STAPHYLOCOCCUS AUREUS ISOLATED FROM INTRAMAMMARY INFECTION IN IMMUNOLOGICALLY PROTECTED GOATS

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Abstract
In a goats farm with 86 goats immunologically protected by vaccination against gangrenous mastitis, evolved clinical and subclinical intramammary infections. 30 milk samples from goats with clinical mastitis evolving and 16 milk samples from apparently healthy goats were collected. Following bacteriological exams 36 Staphylococcus aureus strains were isolated and identified using standard protocols, 25 (69.44%) being isolated in pure culture and 11 (30,55%) in association with other bacteria: Escherichia coli, Pasteurella haemolytica, Klebsiella oxytoca. Bacterial strains were morphologically identified using Gram staining and biochemical tests. The antibiotic susceptibility was tested for 15 strains of Staphylococcus aureus on 10 antibiotics and showed similar patterns.
Keywords: *goat, mastitis, Staphylococcus aureus*

Introduction

Staphylococcus spp. are the most frequently diagnosed causal microorganisms of intra mammary infection (IMI) in goats. (Perianu T., 2004; Moga Manzat R., 2005) The number of species of the genus *Staphylococcus* is steadily increasing. Some species of this genus cause a variety of diseases by production of a series of enzymes and toxins, invasion of host cells and tissues (Gots F.Bannermann, Schleifer K.H., 2006; Azizollah Ebrahimi, 2010; Euzeby, J.P., 2011). *Staphylococcus aureus* is the etiological agent of gangrenous mastitis, a disease with high mortality rates. However, there is a low prevalence in caprine herds and its transmission between does is infrequent.

Gangrenous staphylococcal mastitis is characterized by severe general disorders and quantitative and qualitative changes of breast milk that is produced after breast necrosis. Infections occur in lactating goats and are influenced by various factors: race, age, period of lactation,lambs weaning, hygiene, etc. (Perianu T., 2004) The main sources of infection are sick animals that may contaminate food, bedding,milking items, hand of milkers. (Moga Manzat R, 2005) Infection is made through papillary hole or breast skin accidental wounds (Perianu T., 2004, Moga Manzat R., 2005 Velescu E. et al, 2009; Tanase O., 2003).

Gangrenous mastitis in Romania was reported in all regions (Perianu T., 2011). It creates important economic losses due to decreased milk production, slaughter females with compromised udder, destruction of large quantities of contaminated milk and high costs for therapy and prophylaxis. (Perianu T., 2011; Vasiu C., 2007). Gangrenous mastitis causes high economic damage, morbidity may reach 15-20% and mortality may be 70- 80%. Females passing through illness lose lactation (Unnerstad, H. E., 2009). However, Some authors affirm that there is a low prevalence in caprine herds and its transmission between is infrequent (Contreras A, 2003; Bravo G.A.C. et al.2011).

The public health significance of *Staphylococcus aureus* isolated from milk and dairy products is important because these products can be a sorse of toxins and antibiotic-resistans strains for humans (Gundogan N.; 2006, Hennekinne J. A.,2011; Mirzaie, H., 2012; Marwa H., 2014).

Materials and methods

Intra-mammary infection evolved in 2015 from a flock of 86 goats immunologically protected by vaccination against gangrenous mastitis. According to the anamnesis, vaccination was carried out in December of 2014 and mastitis episode was triggered in March 2015. Screening for subclinical cases was performed immediately before the collection of milk samples for the microbiological diagnosis of mastitis by the California Mastitis Test (CMT). Milk samples were collected from 45 goats: 30 milk samples from goats with clinical mastitis evolving and 15 milk samples from apparently healthy goats were collected. For microbiological examination of milk samples were collected in sterile containers numbered and identified properly.

Laboratory investigations were conducted in the Laboratory of Microbiology of the Faculty of Veterinary Medicine in Iasi.

For isolation and identification of staphylococcus were used the usual media culture for aerobic bacteria (agar nutrient, broth nutrient), plain or supplemented with 10% sheep blood. As special media there were used: Chapmann medium (Oxoid) to test the ability to ferment mannitol wich is selective because the presence of a high salt concentration (7,5%) wich suppresses the growth of most bacterias and Baird Parker agar medium (Oxoid) wich is recommended for the isolation of *Staphylococcus aureus* with supplementation of acriflavine (Sigma) 7 g.mL-1. Supplementation with acriflavine aims selectivity of different types of media for *Staphylococcus aureus* (Devriese L.A., 1981, Roberson et al. 1992, Davis A. et al., 2006). Acriflavina supplemented Bair Parker agar can potentially reduce the time and labour for identifying *Staphylococcus aureus*.The ability to produce coagulase does not influence acriflavine resistance in *Staphylococcus aureus*. Acriflavine is known to inhibit the growth of coagulase-negative staphylococci as well as some coagulase-positive species of staphylococci, *Staphylococcus intermedius* and *Staphylococcus hyicus*, which have been shown to be sensitive to acriflavine (Roberson et al. 1992, Davis A. et al., 2006). All the samples were incubated at 37 C for 24 h.

To determine pathogenicity *in vitro*, haemolysis test has been carried (as betahemolytic *Staphylococcus aureus*) and citrate plasma coagulation test to differentiate coagulase positive staphylococci (*Staphylococcus aureus, Staphylococcus intermedius*) of the coagulase negative (*Staphylococcus epidermidis*) and streptococci. *API Staph identification systems* (BioMerieux, France), was used for confirming the biochemical (Carp-Carare C. et al., 2015).

It was tested the sensibility of 15 *Staphylococcus* strains to 10 antibiotics: penicillin (30 μg), streptomycin (10 μg), erythromycin (30 μg), amoxicillin/ clavulanic acid (30 μg), ampicillin/cloxacillin (30 μg), neomycin (30 μg), lincomycin (15 μg), oxytetracycline (30 μg), cefoperazone(30 μg), cephalexin(30 μg), using the disk diffusion method on Mueller Hinton agar (Oxoid). Interpretation of the inhibiton zone diameters were done instructions of the Clinical and Laboratory Standards Institute (CLSI,2015).

Results and discussions

Intramammary infections have evolved from minor organoleptic changes of milk (subclinical mastitis) to visible changes of the udder and milk (serous aspect, reddish and contains coarse fibrin) (fig.1). During clinical progression, was installed fever and gangrene of the mammary parenchyma, as a consequence of the hemolysins action (prolonged local vasoconstriction) and thrombosis of blood vessels and the lymphatic system. It usually appears in only one half of the mammary gland (Perianu T., 2011) at the level of which, acting in virulence and toxicity staphylococci. (Perianu T., 2011; Moga Manzat R., 2005).

General condition of the animals was aggravated over time, regional lymph nodes have increased in volume and mamelar parenchyma and the affected nipple have lost sensitivity and natural pigmentation. In time, the lesions have became characteristic for gangrenous staphylococcal mastitis (Perianu T., 2011) (fig. 2).

Fig. 1 Gangrenous mastitis – modified mammary secretion

Fig. 2 Nipple necrosis and partial necrosis of the mammary parenchymal

After 2-5 days, at some animals (15-20%) it was observed that the necrotic tissue separated from the healthy one, allowing the appeareance of a wound,wich healed in 1-2 months (fig. 3a,b).

Fig. 3 a, b Detachment of necrotic tissue

Bacteriological investigations allowed the isolation of pure culture in mixed cultures, of some bacterial species that can be incriminated in triggering or maintaining intramammary infections.

Out of 46 samples taken from goat milk, there were isolated 36 strains of *Staphylococcus aureus*. Of these, 25 (69.44%) were isolated in pure cultures and 11 (30.55%) strains associated with other bacterias that have pathogenic potential:*Escherichia coli, Klebsiella oxytoca* and *Pasteurella multocida* (fig. 4).

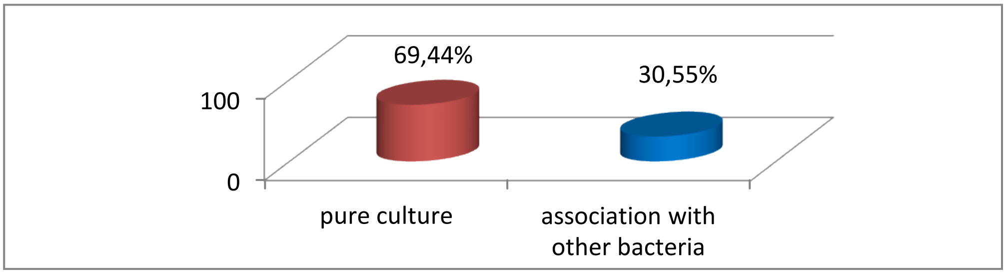

Fig. 4 The frequency of isolation *Staphylococcus aureus* from milk samples

In dairy ruminants, these Gram-negative bacteria, and particularly *Escherichia coli*, are a common cause of mastitis in both lactating and non-lactating animals. (Genaro C. B, 2011). All are considered environmental mastitis pathogens (Hogan J. et col., 1999).

Isolated strains of *Staphylococcus aureus* showed all the cultural and morphological characteristics of the species. On nutrient agar, in 24 hours and aerobically conditions, germs grew abundand creating colonies with a diameter of approx. 3 mm, smooth, round , opaque, creamy,slightly convex and with a yellow-white pigment. On Baird-Parker acriflavin environment and Chapman environment, strains of *Staphylococcus aureus* had a characteristic appeareance (fig. 5). Biochemical confirmation of *Staphylococcus aureus* strains was determined on API Staph System galleries (BioMerieux, France) with 20 microampules containing deshydrated substrates and/or nutrient media (fig. 6).

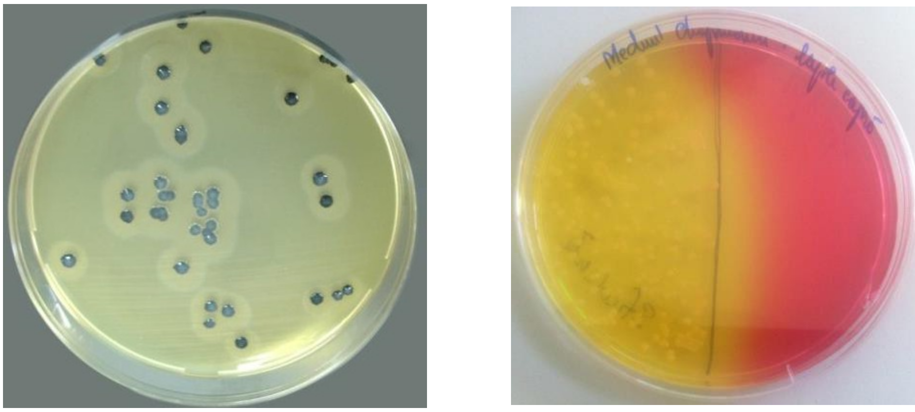

Fig. 5 *Staphylococcus aureus*- cultural characteristic: Baird Parker Agar Medium (left); *Chapman Medium* - *mannitol*-fermenting (right)

Fig.6 Galleries Api-Staph biochemical profile of some species *Stapyhlococcus aureus*

Antibiograme aimed at establishing an appropriate treatment for mastitis outbreak. Tests performed on 15 *Staphylococcus aureus* strains showed a sensitivity profile with moderate oscillations in the panel of used antibiotics. The resistance pattern is given in table 1.

Table 1

Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from goat milk samples

	Antimicrobial agent									
S.aureus n=15	P	S	E	AMC	APX	Ne	My	Te	CFP	CN
Rezistance	8/15	9/15	8/15	10/15	10/15	3/15	3/15	2/15	1/15	2/15
%	53,33	60	53,33	66,66	66,66	20	20	13,33	6,66	13,33

Legend: *P-penicillin* *Te- oxitetraciclina*
S- streptomycin *Ne-neomycin*
E-eritromycin *My-lincomycin*
AMC-amoxicillin/clavulanic acid *CFP-cefoperazon*
Ax- ampicillin-cloxacillin *CN-cefalexin*

Results of antimicrobial susceptibility tests agains *Staphylococcus aureus* showed a special resistance to AMC and APX , 66.66%,of *Staphylococcus aureus* strains being resistant to the two combinations of antibiotics. Also, it is noticeable the resistance to penicillin (53.33%) and streptomycin(60%). This may be the consequence of "*selection pressure*" due to use of beta-lactam antibiotics in first choice intramammary infections treatment, offering the possibility to appear beta-lactamases strains with an extended resistance. Resistance to erythromycin (53.33%) completes the multiple resistance profile. This can be attributed to cross transmitting mechanisms of resistance genes of *Staphylococcus aureus* strains to aminoglycosided, since the incidence is increased to streptomycin also. However, this is not totally certain as each of the aminoglycosides have a slightly different mechanism of resistance due to their different aminoglycoside modifying enzymes chromosomal mutation eg streptomycin and impermeability of membranes (Al Masaudi S.B.,2011).

Antibiotic resistance identified at *Staphylococcus aureus* strains may correlate with the antibiotics used in the treatment of local (intramammary) first intention for intramammary infections. A positive aspect can be the low resistance to tested cephalosporins: cefoperazon(6.66%) and cephalexin (13.33%) wich are marketed as intramammary syringes.

Treatments performed without antibiogram are the main reason for antibiotical resistance in pathogenic bacterial strains. However, treatment is often unprofitable.(J.K. Shearer, 1999, Tudose A., 2013).

An important aspect of the case as a study , the goats were vaccinated against gangrenous mastitis caused by *Staphylococcus aureus*. At best, vaccination against *Staphylococcus* mastitis,(the most common type of mastitis in dairy goats) has been shown to reduce severity and possibly duration of infection by these agents. Their efficacy in goats is unknown(Shearer J.K. 1999).

Therefore, it is possible that immunological protection have been partially established and developed mastitis in the first stage without any obvious clinical signs. It is also possible that vaccination to be done under contraindicatted conditions, with the possibility that animals were immunosuppressed due to environmental conditions or pressure amid a parasite. As a result, the vaccine did not induce an active immune protection againts staphylococcal infection.

We consider that this subclinical development of mastitis amid ensure a false immunological protection, confuses the owners and they do not indentify in optimal time the intramammary infections. Milk is a food consumed by humans at all ages and is a raw material for dairy products and the emergence of staphylococcal mastitis in a herd has serious implications in public health, creating the risk of food poisoning.

Conclusion

Considering the possibility of a failure in immunization of goats against staphylococcal mastitis, the risk of subclinical development of mastitis, difficult treatments and sometimes without results, and other issues that relate to antibiotic resistance of *Staphylococcus aureus* strains, wich evolve outbreaking and also the risk of food poisoning by eating contaminated milk, we can deduce that prevention by vaccination against gangrenous mastitis in goats must be supported by other preventive measures. Very good results can be obtained if good raising of goats skills are respected, milking equipment is correctly set including post-milking-teat-dipping and preventive treatment during the non-lactating period and also elimination of goats with chronic mastitis.

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