Arab Gulf J. scient. Res. **3** (2), pp. 717-726 (1985)

Incidence of Mastitis in the Dairy Herd of the College of Agriculture, King Saud University, Riyadh, Saudi Arabia

E.I. Elnima, M.F. Hussein, A.M. Khalil and E. Elnashar.

Microbiology Section, Department of Pharmaceutics, College of Pharmacy, and Department of Animal Production, College of Agriculture, King Saud University, Riyadh, Saudi Arabia.

ABSTRACT. Milk samples from the dairy herd of the College of Agriculture, King Saud University, Riyadh, were subjected to bacteriological examination, somatic cell counts and qualitative milk tests, namely pH-determination, Whiteside and MBR tests. Coagulase-positive *Staphylococcus aureus* was the only major pathogen isolated. This organism was responsible for subclinical mastitis in 10% of the Friesian and 20% of the Jersey cows in the herd. The corresponding counts of viable bacteria in these two breeds were 6.69×10^2/ml and 5.7×10^3/ml, respectively. The same organism was isolated from all cases of clinical mastitis met with in these cows (10% and 16.7% incidence in Friesian and Jersey cows, respectively). More than 50% of the cows also excreted large numbers of micrococci in the milk ($3.9 \times 10^3 - 6.5 \times 10^3$/ml) but these are known to cause very mild infections and are often disregarded in mastitis surveys. Somatic cell counts averaged 1,622,000/ml in cows infected with *Staph. aureus*, 272,000/ml in those infected with micrococci and 204,000 in non-infected cows. A good correlation was noted between Whiteside and MBR tests and between these tests and bacteriological findings. The pH of the milk was slightly alkaline.

Bovine mastitis is a complex disease which is usually a consequence of different micro-organisms from different sources (Bramley 1981). It is characterized by pathological changes in the udder tissue, physical and chemical changes in the milk and a decrease in milk production. The condition, however, occurs either as a clinical or subclinical disease. In the former case, signs of udder inflammation such as swelling, heat and tenderness as well as abnormalities in the milk, *e.g.* discolouration or clots, are grossly visible. Subclinical mastitis, on the other hand, is not accompanied by gross changes and a diagnosis can, therefore, be established only by the laboratory examination of milk samples (Wilson 1981).

In cows, the major pathogens involved include *Staphylococcus aureus*, the commonest cause of subclinical mastitis, (Wilson 1981), *Streptococcus agalactiae,*

Strept. dysgalactiae and *Strept. uberis*, although coliforms and pseudomonads may also cause udder infections. Infection with these major pathogens usually produces a marked increase in somatic cells in the milk of the affected quarters. This indicated that they are of intramammary origin (Neave 1975). On the other hand, minor pathogens such as *Corynebacterium bovis* and micrococci, consisting of members of staphylococcus and micrococcus, can also get established in the bovine udder. These, however, rarely cause clinical mastitis (Dodd 1981) and are usually associated with a mild form of leucocytosis (Kingwill 1981). The somatic cell count for quarters infected with minor pathogens thus falls between ranges shown by uninfected and major pathogen infected quarters (Bramley 1975). Cows with healthy quarters have low somatic cell counts in their milk. On the other hand, it has been shown experimentally that cows with high cell counts were more able to withstand infections than cows with a low number of phagocytes, and this resistance has been attributed to the elevated neutrophil counts (Schalm *et al.* 1964). However, there is no evidence that the level of cell count of uninfected quarters has any influence on new infection rates. There are no published reports on mastitis in the Kingdom of Saudi Arabia, despite the rapid development of dairy farming in this country. At present, around 23,000 dairy cows (predominantly Holstein/Friesians and few Jerseys imported from Europe or USA) are being kept on some 27 modern type commercial farms around the country (Basmaeil 1984).

Mastitis is a common problem on those farms, but, apparently, only the clinically visible disease is recognized and that the true incidence is still unknown. The present study constitutes the first attempt to investigate the incidence of mastitis on one such modern type farm; in the College of Agriculture, King Saud University. Hopefully this will stimulate further research into the problem among these exotic cattle which are performing under the locally prevailing arid conditions of the Gulf Area.

Material and Methods

History of the herd

This herd was established in November 1978 by importing pregnant heifers (aged 27-30 months) from Europe (Friesians and Jerseys). The cows studied (61/ lactating cows) consisted of these cows and their daughters (Ten in the first season, and those in 2*nd* to 4*th* lactation). The cows were tested annually for brucellosis and they were brucellosis free. There were also vaccinated against major epizootic diseases in the area, such as, rinderpest, foot and mouth disease, clostridial infections and pasteurellosis. Clinical and production records are kept on the premisis and the samples analysed through the years 1980-1983. The culling rate in this herd was 1-5%. The cows were machine-milked twice daily, at 5 a.m. and 4 p.m., employing a simple herring-bone type system. During the process, standard sanitary procedures were followed, the milkers wore gloves disinfected with iodophor

solution (0.5% available iodine) and the teats were dipped after milking in the same solution.

Aseptic collection of milk samples

Before sampling, the udder was physically examined for tenderness, abrasions heat,... etc. As the healthy udder is normally sterile (Neave 1975), aseptic technique is essential for sample collection in order to exclude contaminants. The method used was a modification of that described by Higgs and Bramley (1981). Milk samples were collected immediately before morning milking. The teats were washed with water then treated with 70% alcohol and left to dry. Sterile disposable plastic containers were used for sample collection (Sterilin products, Sterilin Ltd., Teddington, Middlesex, England) and the sample was squeezed out by hand, applying minimum pressure. The technician was wearing sterile disposable gloves during the process. The samples were immediately transported to the laboratory for examination. The following tests were carried out on freshly collected samples:

1. pH Measurement

The pH of each milk sample was determined using a pH-meter.

2. The Whiteside Test

This test is an indirect measure of the cell (leucocyte) content of milk, and is thus useful for detecting samples which are abnormal in this respect. The method employed was essentially that described by Schalm *et al.* (1971). Five drops of the milk sample were mixed with two drops of 1 *N* NaOH on a glass plate. After stirring briskly for 15 sec, the result was immediately read against a dark background. If the milk was normal, no change in the consistency or appearance of the mixture occurred and the result was recorded as negative. Positive results were recorded according to the intensity of the change in the mixture.

3. Somatic Cell Count

Several indirect methods are available for the detection of the cell content of milk, *e.g.* Whiteside test, California mastitis test, ... etc., however, a direct count of somatic cells in milk is superior. The method followed was that described by Schalm *et al.* (1971). One drop of formalin solution was added to 10 ml of milk in a sterile stoppered bottle and inverted once to mix. The formalised sample was used to prepare a film on a haemocytometer slide. When dry, the slide was stained with Newman's stain and examined microscopically. The total number of cells in 30 squares was determined and the average per square was used to calculate the number of cells per 1 ml of milk.

4. Methylene Blue Reduction Test

The methylene blue reduction method indirectly measures bacterial densities in milk in terms of the time interval required for dye reduction 'methylene blue reduction time'. This permits rapid grouping of milk samples into different grades. One ml of methylene blue solution was added aseptically to 10 ml of well mixed sample in a sterile stoppered bottle. After gentle mixing, the mixture was incubated at 36 ± 1°C. The time taken for complete decolourization of the mixture was recorded (Schalm *et al.* 1971). In general, reduction time is inversely related to the bacterial content of milk and generally correlates with estimates of bacterial populations by the agar plate method (Abelle 1945).

5. Bacteriological Examination

The method followed was that described by Higgs and Bramley (1981). This method specifies the isolation and identification of the following micro-organisms: *Staph. aureus*, *Strept. agalactiae*, *Strept. dysgalactiae*, *Strept. uberis*, *E. coli*, *Corynebact. pyogens* and others in udder quarter milk samples.

Media

Aesculin blood agar was used to support the growth of all the pathogens. The medium allows good differentiation between staphylococci, streptococci and micrococci according to their colonial appearance and type of haemolysis.

Calf blood (7%) was added to the molten base at 45°C. After mixing, 10 ml of medium were poured into each of 9 cm sterile plastic petri dishes.

Plate count agar (Oxoid Ltd., UK) was used for the determination of viable count using Miles and Misra's (1938) method with a 50 dropper. The milk samples, neat, 1 in 10 and 1 in 100 dilutions in quarter strength ringer solution were plated out on the surface of overdried plate count agar. After incubation at 37°C for 24 hr, the number of colonies in each of six drops at each dilution were counted. The average was used to determine the viable count per ml of the milk sample.

The pathogens isolated on aesculin blood agar were then identified using biochemical tests, *e.g.* coagulase test for *Staph. aureus*.

Results and Discussion

The percent incidence of clinical mastitis, revealed by abnormal changes in the udder or milk, over the years 1980-1983 is shown in Table 1. Over that period there was a stable rate of infection except in the last year when there was a moderate increase in the incidence of clinical mastitis, particularly in Jersey cows. Figures for subclinical cases of mastitis for the years 1980-1982 are lacking since no previous surveys were carried out on this herd. However, in the year 1983, in

addition to the clinical cases of mastitis shown in Table 1, the rate of subclinical mastitis determined in the present survey was 20% and 10% for Jersey and Friesian cows, respectively. For the Friesian cows showing subclinical mastitis only one quarter per cow was infected with *Staph. aureus*, whereas for Jersey cows, two of them had three infected quarters and the rest had the pathogen in one quarter only. During the present survey, the tests performed on the milk samples included qualitative assessments, *viz.* pH measurement, the Whiteside test, methylene blue reduction test and somatic cell count. In addition, bacteriological examination of milk was done in order to isolate and identify any of the pathogens normally encountered in udder infection. The viable count of bacteria in the samples was also determined.

Table 1. The proportion (%) of udder quarters slowing clinical mastitis for each year (1980-83) in groups of Jersey (J) and Friesian (F) cows in the herd.

Year / Breed	1980	1981	1982	1983
J	10.00	8.22	9.22	16.66 (20%)*
F	6.32	7.17	6.93	10.00 (10%)*

* Figures in brackets represent per cent cases of subclinical mastitis revealed by microbiological examination of milk samples.

Table 2. Proportion of udder quarters (%) infected with *Staphylococcus aureus* and/or Micrococci and the arithmetic mean Bacterial count per ml of milk examined in groups of Jersey (J) and Friesian (F) cows in the herd.

Organism	*Staphylococcus aureus*		**Micrococci**		**Mixed** *(Staph. Aureus* **& Micro-cocci)**	
Breed	J	F	J	F	J	F
% udder quarters infected	16.66	6.66	53.33	54.84	3.33	3.23
Mean Bacterial count/ml $\times 10^3$	5.71	0.67	3.91	6.53	11.50	0.54

The result of the bacteriological examination of milk is shown in Table 2. The only major pathogen isolated from this herd was coagulase positive *Staph. aureus*, infecting 16.66% of Jersey and 6.66% of Friesian cows, the average bacterial count per ml of milk was 6.69×10^2 and 5.71×10^3 for Friesian and Jersey cows, respectively. There were two cases of mixed infection, one quarter infected with *Staph. aureus* and another with a micrococcus. These results emphasize the importance of *Staph. aureus* as the most common cause of subclinical bovine mastitis (Wilson 1981). Table 2 shows that more than half of the cows, irrespective of breed, excrete large numbers of micrococci ranging from 3.9×10^3 to 6.5×10^3 per ml of milk. In mastitis studies, the minor pathogens, *C. bovis* and coagulase negative staphylococci (micrococci), are normally ignored because they commonly cause mild infections (Black *et al.* 1972). In this survey, *C. bovis* was not encountered and the minor pathogens isolated were micrococci. Table 3 shows the relationship between mean bacterial count, mean somatic cell count, Whiteside test, methylene blue reduction and pH of milk from udder quarters infected with *Staph. aureus* and micrococci and from non-infected udder quarters. There was good correlation between the indirect tests performed on the milk samples and the bacteriological findings. The methylene blue reduction time was found to be shorter with milk samples from quarters excreting *Staph. aureus* or micrococci. The pH of the milk was found to be slightly alkaline (7.02 – 7.78) mainly, in samples from quarters harbouring *Staph. aureus*. According to Shommein (1975), the pH of normal milk lies between 6.3 to 6.9 but in mastitis it tends to become alkaline. Hence, its measurement is a useful index of milk quality. However, it is generally accepted that adequate diagnosis of subclinical infection requires a combination of bacteriological examination and cell determination of all four quarters (Neave 1975). According to the latter author, even a bacteriological examination alone is acceptable, provided that the cause of the condition is bacterial and the pathogen

Table 3. The relationship between various tests performed on milk from udder quarters infected with *Staph. aureus* and micrococci & from non-infected udder quarters.

Udder quarter	Mean bacterial count per ml $\times 10^3$	Mean somatic cell count per ml $\times 10^5$	Whiteside score	Methylene blue reduction time (hr)	pH
Staph. aureus infected quarters	5.076	16.220	2	2	7.02-7.78
Micrococci infected quarters	3.883	2.72	0	3	6.68-7.10
Non-infected quarters	–	2.04	0	>3	6.45-6.69

involved could be readily isolated by simple methods. The mean somatic cell count per ml is highest in milk from quarters infected with *S. aureus*, 16.222×10^5 and milk from uninfected quarters showed the lowest count, 2.04×10^5, whereas that from micrococci infected quarters fell in between, 2.72×10^5 (Table 3). This is in good agreement with previous reports (Bramley 1975). An injured quarter, infected with *Staph. aureus*, revealed a high somatic cell count in the order of 1.18×10^7. The Whiteside scores were found to agree with the somatic cell count.

This study constitutes the first mastitis surveillance of the dairy herd of the College of Agriculture, King Saud University. It does not pretend to be a major investigation of the problem, yet it is considered of value since it is the first attempt to examine this problem among exotic breeds of dairy cows in the Kingdom of Saudi Arabia and should arouse interest in mastitis control in its early stages. Since the completion of the present survey, we have initiated dry-cow therapy in this herd, but it is still too early to evaluate its results. We will continue to monitor the herd to see whether staphylococcal infections would decline and if infections with other pathogens such as *Strept. uberis* or *Strept. dysgalactiae* would occur. The results of this study show that sanitary conditions on this farm are satisfactory.

Acknowledgement

The authors wish to thank Mr. Mohammed Al-Mubarak and Mr. Ibrahim El-Najar for their unfailing technical assistance. Thanks are also extended to Mr. K. Abbas for typing the manuscript.

References

Abelle, I.C.A. (1945) The methylene blue reduction test as a means of estimating the bacterial content of milk to determine its suitability for pasteurisation or as a basis for grading, *J. Milk Technol.* **8**: 67-69.

Basmeil, S.M. (1984) The present and future production of ruminants in Saudi Arabia (Text in Arabic), *The Arab Symposium on Intensive Production of Ruminants in the Arab World, Riyadh 10-14 Nov. 1984, Organized by the Arab Organization for Agricultural Development.*

Black, R.T., Marshal, R.T. and **Bourland, C.T.** (1972) Locus of mammary gland infections of *Corynebacterium bovis, J. Dairy Sci.* **55**: 413-416.

Bramely, A.J. (1975) Infection of the udder with coagulase negative micrococci and *Corynebacterium bovis, Bull. int. Dairy Fed.* (Document **85**): 337-381.

Bramely, A.J. (1981) The role of hygiene in preventing intramammary infections, *in*: **Bramely, A.J., Dodd, F.H.** and **Griffin, T.K. (eds.)** *Mastitis Control and Herd Management* Technical Bulletin 4. National Institute for Research in Dairying, Reading, England. Hannah Research Institute, Ayr, Scotland. pp. 53-66.

Dodd, F.H. (1981) Mastitis control, *in*: **Bramely, A.J., Dodd, F.H.** and **Griffin, T.K. (eds.)** Mastitis Control and Herd Management, Technical Bulletin 4. National Institute for Research in Dairying, Reading, England. Hannah Research Institute, Ayr, Scotland. pp. 11-23.

Higgs, T.M. and **Bramely, A.J.** (1981) Laboratory techniques for the examination of milk samples, *in*: **Bramely, A.J., Dodd, F.H.** and **Griffin, T.K. (eds.)** *Mastitis Control and Herd Management*, Technical Bulletin 4. National Institute for Research in Dairying, Reading, England. Hannah Research Institute, Ayr, Scotland. pp. 95-109.

Kingwill, R.G. (1981) The NIRD-CVL mastitis control method, *in*: **Bramely, A.J., Dodd, F.H.** and **Griffin, T.K. (eds.)** *Mastitis Control and Herd Management*, Technical Bulletin 4. National Institute for Research in Dairying, Reading, England. Hannah Research Institute, Ayr, Scotland. pp. 24-39.

Miles, A.A. and **Misra, S.S.** (1938) The drop plate Method, *J. Hyg. Camb.* **38**: 732-742.

Neave, F.K. (1975) Diagnosis of mastitis by bacteriological methods alone, *Bull. int. Dairy Fed.* (Document **85**): 19-36.

Schalm, O.W., Carrol, E.J. and **Jain, N.C.** (1971) *Bovine Mastitis*, Lead and Fibiger, Philadelphia, 94 p.

Schalm, O.W., Lasmanis, J. and **Carrol, E.J.** (1964) Effects of pre-existing leukocytosis on experimental coliform (Aerobacter aerogenes) mastitis in cattle, *Am. J. Vet. Res.* **25**: 83-89.

Shommein, A.M. (1975) The diagnosis of bovine mastitis-review, *Sudan J. vet. Sci. Anim. Husb.* **13** (2): 74-81.

Wilson, C.D. (1981) Prevalence of udder infection in the U.K., *in*: **Bramely, A.J., Dodd, F.H.** and **Griffin, T.K. (eds.)** *Mastitis Control and Herd Management*, Technical Bulletin 4. National Institute for Research in Dairying, Reading, England. Hannah Research Institute, Ayr, Scotland. pp. 40-49.

(Received 18/02/1984;
in revised form 24/12/1984)

التهاب الضرع بين أبقار الحليب في كلية الزراعة، جامعة الملك سعود، الرياض

الأمين إبراهيم النعمة، منصور فارس حسين، علي محمد خليل والسيد النشار

قسم الصيدلانيات - كلية الصيدلة وقسم الانتاج الحيواني - كلية الزراعة - جامعة الملك سعود - الرياض - المملكة العربية السعودية

أجريت تحاليل بكتريولوجية بالإضافة إلى تقدير أعداد الخلايا وبعض الاختبارات النوعية كتقدير درجة تركيز أيونات الهيدروجين واختبارات هوايتساید واختزال أزرق المثيلين في عينات الحليب من مزرعة الإنتاج الحيواني بكلية الزراعة، جامعة الملك سعود، الرياض. وقد لوحظ أن البكتريا من نوع Coagulase + ve *Staph. aureus* هي البكتريا الممرضة الوحيدة التي تم عزلها من هذه الأبقار، وقد تسببت في حدوث التهاب الضرع تحت الحاد بنسبة ١٠٪ بين أبقار فريزيان و ٢٠٪ بين أبقار جيرسي. وكانت أعداد البكتريا الحية في هذه الأبقار ٦,٦٩ × ١٠^{٢}/مل و ٥,٧ × ١٠^{٣}/مل على التوالي. كما عزلت نفس البكتريا من جميع حالات التهاب الضرع الحاد التي سجلت أثناء الدراسة.

كذلك وجدت البكتريا من نوع Micrococci بكميات كبيرة (٣,٩ × ١٠^{٣} - ٦,٥ × ١٠^{٣}/مل) في أكثر من ٥٠٪ من أبقارالقطيع إلا أن هذه الميكروبات عادة تسبب إصابات طفيفة جداً ولا تؤخذ في الاعتبار عند إجراء المسح لا لتهاب الضرع. أما أعداد الخلايا في عينات الحليب، فقد قُدِّرت في

المتوسط بحوالي ١,٦٢٢,٠٠٠/مل في الأبقار المصابة بـ *Staph. aureus* و ٢٧٢,٠٠٠/مل في الأبقار المصابة بـ *Micro-cocci* و ٢٠٤,٠٠٠/مل في الأبقار غير المصابة.

وقد لا حظنا العلاقة بين اختبارى هوايتساید واختزال أزرق المثيلين وبين هذين الاختبارين ونتائج الفحص البكتريولوجي، أما درجة تركيز أيونات الهيدروجين في الحليب فقد تميزت بقلوية طفيفة.