Dicle Üniversitesi Veteriner Fakültesi Dergisi
http://www.dicle.edu.tr/veteriner-fakultesi-dergisi

Araştırma Makalesi

ISSN:1307-9972 e-ISSN:1308-0679

Investigation on the effectiveness of mastitis vaccine (Startvac®) in sheep and goat gangrenous mastitis cases caused by *Staphylococcus aureus*

Simten YEŞİLMEN[1], Nurettin IŞIK[2], Nihat ÖZYURTLU[3], İbrahim KÜÇÜKASLAN[3*], Zafer CANTEKİN[4], Yaşar ERGÜN[5]

[1]Dicle University, Faculty of Veterinary Medicine, Department of Microbiology, 21280 Diyarbakır, Turkey,
[2]Diyarbakır Food Control Laboratory Directorate, 21100 Diyarbakır, Turkey,
[3]Dicle University, Faculty of Veterinary Medicine Department of Obstetrics and Gynaecology, 21280 Diyarbakır, Turkey,
[4]Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Microbiology, Tayfur Sokmen Campus 31000 Hatay, Turkey,
[5]Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Tayfur Sokmen Campus 31000, Hatay, Turkey

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
31.10.2017	06.12.2017	25.12.2017

Abstract

Mastitis is the inflammatory reaction of the udder to bacterial, chemical, thermal and mechanical effects. It causes significant production losses. The purpose of this study is to determine the effectiveness of Startvac® (Hipra), vaccine in sheep and goats with gangrenous mastitis caused by *Staphylococcus aureus*. The research sample consisted of eight sheep and four goats. The sheep and goats were divided into two groups. The first group was administered classical treatment procedure (parenteral and intramammary antibiotics with nonsteroidal anti-inflammatory drug). In the second group, 1 ml Startvac® vaccine was used with classical treatment procedure. Patients returned to normal in the first group after 48 hours, although lactation ceased on day six and hardness was detected in the udder tissue. On day 10 of treatment, the clinical findings of the animals returned to normal, although the milk yield was much lower when compared to the other treatment group. The clinical appearance of in Group 2 returned to normal after 48 hours, and the animals recovered on Day 6 of treatment. On Day 10, the clinical symptoms disappeared and milk yield increased significantly compared to Group 1. It was concluded that Startvac® mastitis vaccine could increase treatment success in gangrenous mastitis cases in sheep and goats when administered in addition to the classical clinical treatment.

Keywords: Gangrenous mastitis, mastitis vaccine, small ruminant

Koyun ve keçilerde *Staphylococcus aureus* kaynaklı gangrenli mastitis olgularında mastitis aşısının (Startvac®) etkinliğinin araştırılması

Öz

Mastitis; memenin bakteriyel, kimyasal, termal ve mekanik etkilere verdiği yangısal reaksiyondur. Önemli üretim kaybına neden olur. Bu çalışmanın amacı; *Staphylococcus aureus* kaynaklı kangrenli mastitisli koyun ve keçilerde Startvac® (Hipra) aşısının etkinliğini belirlemektir. Araştırma materyalini sekiz koyun ve dört keçi oluşturmaktadır. Koyun ve keçiler iki gruba ayrıldı. İlk gruba klasik tedavi prosedürü (parenteral ve meme içi antibiyotik tedavisi ile birlikte nonsteroidal antienflamatuar ilaç), ikinci grupta klasik tedavi ile birlikte 1 ml Startvac® aşısı uygulandı. İlk grupta her ne kadar 6. günde laktasyon durmuş ve meme lobunda sertlik oluşmuşsa da hastalar 48 saat sonrasında normale döndü. Tedavinin 10. gününde süt verimi diğer tedavi grubuna göre daha az olmasının yanı sıra hayvanların klinik bulguları normale döndü. Grup 2 de klinik görünüm 48. saatte normale döndü ve tedavinin 6. gününde iyileştiler. Onuncu günde klinik semptomlar yok oldu ve süt verimi Grup 1'e göre önemli düzeyde arttı. Koyun ve keçilerde kangrenli mastitis olgularında klasik tedaviye ek olarak uygulanan Startvac® mastitis aşısının tedavi başarısını arttırabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Gangrenöz mastitis, mastitis aşısı, küçük ruminant

Introduction

Mastitis is the inflammatory reaction of the udder to bacterial, chemical, thermal and mechanical effects. It causes significant production losses (1, 2, 3, 4). Gangrenous mastitis in sheep is observed predominantly in the first five weeks of lactation and is associated with such symptoms as an elevated body temperature (40.5-42ºC), loss of appetite and depression. While the udder is hot and sore in the early periods of infection, it hardens, swells and reddens as the infection progresses. Within 24–48 hours following infection, the udder becomes cyanotic and cold, and a high mortality rate (1, 5, 3). Many infectious agents lead to gangrenous mastitis in sheep, although the most common is *Staphylococcus aureus* (1, 6).

Treatment of the disease, which is very important and challenging in sheep breeding, due to the fact that it is a peracute disease. Many vaccination trials have been performed in the past (7). Commercial mastitis vaccines have been used in the dairy cattle sector for many years, and have been reported as contributing significantly to reducing the severity of infection in cases of clinical staphylococcal mastitis (8). A vaccine named Startvac® (Hipra), which came to the market in recent years and is being used in cattle, contains inactive *Escherichia coli* and *S. aureus*, and prevents the formation of slime factor of Staphylococcus and the formation of capsules by coliform bacteria (9).

The purpose of this study is to determine the effectiveness of Startvac® (Hipra), which is a commercial vaccine used in cases of gangrenous mastitis that are associated with a high mortality rate, in sheep and goats in Diyarbakır when used in addition to the classical treatment in cases of gangrenous mastitis caused by *S. aureus*.

Material and Methods

Samples

The research sample consisted of eight sheep and four goats from different herds in the province of Diyarbakır and its surrounding villages, which were in the first six weeks of lactation and which were brought to the clinic with symptoms of gangrenous mastitis (swelling, redness, hardening, hemorrhagic exudate, change of color, loss of appetite and depression) (Figure 1).

Bacterial isolation and antibiogram

For the bacterial isolation from the milk, obtained from the sheep under aseptic conditions, the samples were inoculated into 5% sheep blood agar and were left to incubate at 37ºC for 24–48 hours. The *S. aureus* suspected colonies that were isolated at the end of the isolation process were subjected to catalase and coagulase tests following Gram staining. Isolated *Staphylococcus* spp. strains were identified using Vitec GP cards with a Vitec-2 (Biomériux) device (10), while the nucleic acid isolation of the isolates was carried out using the Phenol/Chloroform/Isoamyl alcohol method (11). The genetic identification of the *S. aureus* isolates was confirmed with PCR analyses using specific primers (Table 1).

The antibiotic sensitivities of isolated and identified bacteria were determined using the Disk Diffusion technique (13), *S. aureus* suspensions, which were produced during one night at 37^0C using Nutrient Bouillon and prepared according to the 0.5 McFarland turbidity standard. These were then spread onto Mueller-Hinton Agar in an amount of 0.1 ml, after which 30µg Amoxycillin+Clavulanic Acid, 5µg Kanamycin, 15µg Eritromycin, 5µg Enrofloxacin, 30µg Oxytetracyclin, 30µg

Florfenicol, 75µg Cefoperazone and 5 µg Cloxacillin antibiotic disks were placed over them and were incubated for 24 hours at 37^0C. The sensitivity/resistance of the bacteria to these antibiotics were assessed by measuring the diameters of the zones formed around the disks. The sensitivity patterns of isolates to different antibiotic discs were read by measuring the diameter of zone of inhibition in millimeter as per the chart provided by manufacturer and classified as Sensitive, Intermediate and Resistant based on CLSI guidelines (14).

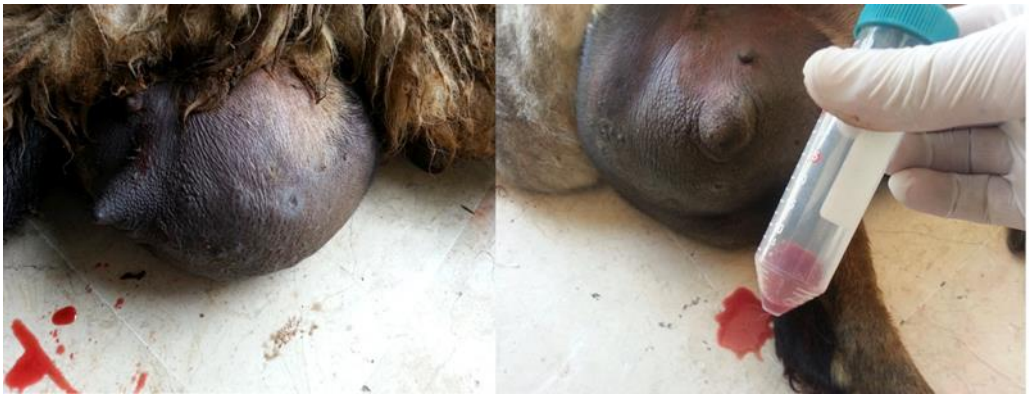

Figure 1. Clinical appearance of gangrenous mastitis.

Table 1. *S. aureus* specific primers and characteristics.

Primer Names	Sequences of Primers	Length of amplicons	Reference of Primers
Sau 327	5'-GGA CGA CAT TAG ACG AAT CA-3'	1318 bp	Riffon *et al.* (12)
Sau 1645	5'-CGG GCA CCT ATT TTC TAT CT-3'		

Table 2. Origin of samples, types of animals

Sampling Locations	Type of animal
Diyarbakır/Kulp/Narlıca	2 goats
Diyarbakır /Lice/Tepe	2 goats
Diyarbakır /Çınar/Mollapolat	2 sheep
Diyarbakır /Ergani/ Alitaş	2 sheep
Diyarbakır / Merkez/Ovabağ	1 sheep
Diyarbakır/Merkez/Beneklitaş	1 sheep
Diyarbakır/Merkez/Mermer	2 sheep

Groups and Treatments

The sheep and goats were divided into two groups for the different treatment methods. The first group, which consisted of three sheep and one goat, was administered parenteral antibiotics (Cephalexin monohydrate, kanamycin) with intramammary antibiotics (Cephalexin+Kanamycin) and a parenteral nonsteroidal anti-inflammatory drug (Flunixin meglumine), while the second group, which consisted of 5 sheep and 2 goats, was administered 1 ml Startvac® vaccine in addition to parenteral (Cephalexin monohydrate, kanamycin) and intramammary antibiotics (Cephalexin+Kanamycin) and a parenteral nonsteroidal anti-inflammatory drug (Flunixin meglumine). Bacteriological sampling was not repeated after treatment, as the animals had been administered antibiotics, however the clinical findings and increases in milk yield of the udder lobes with mastitis were taken into account as signs of recovery criteria.

Results

S. aureus was isolated and identified in all samples, and the origins of the isolates which were identified are shown in Table 2. Antibiogram results of isolated strains ware shown in Table 3.

One goat in the first group died three hours following treatment, while the body temperature of the other goat returned to normal after 48 hours, although lactation ceased on day six and hardness was detected in the udder tissue. The body temperatures of 3 sheep in the first group returned to normal 48 hours after treatment and color of the milk was lighter in comparison to Day 1. On Day 6 of the treatment, there was a significant reduction in milk yield, despite the clinical findings of all 3 sheep having returned to normal. On day 10 of treatment, the clinical findings of the sheep returned to normal and the color of the milk had improved, although the milk yield was much lower in comparison to the other treatment group (Table 4).

The clinical appearance of 2 goats and 5 sheep in Group 2 returned to normal after 48 hours, and the animals recovered and clinically returned to normal on Day 6 of treatment. The milk color became lighter and udder hardness decreased significantly on Day 6 when compared to Group 1. On Day 10 of treatment, the clinical symptoms disappeared and milk yield increased when compared to Group 1 (Table 5).

Table 3. Sensitivity of strains to different antibiotics

Antibiotic Disks	Sensitive n, (%)	Intermediate n, (%)	Resistant n, (%)
Amoxycillin+Clavulanic Acid (30µg)	12, (100)	-	-
Kanamycin (5µg)	12, (100)	-	-
Eritromycin (15µg)	7, (58.33)	5, (41.67)	
Enrofloxacin (5µg)	6, (50)	5, (41.67)	1, (8.33)
Oxytetracyclin (30µg)	4, (33.33)	5, (41.67)	3, (25)
Florfenicol (30µg)	12, (100)	-	-
Cefoperazone (75µg)	12, (100)	-	-
Cloxacillin (5 µg)	3, (25)	4, (33.33)	5, (41.67)

Table 4. Clinical scores of Group 1

Animals	Day 0		48 h		96 h	UTHS	Day 10
	BT (°C)	MY (ml)	BT (°C)	MY (ml)	MY (ml)	+, ++, +++	MY (ml)
Goat 1.1	39.2	Hemorrhagic exudate	38.2	40	70	+	x
Goat 1.2	38.0	Hemorrhagic exudate	x	x	x	x	x
Sheep 1.3	38.7	Hemorrhagic exudate	38.3	70	80	-	120
Sheep 1.4	38.8	Hemorrhagic exudate	38.2	60	90	+	110
Sheep 1.5	39.1	Hemorrhagic exudate	38.3	80	100	+	130

BT: Body temperature, **MY:** Milk yield, **UTHS:** Udder Tissue Hardness Score

Table 5. Clinical scores of Group 2

Animals	Day 0		48 h		96 h	UTHS	Day 10
	BT (°C)	MY (ml)	BT (°C)	MY (ml)	MY (ml)	+, ++, +++	MY (ml)
Sheep 2.1	38.9	Hemorrhagic exudate	38.3	80	130	-	240
Sheep 2.2	39.3	Hemorrhagic exudate	38.2	40	80	-	150
Sheep 2.3	39.1	Hemorrhagic exudate	38.2	100	160	-	240
Sheep 2.4	38.7	Hemorrhagic exudate	38.2	70	120	-	270
Sheep 2.5	39.0	Hemorrhagic exudate	38.3	85	140	-	200
Goat 2.6	38.7	Hemorrhagic exudate	38.3	50	90	-	190
Goat 2.7	38.8	Hemorrhagic exudate	38.0	30	60	-	210

BT: Body temperature, **MY:** Milk yield, **UTHS:** Udder Tissue Hardness Score

Discussion

It has been reported in several studies that the main agent causing gangrenous mastitis is S. aureus (1, 3). In the present study, all isolated bacteria were found to be S. aureus using conventional techniques, with their identification confirmed using PCR.

In this study, the milk composition of sheep and goats that were brought to the clinic were in the form of hemorrhagic exudate at the moment of sampling, which is in line with gangrenous mastitis symptoms (3). Taking into consideration the post treatment continuation of lactation and change in milk yields, only one goat in Group 1, which received classical treatment, ceased lactating, however no death or cease in lactation occurred in Group 2, where a vaccine was administered as well as the classical treatment. This suggests that the administration of a vaccine may reduce the severity of infection and tissue damage. Furthermore, the increase in milk yield was higher in Group 2, which is in line with the findings of Watson et al. (7), who suggested that vaccine-administered sheep produced higher milk yields following administration. With udder tissue scoring, 75% of the animals in Group 1 still had hardness in the udder tissue after 96 hours, although with in this time frame the hardness had disappeared in all off the Group 2 animals. This was significant due to the fact that vaccine administration at the beginning of the treatment increased the treatment success. This positive development in clinical recovery and the increase in milk yields suggest that the administration of the vaccine contributed to the suppression and elimination of infections in cases of mastitis in small ruminants (7).

Startvac® is a polyvalent, subunit vaccine that provides protection against Staphylococcus and coliform bacteria in cases of mastitis in cattle. It prevents biofilm formation in Staphylococcus, while ensuring opsonization of bacteria before the formation of capsules, thus contributing to their elimination (9). It has also been reported that the subunit vaccines that are used in the study can prevent the colonization of bacteria, and may increase the success rate against homologous and heterologous bacteria by activating the immune system within 1–2 days. It has further been reported that such practices accelerate the elimination of bacteria by stimulating and increasing non-specific immune response (15). Exposing the organism to a specific antigen in very low or very high doses could lead to a situation where no immune response against this antigen occurs (immunological paralysis) (16). In the present study, immunological paralysis may have occurred as a result of high doses of S. aureus and its toxins. It is believed that the vaccine that was administered in addition to the classical treatment in Group 2 stimulated the paralyzed immune system and initiated an immune response due to the fact that the vaccine was inactive and contained a lower dose of S. aureus.

In the antibiotic sensitivity tests, the 12 isolates used in the study were found to be most sensitive to Amoxycillin+Clavulanic Acid (100%), Kanamycin (100%), Florfenicol (100%) and Cefoperazone (100%), while the least sensitivity was observed against Cloxacillin (25%), Oxytetracyclin (33.33%), Enrofloxacin (50%) and Eritromycin (58.33%)

França et al. (17) found that bacterial isolates from cases of mastitis in small ruminants in Brazil were sensitive to amoxicillin (50.0%), streptomycin (42.8%), tetracycline (40.4%), lincomycin (39.0%) and erythromycin (33.8%); while Kunz et al. (18) reported that 67 S. aureus and 208 CNS strains, from cases of mastitis in small ruminants, were resistant to penicillin (31.3%), ampicillin (29.9%),

erythromycin (1.5%) and tetracycline (3.0%). Virdis et al. (19) reported that 14 of 25 S. aureus isolates (56.0%) were resistant to one or more antibiotics, and the highest level of resistance was observed against kanamycin (28.0%), oxytetracycline (16.0%) and ampicillin (12.0%). Tel et al. (20) reported that resistance to penicillin G, ampicillin and erythromycin were 27.2%, 25.4% and 6.3% respectively in S. aureus strains isolated in Sanliurfa Turkey (n = 110).

In conclusion, it was found that the administration of a single dose of mastitis vaccine at the beginning of treatment contributed to the success of the clinical recovery in routine clinical treatment practices in cases of gangrenous mastitis in sheep and goats, and the milk yields of vaccinated animals increased to normal levels after treatment. It can thus be concluded that mastitis vaccine could improve treatment success when administered in addition to the classical clinical treatment.

References

1. Alaçam E. (1990). Mastitis. in: Koyun-Keçi hastalıkları ve Yetiştiriciliği. Tüm Vet. Hayvancılık Hizmetleri Yayını. Teknografik Matbaası, İstanbul, pp.375-388.

2. Baştan A. (2013). İneklerde Meme Sağlığı ve Sorunları. 2. Baskı, Kardelen ofset ve matbaacılık, Ankara.

3. Pir Yağcı İ. (2005). Koyunlarda Mastitisin Etiyopatogenezi ve Tanısı. Veteriner Hekimler Derneği Dergisi, 76(3-4),30-34.

4. Tel OY, Aslantaş Ö, Keskin O et al. (2011). Investigation of Toxin Genes of Staphylococcus Aureus Strains Isolated from Gangrenous Mastitis in Ewes. Kafkas Univ Vet Fak Derg. 17(5):869-871.

5. Büyükpamukçu M. (1980). Veteriner Patoloji. 3. Cilt, Ankara Üniversitesi Basımevi, Ankara, pp.174-191.

6. Belschner AP, Hallberg JW, Nickerson SC et al. (1996). Staphylococcus aureus mastitis therapy revisited. Proceedings, National Mastitis Council Annual Meeting, Madison, Wisconsin, 116-122.

7. Watson DL. (1988). Vaccination against experimental staphylococcal mastitis in ewes. Res Vet Sci. 45(1):16-21.

8. Pereira UP, Oliveira DGS, Mesquita LR et al. (2011). Efficacy of Staphylococcus aureus vaccines for bovine mastitis: A systematic review. Vet Microbiol. 148(2–4): 117-124.

9. Anonymous. (2014). What is Starvac®? Access Url: www.startvac.com Date of access: 8.12.2014.

10. Quinn PJ, Carter ME, Markey BK et al. (1994). Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, Lynton House, London, 209-236.

11. Sambrook J, Russell W (2002). Molecular cloning: a laboratory manual (3rd ed.), Cold Spring Harbor Press, New York, NY.

12. Riffon RS. (2001). Development of a Rapid and Sensitive Test for identification of major pathogens in bovine mastitis by PCR. J Clin Microbiol. 39(7): 2584–2589.

13. Bauer AU, Kirby WM, Sherris JC et al. (1966). Antibiotic susceptibility testing by a standardized single disc method. J Clin Pathol. 45:493-494.

14. CLSI. (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute.

15. Foster N, Berndt A, Lalmanach AC et al. (2012). Emergency and therapeutic vaccination is

stimulating innate immunity an option? Res Vet Sci. 93(1):7–12.

16. Diker KS. (1998). İmmunoloji. Medisan Yayınevi Ltd. Şti. 1. Baskı, Ankara.

17. França CA, Peixoto RM, Cavalcante MB et al. (2012). Antimicrobial resistance of Staphylococcus spp. from small ruminant mastitis in Brazil. Chirles Pesq Vet Bras. 32(8):747-753.

18. Kunz F, Corti S, Giezendanner N et al. (2011). Antimicrobial resistance of Staphylococcus aureus and coagulase negative staphylococci isolated from mastitis milk samples from sheep and goats. Schweiz Arch Tierheilkd. 153(2):63-69.

19. Virdis S, Scarano C, Cossu F et al. (2010). Antibiotic resistance in staphylococcus aureus and coagulase negative staphylococci İsolated from goats with subclinical mastitis. Vet Med Int. Article ID 517060, 6 pages doi:10.4061/2010/517060.

20. Tel OY, Aslantaş O, Keskin O et al. (2012). Investigation of the antibiotic resistance and biofilm formation of Staphylococcus aureus strains isolated from gangrenous mastitis of ewes. Acta Vet Hung. 60(2):189-197.

Corresponding Author:

*Assoc. Prof. Dr. İbrahim Küçükaslan
Department of Obstetrics and Gynecology Faculty of Veterinary Medicine, Dicle University, 21280, Diyarbakır, TURKEY,
E-mail:i.kucukaslan@gmail.com