Assessment of the duration of maternal-derived antibodies specific to the *Mycoplasma agalactiae* vaccine in goat kids

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Abstract

**Background:** Contagious agalactia (CA) is one of the most important diseases in the small ruminant industry in Iran. The historical aetiology of this disease is *Mycoplasma agalactiae* (Ma). The main way to control this disease, in addition to management measures, is vaccination. In ruminant newborns, determining the age of first vaccination against Ma is a challenge due to the interference between colostrum-derived maternal immunity and vaccination-induced immunity. The aim of this study was to evaluate the consistency of maternal-derived antibodies specific to the Ma in goat kids blood serum born from the vaccinated does.

**Objectives:** Determination of level of antibody against Ma in goat kids born from vaccinated dams against Ma. Assessment of duration of protective level of maternal derived antibody in goat kids serum, after receiving colostrum from vaccinated mother with Ma vaccine. Determination the best time vaccination against Ma in goat kids receiving colostrum from vaccinated dams.

**Methods:** 20 Saanen goat kids were studied in two groups of 10 animals including control (receiving colostrum from unvaccinated does) and treatment (receiving colostrum from vaccinated does). Indirect Elisa was used to evaluate serum specific antibodies to Ma in goat kids (control and treatment groups) from birth to 100 days of age.

**Results:** After receiving a sufficient amount of colostrum, the goat kids in the treatment group had a significantly higher S/P% than the control group until 56 days after birth (p < 0.05) and at 70–100 days after birth, there was no significant difference between the treatment and control groups (p > 0.05).

**Conclusions:** This study showed that 56–70 days of age could be a good age to give the first dose of CA vaccine in goat kids, but more studies are needed on the effectiveness of this vaccine at this age.

**KEYWORDS**
goat kid, immunity, *Mycoplasma agalactiae*, vaccine
Contagious agalactia (CA) was first reported in Italy in 1816 as mal di sito (disease of the place), due to disease ability to persist in one place and cause disease in new herds entering the same place (Lambert, 1987). Due to its contagious nature, the disease was named Contagious agalactia in 1871 by the municipality of the Italian metropolitan area (Brusasco) (Madanat et al., 2001). Clinical signs of the CA include mastitis, arthritis, keratoconjunctivitis and occasionally abortion (Thiaucourt & Boks, 1996; Migliore et al., 2021). The causative agent of the historical aetiology of CA is Mycoplasma agalactiae (Ma), which was first isolated and identified in 1923 (Jey & Trady, 2019). In sheep, Ma is still the most important cause of the disease, but in goats the other three pathogens, which include Mycoplasma mycoides subspecies capri (Mmc) (older name: Mycoplasma mycoides LC), Mycoplasma putrefaciens (Mp) and Mycoplasma capricolum subspecies capricolum (Mcc), can cause CA to a lesser extent (Nicholas et al., 2008; Hajizadeh et al., 2018).

The prevalence of infection to CA causing Mycoplasmas in small ruminants in Iran is estimated 20% (Pirali Kheirabadi & Ebrahimi, 2007), 51% (Ashstari et al., 2015), 36% (Shamsaddini Bafti et al., 2017) and 25% (Hajizadeh et al., 2018). Economic losses caused by CA in small ruminant breeding industry can be categorised in three areas including: high morbidity of the disease, reduced production and treatment costs (Jey & Trady, 2019). The main action to control CA in endemic areas and Iranian sheep and goat herds is regular vaccination with the killed vaccine (Hajizadeh et al., 2018; Constable et al., 2016). Prevention of CA in goat kids, which have been born from vaccinated does, depends on receiving enough maternal immunity through colostrum (Constable et al., 2016). Studies showed feeding 200 ml of colostrum immediately after birth and 210 ml/kg in indoor situation and 280 ml/kg in outdoor raising, during first 24 h after birth would be enough for receiving good passive immunity in goat kids (Matthews, 2016). To date, no studies have been performed on the appropriate age for the first vaccination against CA in goat kids, and the only suggestion is early vaccination of these newborns after the age of 70 days (Constable et al., 2016). There are currently two types of vaccines against Ma in the Iranian market. One is the monovalent vaccine: Agalactia Vac (killed vaccine inactivated by formaldehyde, contains saponin adjuvant, for all age groups of animals, Razi Institute, Alborz, Iran) and the other is the polyvalent vaccine: Agalaksivac (killed vaccine inactivated by phenol, contains Montanid ISA 206 adjuvant, for all age groups of animals, Vetal Company, Gaziantep, Turkey). Studies in Iran have shown that the main cause of CA in goats is Ma, but Mcc, Mp and Mmc are also among the etiological agents of CA in goats (Shamsaddini Bafti et al., 2017; Hajizadeh et al., 2018). Therefore, in the Iranian goat breeding industry, the use of the polyvalent vaccine (Agalaksivac) seems to be a better choice.

Due to some high-risk diseases of small ruminants in Iran such as foot and mouth disease (FMD) (Nikvand et al., 2019), Peste des petits ruminants (PPR) (Bazarghani et al., 2006), sheep pox (Mirzaie et al., 2015), goat pox (Mirzaie et al., 2015), Contagious ecthyma (Davari et al., 2015), Enterotoxemia (Ahmadi Rahnemoon et al., 2020), Pasteurellosis (Valadan et al., 2014), CA (Hajizadeh et al., 2018), there is a heavy traffic of newborn vaccines in the sheep and goat herd health program up to the age of 100 days. A proper order based on scientific findings in this heavy traffic is needed to achieve a better health index in small ruminants herds.

The aim of this study was to evaluate the duration of maternal immunity against Ma in goat kids after receiving colostrum from mother vaccinated with Agalaksivac oil vaccine, from birth to 100 days of age.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was conducted for one year (September 2020 to September 2021) in a farm with 1500 Saanen goats (Ajdad Sepidan Kosar located in Qarchak city, Tehran Province, Iran). There was no history of CA on this farm. Immunization of all animals against CA was performed on the farm by vaccination with Agalaksivac vaccine (Vetal Company, Gaziantep, Turkey) every 6 months. The sample size was determined based on previous studies (Sotoodehnia et al., 2005; Ozdemir et al., 2019; Efif & Negida, 2017).

2.2 | Animals

Twenty seronegative Saanen female goats, aged 10 months, were included in study. Indirect Elisa test (Ma indirect Elisa commercial kit, MAGALS-5SP, ID-Vet, Grabels, French) was used for detection of antibody against Ma in blood serum of chosen animals. Animals were allocated to two groups of 10 heads. Animals in one group received Agalaksivac (killed vaccine, Vetal Company, Gaziantep, Turkey) subcutaneously (treatment group) and in the other group including 10 goats 0.9% sodium chloride was injected as placebo (control group). The vaccine and placebo were injected under the skin of the neck. Singleton pregnancy of the study animals was confirmed by ultrasound (Buckrell, 1988). Grouping was done by simple random method (lottery method) (Efif & Negida, 2017).

2.2.1 | Newborn kids

After birth, kids with appropriate weight range (2.5–3.5 kg) (Ince, 2010) were included in the study and fed with enough colostrum (200 ml immediately after birth and 210 ml/kg in the first 24 h) (Matthews, 2016). Colostrum was prepared from the mother of each newborn kids, without measuring IgG. To determine the absence of failure of passive transfer (FPT) in kids, a blood sample was taken from each one 48 h after birth and serum total IgG concentration was measured using Elisa method (DEIA640, CD Creative Diagnostics, Shirley, USA) (Batmaz et al., 2019). In this study, all chosen does had singleton pregnancy and have given birth to one kid, then 20 newborn kids participated in two groups of 10 heads. Infants born to mothers receiving the vaccine
were in the treatment group and infants born to mothers receiving the placebo were in the control group.

2.3 | Vaccination

Due to the permissibility of using the vaccine in the second half of pregnancy in the vaccine manufacturer’s instructions, the animals received a dose of vaccine or placebo on the 105th day of pregnancy according to the group and this administration was repeated on the 120th day of pregnancy (Constable et al., 2016). For vaccination, the polyvalent Agalaksivac (killed vaccine, Vetal Company, Gaziantep, Turkey) with three strains including Ma, Mcc and Mycoplasma mycoides subsp. mycoides (Mmmm) was used. This vaccine is oily in nature, inactivated by phenol and contains Montanid ISA 206 adjuvant. Each dose (2 ml) of the vaccine contains $1 \times 10^9$ of each of the three organisms. The exact concentration of these microorganisms per millilitre of vaccine is not available.

2.4 | Serum collection

In does, blood samples were taken on the 105th day of pregnancy (45 days before parturition) and continued weekly until parturition (day 150 of pregnancy) (Bodjo et al., 2006). In newborn kids, the first blood sample was taken immediately after birth (15 min after birth and 10 min before eating the first meal of colostrum) and then on days 3, 7, 14, 21, 28, 42, 56, 70 and 100 after birth (Bodjo et al., 2006; Veschi et al., 2015).

2.5 | Entry and exit criteria

Inclusion criteria were considered for mothers (complete clinical health, singleton pregnancy, having a good body condition score [3 on the 5-point scale]) and for newborn kids (complete clinical health, delivery without intervention, appropriate weight) (Vries et al., 2014). Exclusion criteria were considered for mothers (disease incidence) and for infants (failure of passive transfer, disease incidence) (Vries et al., 2014).

2.6 | Serological analysis

Ma indirect Elisa commercial kit (MAGALS-SSP, ID-Vet, Grabels, French) was used to determine the presence of Ma antibody in serum of all studied animals. Goat IgG Sandwich Elisa commercial kit (DEIA640, CD Creative Diagnostics, Shirley, USA) was used to determine the serum total IgG concentration in all studied newborn kids. These tests were performed according to the manufacturer’s instructions, by an expert in the Laboratory of Immunology, Department of Microbiology and immunology, University of Tehran, Iran.

2.7 | Statistical analysis

The normal distribution of data in each group was confirmed using Shapiro–Wilk test. After plotting the S/P%-time model in GraphPad Prism software (Version 7) for S/P% obtained in indirect Elisa, independent T-test in SPSS software (Version 22) was used for statistical analysis of data. Statistically significant differences were declared at a $p$ value of less than 0.05.

3 | RESULTS

No clinical signs of any disease were observed during the study in does and newborn kids. FPT did not occur in any of the neonates studied. In mothers, as shown in Table 1 and Figure 1, on days 45 and 35 before parturition, there was no significant difference in Elisa S/P% between the control and treatment groups ($p > 0.05$). On 28, 21, 14, 7 and 2 days before parturition, the Elisa S/P% were significantly higher in the treatment group as compared with S/P% in the control group ($p < 0.05$).

In newborn kids, as shown in Table 2 and Figure 1, before receiving colostrum, the Elisa S/P% were not significantly different between the control and treatment groups ($p > 0.05$). On days 3, 7, 14, 21, 28, 42 and 56 after birth, the Elisa S/P% in kids in the treatment group were significantly higher than kids in the control group ($p < 0.05$). At 70 and 100 days after birth, there was no significant difference in Elisa S/P% between the kids in the control and treatment groups ($p > 0.05$).

4 | DISCUSSION

CA is one of the most important diseases in the small ruminant breeding industry in Iran (Pirali Kheirabadi & Ebrahimi, 2007; Ashtari et al., 2015). During a study by Pirali Kheirabadi and Ebrahimi (2007), 101 healthy sheep were studied in the west of Iran. The samples included milk and conjunctival swab. PCR test was used. Twenty per cent of the studied animals were positive for Ma infection. During this study, it was found that in the west of Iran, 77% of sheep herds have at least one ewes infected by Ma (Pirali Kheirabadi & Ebrahimi, 2007). In a study by Ashtari et al. (2015), 91 sheep (suspected CA) were studied in Khuzestan province of Iran. The samples included milk, conjunctival swab, ear swab and joint fluid. PCR test and Mycoplasma culture were used. In Mycoplasma culture method, 37% of the samples were positive. In PCR test, 51.65% of the samples were positive for Mycoplasma, of which 8.79% were related to Ma. During this study, it was found that the conjunctival swab is the best sample for identification of Mycoplasma and other etiological agents are involved in the occurrence of CA, in Khuzestan province of Iran (Ashtari et al., 2015). In a study by Shamssaddini Bafti et al. (2017), 132 sheep and goats (suspected CA) were studied in the southeast of Iran. The samples included milk, conjunctival swab, ear swab and joint fluid. PCR test and Mycoplasma culture were used. In Mycoplasma culture method, 14.8% of the samples were positive. In PCR test, 36% of the samples were positive for Mycoplasma.
### Table 1

| Day (before parturition) | Control group | Treatment group | p Value | Interpretation |
|--------------------------|---------------|-----------------|---------|----------------|
| -45                      | 5.4 ± 1.9     | 4.4 ± 1.4       | =0.191  | µ<sub>C</sub> = µ<sub>T</sub> |
| -35                      | 6.1 ± 1.2     | 6.6 ± 4.5       | =0.738  | µ<sub>C</sub> = µ<sub>T</sub> |
| -28                      | 3.9 ± 0.8     | 8.7 ± 2.5       | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| -21                      | 7.3 ± 2.0     | 34.6 ± 2.8      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| -14                      | 4.8 ± 0.9     | 40.8 ± 7        | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| -7                       | 5.3 ± 1.6     | 58.3 ± 8.5      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| -2                       | 6.6 ± 0.6     | 63.9 ± 3.3      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |

µ: mean, C: control group, T: treatment group.

### Figure 1

S/P%–time diagram in mothers and newborn kids participating in the study

### Table 2

| Day (after birth) | Control group | Treatment group | p Value | Interpretation |
|-------------------|---------------|-----------------|---------|----------------|
| 0                 | 4.4 ± 1.3     | 4.2 ± 0.9       | =0.694  | µ<sub>C</sub> = µ<sub>T</sub> |
| 3                 | 4.5 ± 0.5     | 63.4 ± 3.0      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 7                 | 5.7 ± 2.0     | 44.5 ± 6.8      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 14                | 5.5 ± 2.1     | 35.9 ± 4.2      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 21                | 4.8 ± 1.4     | 32.1 ± 6.4      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 28                | 4.6 ± 0.4     | 18.0 ± 1.1      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 42                | 4.0 ± 1.3     | 12.2 ± 4.1      | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 56                | 3.8 ± 0.8     | 8.9 ± 1.2       | ≤0.001  | µ<sub>C</sub> < µ<sub>T</sub> |
| 70                | 3.0 ± 0.7     | 2.5 ± 1.5       | =0.352  | µ<sub>C</sub> = µ<sub>T</sub> |
| 100               | 2.9 ± 0.6     | 2.8 ± 0.6       | =0.714  | µ<sub>C</sub> = µ<sub>T</sub> |

µ: mean, C: control group, T: treatment group.
of which 6.1% were related to Ma. During this study, it was found that in the southeast of Iran, other etiological agents are involved in the occurrence of CA, which are unknown (Shamsaddini Bafti et al., 2017). In a study by Hajizadeh et al. (2018), 132 sheep and goats (suspected CA) were studied in northwest Iran. The samples included milk, conjunctival swab and joint fluid. PCR test was used. Twenty-five per cent of the studied animals were positive for Mycoplasma infection. Among infected animals, 54% had infection with more than one Mycoplasma species. Among the infected animals, 47.2% had Ma infection, 43.4% had Mp infection, 7.5% had Mcc infection and 1.9% had Mmc infection. During this study, for the first time in Iran, Mp and Mmc were isolated from small ruminant flocks (Hajizadeh et al., 2018).

According to the instructions of the used Elisa kit in this study, S/P% ≥ 60% is equal to the natural infection with Ma. Agalaksivac vaccine caused the S/P% ≥ 60% in all vaccinated female goats (63.9 ± 3.2 days two days parturition) as well as goat kids (63.4 ± 3.0 three days after birth) that were fed enough colostrum of vaccinated does (200 ml immediately after birth and 210 ml/kg in the first 24 h) (Figure 1). On day 70 after birth, there was no difference in maternal immunity levels against Ma among goat kids born to vaccinated and non-vaccinated mothers. According to the results, the persistence of maternal immunity against Ma ends at the age of 56–72 days after birth.

During the first one-third of pregnancy, primary lymphoid tissue (bone marrow and thymus) forms in the fetus, and B and T cells become apparent in the foetal blood. During the middle and final one-third of pregnancy, secondary lymphoid tissues development occurs, such as MALT (mucosa-associated lymphoid tissue) (Marchant & Kollmann, 2015). At this time, the fetus is able to produce an immune response to a range of pathogens. The immune system in animals develops significantly during the first months of life and continues until the animal matures (Dowling & Levy, 2014). Although not determined in goat kids, in calves, the immune system matures at 6 months of age (Chase et al., 2008).

In ruminants, the placenta is syndesmochorial. It is not possible to transmit antibodies throughout the blood from mother to infant, and the maternal immunity is transmitted to infants only through colostrum feeding (Hernandez-Castellano et al., 2015). One of the important principles in the livestock breeding industry is the reception of colostrum by the newborn at a limited time after birth (up to 24 h after birth) (Matthews, 2016). Although maternal immunity is important for the survival of the infants, this immunity is like a double-edged sword because it inhibits the humoral endogenous immune response (such as the vaccine response) until it is sufficiently degraded (Day & Schultz, 2014).

Due to the delayed maturation of the immune system in newborns, vaccination at an early age may be less effective than in adult animals (Bodjo et al., 2006). In this study, we found that suitable time for vaccination against Ma in goat kids which received enough amount of colostrum from vaccinated does is 56–70 days after birth. Vaccines are an important tool for the prevention of CA (Constable et al., 2016). To date, several vaccines have been developed for the CA, and there is still no single vaccine for the disease universally (Nicholas et al., 2008).

To date, few attempts have been made to develop a preventive vaccination for infection with Mmc and Mp (Nicholas et al., 2008). Although the disease caused by these pathogens can be severe, their prevalence is relatively low (Bajmocy et al., 2000). Studies have been performed to identify the immunogenic proteins of Mycoplasmas that cause CA. In addition, advances in epitope mapping have facilitated the identification of immunogenic proteins that can be used to make subunit vaccines (Nicholas et al., 2008). A strongly immunogenic surface lipoprotein (AvgC) has been identified in Ma that is exposed to the surface and stimulates the production of specific antibodies in sheep serum (Santona et al., 2002). In Mycoplasma capricolum subsp. capripneumoniae (Mccp), 20 immunogenic proteins have been identified in the whole cell, 9 of which are located in the cell membrane. Membrane proteins appear to be primarily immunogenic proteins for Mccp (Zhao et al., 2012). In Mmc, 20 immunogenic proteins have been identified in the whole cell (Churchward et al., 2015). These proteins may have good potential for making subunit vaccines.

5 | CONCLUSION

Based on the results obtained and discussion, it can be said, if enough colostrum (21% of body weight) is fed to goat kids at the appropriate time (the first 24 h of life), vaccination of goat kids against Ma at the age of 56–70 days after birth can be a good option in goat herds. During the search of authors in scientific references, no other studies were found on the stability of maternal immunity against Mycoplasmas in goat kids, and it seems that this is the first study on the stability of maternal immunity against Ma in goat kids and determination of right time for vaccination against Ma in goat kids.

ACKNOWLEDGEMENT

Ajdad Sepidan Kosar Company is appreciated for its cooperation in this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Mostafa Abdollahi: Conception, performing the experiment, analysing the data, writing the article. Samad Lotfollahzadeh: Conception and design, collecting the data, writing and revising the article. Taghi Zahraei Salehi: Conception. Farhad Moosakhan: Conceived the experiment, assistance in laboratory work. Afshin Raoofi: Study advice.

FUNDING

There is no source of fund to declare.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.
ETHICAL CONSIDERATION
Before starting the study, the proposal was sent to the research committee of Ajjad Sepidan Kosar Company and the code of ethics in the research was taken (ethics code: 1400/471).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available within the manuscript and also are available from the corresponding author upon reasonable request.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.888

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How to cite this article: Abdollahi, M., Lotfollahzadeh, S., Salehi, T. Z., Moosakhani, F., & Raoofi, A. (2022). Assessment of the duration of maternal-derived antibodies specific to the *Mycoplasma agalactiae* vaccine in goat kids. *Veterinary Medicine and Science, 8*, 2119–2125. https://doi.org/10.1002/vms3.888