

THE EFFECTS OF HEAT STRESS ON THE EXISTENCE OF STAPHYLOCOCCI IN MILK PRODUCED BY DAIRY COWS

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ABSTRACT

Mastitis is an inflammation of the mammary gland affecting the dairy cattle. This disease is prevalent in our farms at a frequency estimated at 20% of the clinical pathology in dairy cows. Mastitis causes considerable economic losses due to the decrease in quantity and the quality of milk produced. This must include the cost of therapies and reforms. The germs responsible for mastitis are very numerous; *Staphylococci* are the most important pathogens causing subclinical mastitis. This study tends to assess the heat stress experienced by lactating cows and its effect on *Staphylococcal* mastitis. Therefore, we observed 15 milking dairy cows with an average age of 3.5 years after calving. Temperature-humidity index (THI) was calculated after recording temperatures and humidity, to test the effects of three levels of THI (<68; (72- 79); >80) on *Staphylococcal* mastitis. The results indicated that the number of coagulase-positive *Staphylococcus aureus* was higher during the winter and spring seasons at values of 1.03×10^2 and 1.19×10^2 , respectively, compared to the summer season at value of 0.75×10^2 . The result of the antibiogram showed that *Staphylococcus aureus* was resistant to Lyncomycin and oxacilin. In conclusion, heat stress causes respiratory alkalosis that has a bacteriostatic effect on *Staphylococcus aureus* growth, and the best timing for the treaty is the summer season using one of the following antibiotics (Amoxicilin, Rifampicin, Levofloxacin, Erythromycin, Chloronphennicol).

Keywords: Antimicrobial use, Heat stress, Lactation cow, Mastitis, *Staphylococcus aureus*.

INTRODUCTION

The dairy industry is a strategic sector for the economies of countries (Lahari, 2023). It is a national priority. In Algeria, as in most countries, bovine mastitis is the main disease of dairy farms. Accurate knowledge of the frequency of mastitis-causing bacteria in dairy cows is essential to determine and adapt mastitis control programs to different epidemiological situations. An average incidence of subclinical mastitis

for 25 percentage of cows was identified in central Algeria (Saidi et al., 2012). *Staphylococci* and *Sreptococci* were the most commonly identified germs in subclinical mastitis (Saidi et al., 2012). *Staphylococcus aureus* is spherical in shape. It measures 1 μm of diameter, it is immobile, it produces an enzyme called coagulase and it may cause udder infection (Sinha et al., 2010). *Staphylococcus aureus* can form a biofilm that can cause a less severe form of mastitis, but it exhibits greater resistance to antibiotics. (Cucarella et al., 2004). Heat stress can affect productivity and animal welfare (Collier et al., 2006 ; Nikkhah and Alimirzaei, 2023). The thermal discomfort occurs when the temperature–humidity index (THI) exceeds 68. (Attia et al., 2022).

Bertocchi et al. (2014), showed that the temperature-humidity index (THI) correlates positively with the somatic cell score (SCS), According to Zoghalmi et al in (2022) a low bacterial load was recorded for $\text{THI} < 72$. The objective of present study was to evaluate the effect of THI on *Staphylococcal* mastitis in lactating cows.

MATERIALS AND METHODS

This study was conducted on the farm «SARL El Asnam» level in Chlef city, Algeria, during the period of December 2022 to July 2023, on a population of 15 dairy cows of the breed Prim Holstein, Montbéliard and Fleckvieh, with an average age of 3.5 years. These cows were followed just after calving. Cows udders studies did not present any visible inflammation or clinical signs.

Three hundred samples were collected for each season, with an average of 60 samples per visit for four quarters of the 15 udder. The analyses were carried out at the laboratory of the University of Hassiba Ben bouali, ouled Fares, Chlef, Algeria. The use of test paper confirmed that the 15 cows had subclinical mastitis. The samples were taken at the four quarters of each udder just before milking. The milk is collected in a sterile test tube after disinfection of the teats, and elimination of the first jet. Milk samples are immediately placed at $+4^{\circ}\text{C}$. The temperature and humidity measurement was performed using an Operation manual for temperature and humidity meter (HTC-1 Digital Thermometer Hygrometer Clock, Fujian, China)

Isolation and identification of *Staphylococcus aureus*

The bacterial culture was carried out on Chapman medium (Pasteur Institute, Algeria). *Staphylococcus aureus* was identified using catalase and coagulase tests, along with gram staining, (HIMedia Laboratory, India)

The diffusion method with Mueller-Hinton agar was utilized to investigate antibiotic susceptibility.

According to the Europran committee (2023) on antimicrobial susceptibility testing, the recommended dose of 8 antibiotic discs (Bio-Rad, France) for *S. aureus* has been set for erythromycin (15 μg), oxacillin (5 μg), gentamicin (30 μg), lincomycin (15 μg), chloramphenicol (30 μg), rifampicine (30 μg), Amoxiciline (25 μg) and levofloxacin (5 μg). Accordingly, After 24 hours of incubation at 37°C , the inhibition diameters were measured, allowing the interpretation in categories S (sensitive), I (intermediate), R (resistant). (EUCAST, 2023).

Statistical analysis

Data analysis was performed by SPSS program, by applying the Student test (comparison of two means). The significant level was set at $p < 0.05$. The results of the antibiogram were expressed as percentages.

RESULTS AND DISCUSSION

It was referred to in the Table 1 that the number of *Staphylococcus* is higher during the winter and spring season at (1.03×10^2 ; 1.19×10^2), respectively than during the summer season at a value of (0.75×10^2) with $p < 0.05$. Knowing that the number of *staphylococci* has reached the microbiological infective limit (10^2 - 10^3) ufc/g (JORA, 2017).

Table 1. Evolution of *Staphylococci aureus* by season in dairy cows of Chlef city, Algeria

Microbiological limits	<i>Staphylococcus aureus</i>	Season
(10 ² - 10 ³) ufc/g	M=1.03x 10 ²	Winter T=16.9 °C H= 47.75%
	M=1.19 x 10 ²	Spring T=27.62°C H=46%
	M=0.75 x 10 ^{2***}	Summer T=33.1*** °C H= 47

M= mean ; T= temperature ; H= humidity ; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

In a cold season, the frequency of mastitis is higher when animals are confined with inadequate housing conditions (Bouhroum et al., 2022). Moreover, the periparturient period is marked by an immune deficiency that causes the udder to be vulnerable to environmental and contagious pathogens. (Derakhshani et al., 2018). *Staphylococcus* mastitis is responsible for 80% of the infections in the herd. In subclinical mastitis, *S. aureus* and *S. agalactiae* are the most common bacteria. (Elhaig and Selim, 2015). The indirect impact of heat stress on milk *Staphylococcus* counts during summer season arises from the blood pH becoming alkaline because of pulmonary hyperventilation, a crucial apparatus for removing excess body heat.. Respiratory alkalosis is always coupled with urinary bicarbonate excretion (Bonnefoy et al., 2011). The temperature humidity index is an indicator that can be used to assess the level of heat stress in dairy cows (Bohmanova et al., 2007 ; Attia et al., 2022). Table 2 shows that when the THI has a high value of 81.66 exceeding the thermal comfort zone (severe stress) the number of *Staphylococcus* drops to a value of 0.75×10^2 cfu/g ($p < 0.05$).

Table 2. Evolution of *Staphylococci* as a function of temperature–humidity index in dairy cows in Chlef city, Algeria

Season	Winter	Spring	Summer
Number Staphylococci (ufc/g)	M=1.03x 10 ²	M=1.19 x 10 ²	M=0.75 x 10 ^{2***}
Temperature humidity index	61.11	74.57	81.66***
Critical interval	<68 Thermal comfort zone	(72- 79) moderate to severe stress	> 80 severe stress

M= mean ; *p < 0.05 ; ** p < 0.01 ; *** p < 0.001

Staphylococci can not be developed due to metabolic alkalosis, as demonstrated earlier. Because the pH of their growth is in an interval of (6-7) (Mehwish et al., 2014). The isolates of *Staphylococci aureus* are sensitive to Erythromycin, Levofloxacin, Rifampicin, Chloronphenicol, gentamicin and Amoxicilin (Table 3).

Table 3. The result of the antibiotic susceptibility of the *Staphylococcus aureus* strain in infected dairy cows in Chlef city, Algeria

Antibiotic	Inhibition diameter (mm)	Critical diameter (mm)	Result
Erythromycin	35.66	23 - 29	S
Levofloxacin	24.25	23-29	S
Lincomycin	19.2	27 - 32	R
Gentamicin	14.66	6	S
Oxacillin	30.75	30-38	I
Amoxicilin	22	14-21	S
Rifampicin	37.75	30-36	S
Chloronphenicol	28.33	>=23	S

S= sensitive, R= resistant I= intermediate

Staphylococci have developed resistance to oxacillin through the production of penicillin-binding protein (PLP), for example, PLP2a (Daurel et al., 2008). Peptidoglycan is an important structure of bacteria, it is synthesized by PLP, β -lactam is able to bind itself to PLP by covalent binding and inhibit the activity of

bacteria. This bond causes a rupture of balance between lysis and peptidoglycan synthesis, bacteria become unable to resist the osmotic pressure exerted on their plasma membrane and die by osmotic lysis. A change in the antibiotic target leads to its inactivity. The acquired resistance of *Staphylococcus aureus*, is characterized by the possession of a new PLP, PLP2a by having very little affinity for β -lactams (Quincampoix et al., 2001). The resistance of *Staphylococci* can also be explained by the hyperproduction of penicillinase, an enzyme that hydrolyses β -lactamines (oxacillin, dicloxacillin, meticillin). Lincomycin antibiotics have only bacteriostatic activity on staphylococci (Quincampoix et al., 2001). The extension of antibiotic resistance leads to difficulties in the management of infections due to *Staphylococci aureus* in dairy cow during winter and spring seasons.

CONCLUSION

In conclusion, the role of thermal stress on *Staphylococcal* mastitis is bacteriostatic. Therefore the treatment of mastitis with one of the following antibiotics such as : Erythromycin, Rifampicin, Chloramphenicol, Amoxicillin, Gentamicin, and Levofloxacin during the summer season will give better results for the health of the udder in infected cows.

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