود. در گروه واکسینه، درصد مهار در سن ۷۰ روزگی نسبت به درصد مهار در سن ۵۶ روزگی به صورت قابل توجهی پایین‌تر بود. در نتیجه، واکسیناسیون بزها در ماه آخر بارداری در افزایش تیتراژ پادتن‌های مادری ضد توکسین اپسیلون کستریدیوم پرفرنجنس تیپ D در بزغاله‌ها مؤثر است. همچنین، سن ۵۶ تا ۷۰ روزگی در بزغاله‌های متولد شده از مادران واکسینه و سن ۴۲ تا ۵۶ روزگی در بزغاله‌های متولد شده از مادران غیرواکسینه به نظر می‌رسد زمان‌های مناسبی برای تجویز اولین دوز واکسن انتروتوکسمی باشد.

کلمات کلیدی: کستریدیوم پرفرنجنس، انتروتوکسمی، توکسین اپسیلون، بزغاله، واکسن

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