Intra mammary challenge of dairy goats with a low dose of *Staphylococcus aureus*

UNDERGRADUATE RESEARCH PROJECT

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ABSTRACT

The aim of this pilot study was to induce a subclinical mastitis in dairy goats with a bovine *Staphylococcus aureus (S. aureus)* strain and to investigate the post infection (p.i.) response during the first 48 hours of this infection. The right udder halves of 2 goats were inoculated with approximately 58 Colony Forming Units (CFU) of *S. aureus* in 1 ml phosphate buffered saline (PBS) 45 days after parturition, The other 2 goats served as negative controls. General health examinations and milk samples were collected from all animals just prior to inoculation and 6, 12, 24 and 48 hours after inoculation. Blood samples were taken just before inoculation and 24 hours and 7 days after inoculation. The infected animals were treated with antibiotics 48 hours after inoculation. Bacteriological analysis were performed in the milk samples within two hours after collection. The total white blood cell count and a differentiation of these cells were obtained from the blood samples.

According to the results of the bacterial counts the inoculation of the two goats lead to an infection of inoculated udder halves. One goat showed *S. aureus* in her milk at 6, 12 , 24 and 48 hours after inoculation while *S. aureus* was isolated from the milk of the other inoculated goat only at 24 and 48 hours after inoculation. The uninoculated animals did not show an intra mammary infection. Furthermore none of the animals (inoculated or not inoculated) showed any clinical signs nor significant differences in blood values during the trial.

Based on these results can be concluded that the dose used in this pilot study has successfully induced a subclinical mastitis.

Conclusion: this experiment shows that a subclinical mastitis can be induced in dairy goats with this low dose of *S. aureus*. This information can be used for other clinical mastitis trials. The main advantage of inducing a subclinical mastitis instead of using naturally infected animals is that the first hours after infection can now be studied. This can be very important in the development of practical diagnostic tools for the detection of subclinical mastitis in goats.

INTRODUCTION

Professional dairy goat farming is a growing industry (FAO, Haenlein,1996) all over the world because of an increasing public interest in alternatives for cow milk and goat cheeses. Because a lot of this goat milk is destined for a vulnerable group of people, for example children with an allergy against cow milk, it is very important to ensure the quality of goat milk. An important hazard to milk quality can be the infection of the udder which can result in mastitis. These infections of the udder bring along potential zoonotic risks due to the presence of bacteria in milk for human consumption (LeJeune & Rajala-Schultz, 2009; Oliver et al., 2009). Mastitis is also causing economic losses mainly due to reduced milk production and discarding of milk because it may contain antibiotics due to treatment of the animal (Halasa et al., 2009; Sears & McCarthy, 2003). After coagulase negative *Staphylococci*, *Staphylococcus aureus*(*S. aureus)* is the most important pathogen causing (sub)clinical mastitis in dairy goats (Bergonier et al., 2003). *S. aureus* is responsible for 35.4% of all the mastitis cases in dairy goats (Min et al., 2007). In the United Kingdom it was found that *S. aureus* caused 13% of all subclinical mastitis cases in dairy goats (Hall & Rycroft, 2007). Diagnosis of subclinical mastitis in dairy goats is, unlike the diagnosis of subclinical mastitis in cattle, difficult since there has been considerable controversy on the relationship between somatic cell count (SCC) and the presence of intra mammary infection (Luengo et al., 2004; Min et al., 2007; Petzer et al., 2008). The average SCC in dairy goats of 428.000 cell/ml (Hall & Rycroft, 2007) is significantly higher than in dairy cattle (less than 100 cells/ml in milk derived from a healthy quarter (Hillerton, 1999; Pyörälä, 2003)). The use of SCC as diagnostic tool for intra mammary infection in goats is complicated, not only because variation in SCC is linked to age, parity, the number of conceived lambs and stage in lactation, but also because the type of pathogen infecting the mammary gland influences the height of SCC (Luengo et al., 2004; Min et al., 2007). Furthermore there is some evidence that SCC of uninfected udder halves in goats were not always lower than SCC of infected udder halves (Min et al., 2007). Because of these difficulties with using SCC as a diagnostic tool for mastitis, research has to concentrate on finding practical and useful diagnostic tools. The only definitive diagnosis of subclinical mastitis in goats so far requires bacteriological culture of milk samples.

For food safety and economic reasons *S. aureus* infections in dairy cattle are identified as the most important worldwide (Barkema et al., 2006; Halasa et al., 2009; Sol et al., 2002). The number of studies in dairy cattle investigating the animal's immune responses is limited because intra mammary infection trials in dairy cattle are extremely expensive and diagnostic tools to evaluate the immune response to specific types of pathogens are limited (Smolenski et al., 2007).

A small number of closely related genotypes of *S. aureus* bacteria are responsible for a large proportion of *S. aureus* mastitis cases in cows, ewes and goats (Mørk et al., 2005; Aires-de-Sousa et al., 2007). Clonal type A *S. aureus* was found in both cows and goats (Aires-de-Sousa et al., 2007), suggesting that *S. aureus* isolates from these animals do not represent separate genetic populations. Research involving these specific strains is therefore very important and to accomplish this, clinical infection trials are necessary. To overcome the expensive research in cattle, goats could be infected with this particular strain instead. Because of the similarities between dairy goats and dairy cattle, it is possible that their immune system responds in the same way to an intra mammary infection with *S. aureus.* If this is the case then mastitis research outcomes concentrated on dairy goats can be extrapolated to the dairy cattle industries. Furthermore it is important to study mastitis in dairy goats in general, in order to find good and practical diagnostic tools. This pilot study might encourage further research on dairy goat mastitis. The aim of this research is to induce for the first time a subclinical mastitis with a bovine *S. aureus* in dairy goats and to investigate the reactions in the goat body that occur during the first 48 hours of this infection.

MATERIALS AND METHODS

Project design
For this study, first parity goats with no previous history of intra mammary infections where used for experimental intra mammary infection with *S. aureus* and subsequent bacterial counts.

Four goats were used over a period of 52 days following parturition (Figure 1). The goat kids stayed on their mother. The kids of two goats (number 159 and 94) were separated after peak lactation (day 45 (Gipson & Grossman, 1989; Paape & Capuco, 1997)) and their mothers were experimentally inoculated intra mammary in the right udder half (other udder half internal control) with 58 colony forming units (CFU) of a *S. aureus* field strain derived from a bovine mammary gland (Sydney University). The other two goats were not treated and served as (external) negative controls. 48 hours after inoculation the two infected goats were treated with oxytetracycline.

Animals
The 4 goats used where all maidens who were bred for milk production and were coming from a dairy farm in New South Wales, Australia. The goats number 159 and 94 where both Saanen bred, the goats number 1000 and 188 where British Alpine bred (Table 1). They were all between 24 and 25 months old when they kidded. All goats kidded between 12 September 2009 and 3 October 2009. The kids were allowed to stay on their mothers after birth. Because these goats were bred for high production they were also hand milked once a day. The first two goats that kidded were chosen to be inoculated because of practical reasons. Those goats (159 and 94) were accidentally the two Saanen goats and the control animals were therefore the British Alpine bred goats.

The goats were housed as a group up until the moment of the challenge. During the nights in an open front shed (5,60 m x 4 m) on a sawdust and straw bed and during the day they where allowed to graze/browse and wander on grass paddock (4 acres) with mainly Barley grass and annuals. Town water was available ad libitum through an automatic drinking trough. The goats where fed twice a day. The diet contained 0,388 kg dry matter ruminant pellets (Coprice®, Ruminant Feed) per day per goat and 0,363 kg dry matter lucerne chaff (Bidgee®) per day per goat through multiple feeding places as well as hay.

At inoculation goats 159 and 94 where separated from the others and housed in a closed shed (3 m x 2 m) until the end of infection (48 hours after inoculation). In this shed the goats were also kept on sawdust and straw and they were fed exactly the same diet as before the start of the trial.

The Animal Care & Ethic Committee (ACEC) of Charles Sturt University (CSU) gave approval to use the 4 goats after inspection of the research plan and housing facilities.

Table 1: Individual goat information

Goat ID Number	Breed	date of birth (age)	Date of parturition	Number and sex of lambs
159	Saanen	30-09-2007	12-09-2009	1, ♂
94	Saanen	31-10-2007	15-09-2009	2, ♂ and ♀
1000	British alpine	05-09-2007	29-09-2009	2, ♂ and ♀
188	British alpine	26-09-2007	03-10-2009	1, ♀

Table 1. Goat ID numbers, breed, age, date of parturition and the number of lambs.

Data collection

Milk

Milk samples were collected from all goats from both udder halves just prior to the challenge and 6, 12, 24 and 48 hours after inoculation.

Bacterial counts were performed on samples taken just before the challenge to determine if the milk contained any viable bacteria prior to the challenge. Then 6, 12, 24 and 48 hours after the challenge, bacterial counts were performed again to establish changes in the number of CFU of *S. aureus* in the milk due to the infection trial.

Milk sampling was performed under strict hygienic conditions. Disposable latex gloves which were disinfected with 70% alcohol prior to the sampling, were worn during the sampling.

Teats were wiped with the 70 % alcohol, and after the first streams of milk were discarded, 20 mL of sample milk was collected from each udder half into two sterile 10 ml tubes. One of the tubes was used for the bacterial counts that all were performed within two hours after sampling, while the other tube of milk was stored at -20ºC immediately after sampling for later processing (not in the scope of this article).

The milk samples intended for the bacterial counts were placed into the incubator within one hour after collection. Samples were incubated at 37ºC for about one hour. Hundredfold dilutions (to 10^{-6}) were made of the milk in sterile PBS, and 0.1 mL of each dilution was inoculated on a horse blood agar plate and incubated for 12 hours at 37ºC. Bacterial colonies of *S. aureus* were counted (looking at colour and hemolysis) and Gram staining was performed on the isolated colonies.

Blood

Blood samples were collected from the jugular vein using a vacutainer system (sleeve, 18G 1"vacutainer needle and 10 ml EDTA vacutainer blood collection tube). Blood samples were taken from all goats just prior to the challenge, 24 hours after inoculation and 7 days after inoculation. All blood samples were processed by the Cell-Dyn®3700 hematology analyzer (Abbott®, United States) which uses impedance with pulse-editing to perform the multi-dimensional cell classification with Multi-Angle Polarized Scatter Separation technology to provide white blood cell count and differentiation and analysis of red blood cells and platelets.

General health examination

During all milk sample collections the goats were also monitored by performing a general health check and udder examination. Before the milk sampling, each goat was examined using the following protocol: general impression of the animal which included demeanour, stance, position, food/water intake and vocalisation, general health check with respiration rate, heart rate and temperature and udder examination with temperature of the udder, swelling, colour, pain, lumps, injuries to the teats/udder, milk extraction, milk colour and milk clots.

Figure 1: Project design
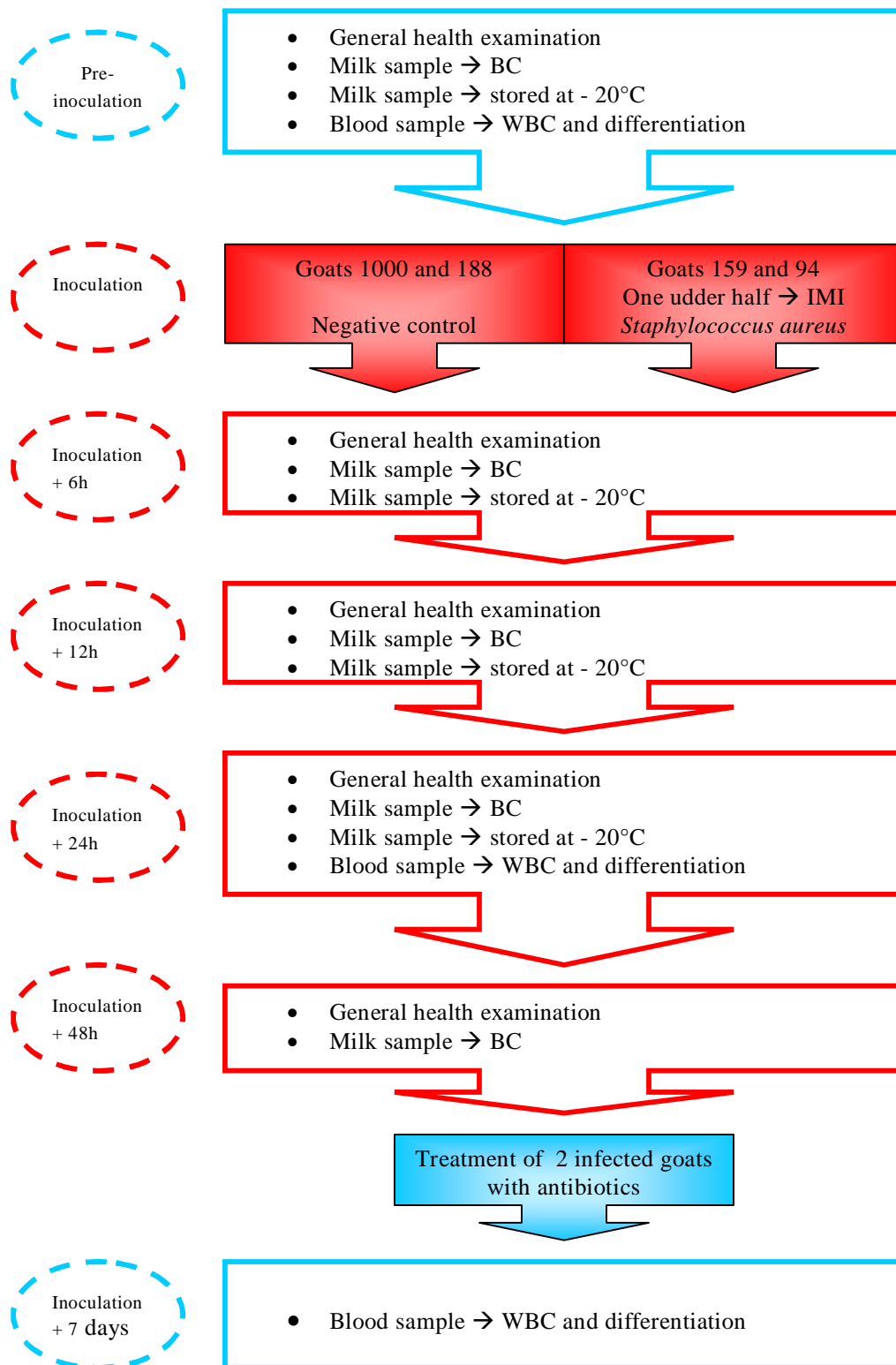
Figure 1. For each sample collection moment it is shown which samples were taken and what was done with the samples. The samples that were stored at - 20°C were destined for later processing with two dimensional electrophoresis and tandem mass spectroscopy of which results have not been processed in this article. BC = bacterial count, WBC = white blood cell count, IMI = intra mammary inoculation.

S. aureus challenge preparations

The *S. aureus* field strain was maintained on a horse blood agar plate. Colonies from the plate stocks were streaked on defibrinated horse blood agar. After 24 hours of incubation at 37°C isolated colonies were inoculated in duplicate into 1 ml of phosphate buffered saline (PBS) with pH 7.4, and incubated for 6 hours at 37°C. Hundredfold dilutions (to 10^{-6}) of the culture in sterile PBS solution were prepared, and 0,1 ml of each dilution was cultured in duplicate on a blood agar plate and incubated for 24 hours at 37°C.

The stock broth culture was maintained at 4°C during the incubation of the plates. After the inoculum density of the stock culture was determined, the broth was diluted sufficiently to provide an inoculum of approximately 58 CFU/ml of PBS. The suspension was placed in an ice chest to be transported to the animal house facilities for the actual inoculation.

Inoculation

Just before inoculation, the goats (159 and 94) were milked. The teats were, after general cleaning of the teats with paper towel, swabbed with 70% alcohol. Surgical sterile gloves were worn during the procedure. 1 ml of the prepared suspension was sucked into a sterile syringe using a sterile needle. Then the needle was replaced by a blunt needle of which the tip was removed in order to make the needle less sharp and which was autoclaved. 1mL of the inoculum was then gently infused into the right mammary glands of goats 159 and 94.

Statistical analysis

Statistical analysis were performed on data collected at the general health examinations just prior to the inoculation and 6, 12, 24 and 48 hours after inoculation. Also data derived from the blood samples that were collected just prior to and 24 hours and 7 days after inoculation, were analyzed. These data were analyzed with the SPSS program PASW Statistics 18 (SPSS inc, Illinois, 2009), using an independent-sample T-test. This was performed both on all data originating from one sample moment and on the collection of data originating from all sample moments after inoculation. The groups to compare were in all cases the two inoculated goats (number 159 and 94) and the control animals (number 1000 and 188).In outcomes with p-values lower than 0.05 the groups were considered to be significantly different. To correct for the large numbers of tests that have been performed a Benjamini and Hochberg False Discovery Rate (©Silicon Genetics, 2003) has been performed.

Statistical analysis of the results from all bacterial counts of the milk samples were performed using the program Graphpad Prism 5.02 (GraphPad Software, inc, 2009). With this program a Fisher's exact test was carried out on data collected from all bacterial counts. Again this test was performed on data originating from one sample moment and on the collection of data originating from all sample moments after inoculation. In outcomes with p-values lower than 0.05 the groups were considered to be significantly different.

RESULTS

Bacteriological results

S. aureus colonies were recovered from milk of the right udder half of one inoculated goat (94) at 6, 12, 24 and 48 hours after inoculation and from milk of the right udder half of the other inoculated goat (159) at 24 and 48 hours after inoculation (Table 2). All agar plates that showed *S. aureus* colonies were pure cultures of this bacteria. S. aureus was not isolated from the milk samples collected from the left udder halves of both the inoculated goats (94 and 159) and from milk from both udder halves of the uninoculated goats (188 and 1000). A significant difference was found between the amount of *S. Aureus* recovered from the milk originating from the right udder halves of the inoculated goats (94 and 159) and the amount of *S. aureus* in the milk originating from the right udder halves of the controls (goat 1000 and 188). The amount of *S. aureus* CFU found ranged from 500 CFU per ml at 48 hours after inoculation in goat 159 to more than 30.000 CFU per ml at 6 and 12 hours after inoculation in goat 94 and at 24 hours after inoculation in goat 159. Unspecified bacteria other than *S. aureus* were found in milk samples deriving from all udder halves except the right udder half of goat 94.

White blood cell results

In all the white blood cells (neutrophils, lymfocytes, monocytes, eosinofils, basofils and total of white blood cells) no significant differences were found between the inoculated and control animals. The found p values for blood analysis all were above 0.05 and ranged (after correction for the number of tests) between 0.835 and 0,108.

General health examination results

No obvious clinical signs (e.g. abnormal temperature or abnormal milk) were observed in the inoculated goats (numbers 159 and 94) after intra mammary infusion of the *S. aureus.*

There were no significant differences in the observations made at the general impression between inoculated animals and control animals. During the infection trial both inoculated and control animals were alert but relaxed or exited. Inoculated goats were not significantly more or less exited during the trial then the control animals. All animals were standing normal on all fours during all sampling moments. All animals did have either a normal vocalisation or were screaming during the infection trial, but again there was no significant difference in vocalisation of inoculated and control animals.

Table 2: Results of bacterial counts of milk samples

Goat ID + Udder half	0 hrs	6 hrs	12 hrs	24 hrs	48 hrs
Goat 94 Left	-	++ UB	+ UB (100CFU/ml)	+ UB (500CFU/ml)	+ UB (200CFU/ml)
Goat 94 Right	-	++ SA	++ SA	+ (4100CFU/ml) SA	+ (1600 CFU/ml) SA
Goat 159 Left	+ (600CFU/ml) Mixed culture UB	-	-	-	-
Goat 159 Right	-	+ UB (100csu/ml)	-	++ SA	+ (500CFU/ml) SA
Goat 1000 Left	-	-	-	-	-
Goat 1000 Right	+ UB (100CFU/ml)	+ UB (100CFU/ml)	+ UB (100CFU/ml)	-	-
Goat 188 Left	+ UB (100csu/ml)	+ UB (100CFU/ml)	-	-	-
Goat 188 Right	-	-	-	-	-

Table 2. Results originating from milk samples taken just prior to inoculation (0 hrs) and 6, 12, 24 and 48 hours after inoculation. Only the results from the agar plates with lowest dilution (1/100) are shown. SA = *Staphylococcus aureus* colonies, UB = unspecified bacterial colonies, ++ = more than 30.000 colonies per ml, + = growth lower than 30.000 colonies per ml with number of colonies given, - = no growth of bacteria. The red boxes represent the inoculated halves. Only growth of SA in right udder half of goat 94 at 6, 12, 24 and 48 hours and in right udder half of goat 159 at 24 and 48 hours. Goat 1000 and 188 both show no growth of SA in both udder halves.

The respiration rates of all goats except goat 188, did rise on the time of inoculation, with the highest value of 100 breaths/minute found in goat 1000 (figure 2). The values ranged between 8 and 100 breaths/minute. There were no significant differences in respiration rates after inoculation between inoculated (mean 36,5 breaths/minute with standard deviation (sd) 11,6) and not inoculated goats (mean 46 breaths/minute, sd 21,274). On the contrary, the highest respiration rates were measured in a not inoculated goat (number 1000). The respiration rates of both the inoculated and the control goats did not stay during the trial within usually used reference values (15 – 20 breaths/minute (Matthews, 1991)) for goats.

Heart rates ranged during the infection period from 108 beats per minute found 6 hours after inoculation in goat 94 to 140 beats per minute on 48 hours after inoculation in goat 159 (figure 3). There were no significant differences in heart rates after inoculation between inoculated (mean 124,13 beats/minute, sd 10,535) and not inoculated goats (mean 125 beats/minute, sd 11,662). The heart rates of both the inoculated and the control goats did not stay during the infection within usually used reference values (70 – 95 beats/minute ((Matthews, 1991))for goats.

Rectal temperatures of the four goats ranged during the infection period between 38,8°C found 24 hours after inoculation in goat 1000 and 40,4°C on 12 hours after inoculation in goat 94 (figure 4). There were no significant differences in rectal temperature after inoculation between inoculated (mean 39,7°C, sd 0,4567) and not inoculated goats (mean 39,413 °C, sd 0,4324). The rectal temperatures found in both the inoculated and the control goats were within usually used reference values (38,6 – 40,6°C (Matthews, 1991)) at all times during the infection trial.

No abnormalities were found in the udder examination in all goats during the trial. No abnormalities were found in the udder, de milk extraction was easy at all times and milk was white and milk clots were absent at all times during the trial.

Figure 2: Respiration rates of the individual goats on different moments during the infection trial
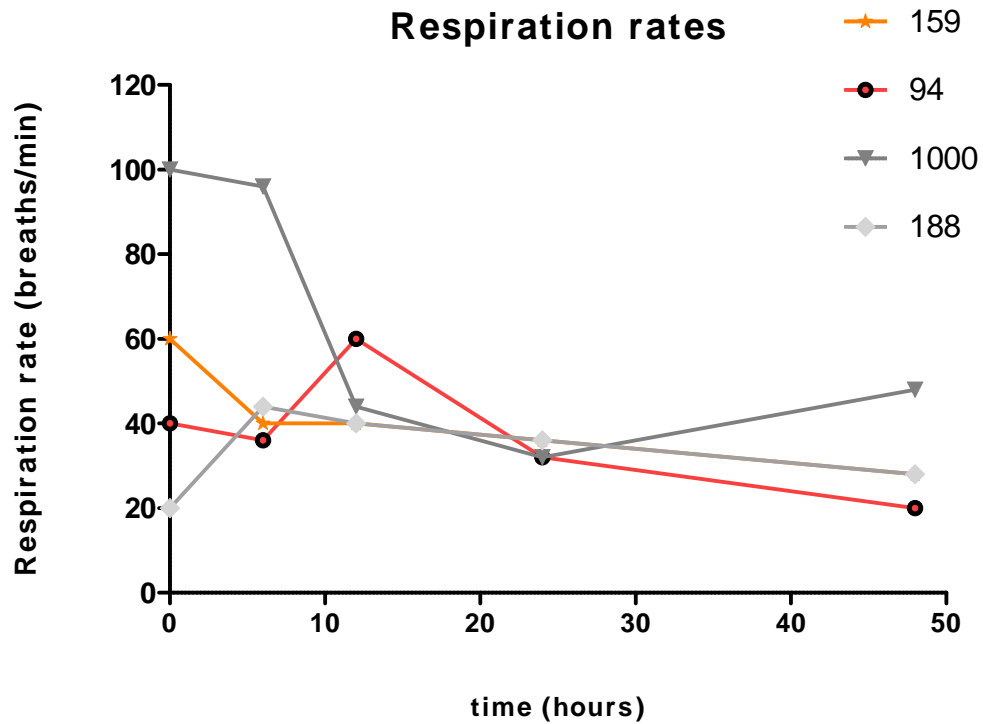
Figure 2. Respiration rates recorded on different moments during the trial in breaths per minute. Each line represents a different goat as shown in the legend. 0 hours = just before inoculation of the goats 159 and 94 with *Staphylococcus aureus*

Figure 3: Heart rates of individual goats on different moments during the trial
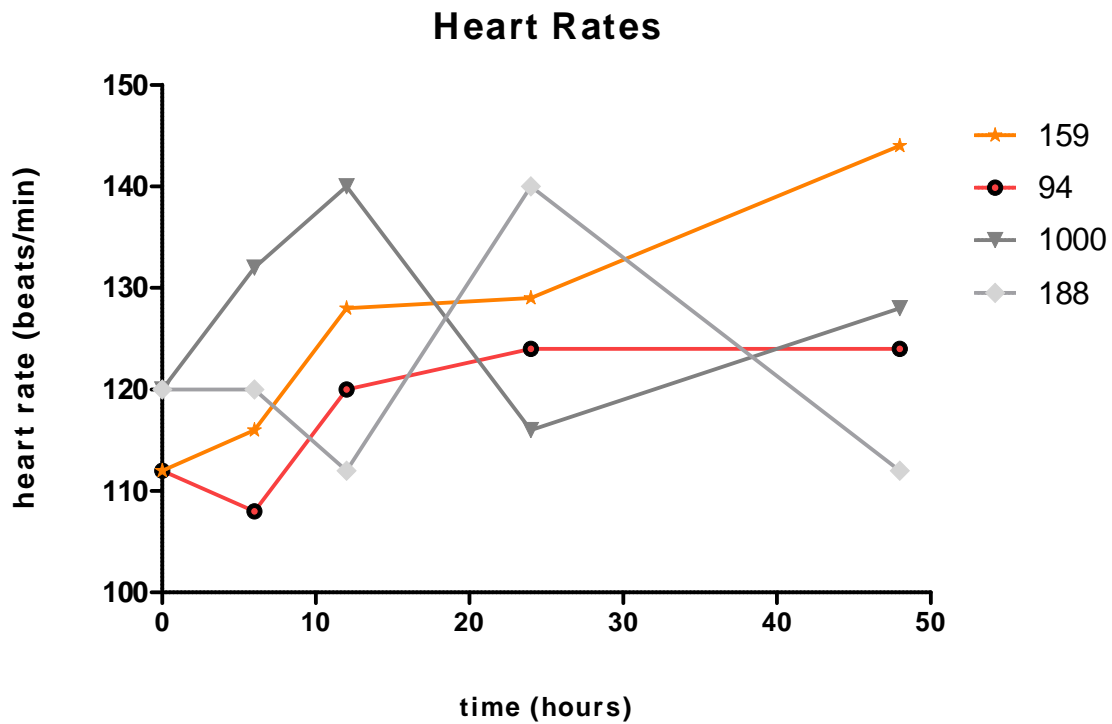
Heart Rates

Figure 3. Heart rates recorded on different moments during the trial in heartbeats per minute. Each line represents a different goat as shown in the legend. 0 hours = just before inoculation of goats 159 and 94 with *Staphylococcus aureus*,

Figure 4: Temperatures of individual goats on different times during the trial
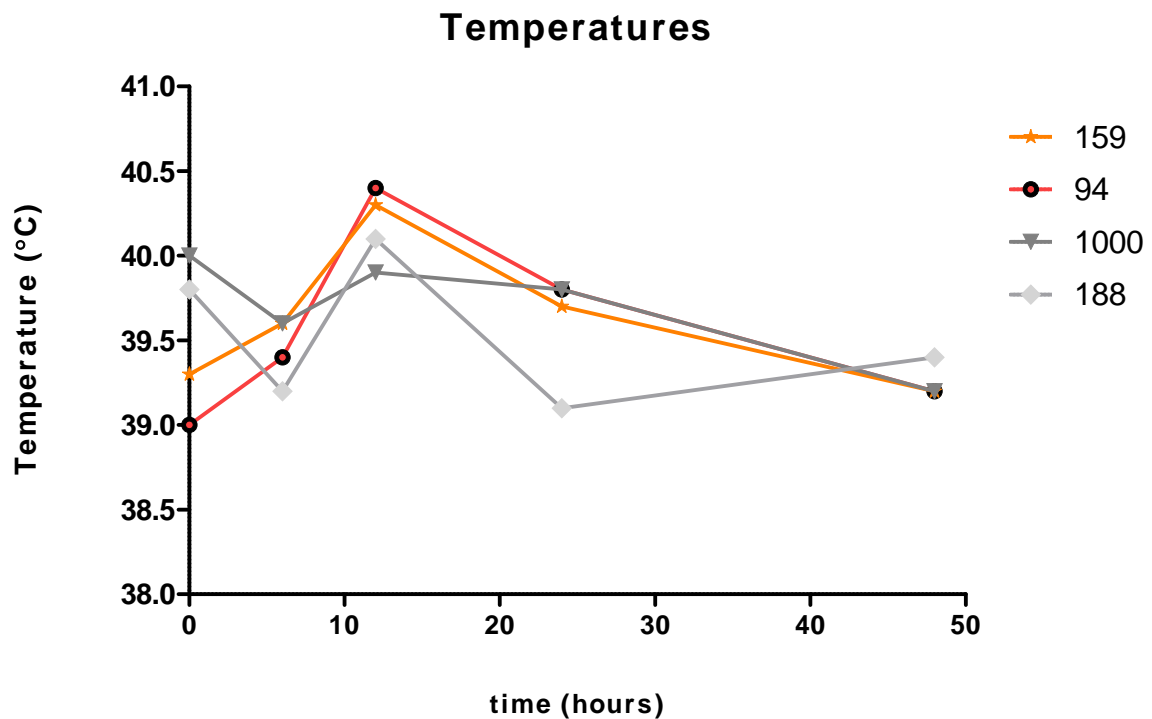
Temperatures

Figure 4. Rectal temperatures recorded on different moments during the trial in degrees Celcius. Each line represents a different goat as shown in the legend. 0 hours = just before inoculation of goats 159 and 94 with *Staphylococcus aureus*,

DISCUSSION AND CONCLUSIONS

Over the past ten years a number of studies have been performed in which animals (mainly cattle) were experimentally infected with *S. aureus*. In these studies several different doses were used depending on the aim of the research. In cattle doses between 8×10^3 CFU (Prenafeta et al., 2009) and 150 CFU (Rivas et al., 2002) of *S. aureus* were used with the aim to induce a clinical infection. Also in dairy goats researchers have tried to induce clinical mastitis using doses of 18×10^3 CFU (Rainard, 2007) and 1×10^4 and 1×10^8 CFU (Ma et al., 2007) of *S. aureus*. However no research has been done on the correct dose of *S. aureus* for introduction of a subclinical mastitis in dairy goats.

In this pilot study therefore has been tried to induce for the first time a subclinical mastitis with *S. aureus* using a dose of 58 CFU.

Bacterial counts

Although both challenged goats showed a clear infection based on bacterial counts at the end of the trial, the course of infection differed between the two goats. Where the milk from the right udder half of goat 94 showed the highest recovery of *S. aureus* at 6 and 12 hours after inoculation, milk from the right udder half of goat 159 showed the highest amount of CFU at 24 hours after inoculation. Moreover goat 159 didn't show any *S. aureus* in her milk at 6 and 12 hours after inoculation. This was not expected since only 6 hours before 58 CFU of *S. aureus* were inoculated in her udder and later in the trial she showed a very high CFU in her milk. In other infection trials researchers found a high amount of CFU of S. aureus in milk directly after the inoculation (Brouillette et al., 2004; Prenafeta et al., 2009) as in goat 94. Furthermore most studies have found the highest CFU at 24 hours after inoculation as in goat 159 (Brouillette et al., 2004; Prenafeta et al., 2009; Rainard, 2007; Reinosos et al., 2002).

The absence of *S. aureus* in the first hours after inoculation in the milk of goat 159 can have several explanations. Firstly *S. aureus* could have been present but was possibly not found because of chance. This could also be the case in all other the negative milk samples that have been found. However all negative findings in the left udder halves of the inoculated goats and in both udder halves of the uninoculated goats are in agreement with the expectations. It is also possible that the infection of the right udder half of goat 159 wasn't successful. Perhaps the bacteria couldn't colonize in the udder, or were killed immediately by the host defence mechanisms of the udder. If this is the case the shedding of bacteria in the milk later (at 24 and 48 hours after inoculation) can only be explained by a new infection that has taken place. The source of this new infection could be the other infected goat (94) that was housed in the same shed and therefore was in close contact with goat 159. A third possible explanation is that the bacteria did colonize the udder but couldn't multiply in the udder because the environment in the udder wasn't adequate for the bacteria. An not adequate environment could for example be a lack of energy sources available in the udder for the bacteria or an incorrect pH in the udder. If this is what happened it took the bacteria longer to multiply to the high level found after 24 hours.

These findings show the individual differences of goats that can occur after a challenge with *S. aureus*. Further research should be conducted using large sample sizes to study normal reaction patterns of bacterial counts in goat milk. This can be important because it can give insights in immunology processes inside the goat udder and in the development of an infection caused by *S. aureus*. Also future studies should perform a control procedure (for example phenotypic or genotype characterisation) to ensure that the isolated *S. aureus* are of the same strain as the one used for inoculation.

In several milk samples other bacteria than *S. aureus* were found. These can either point out a subclinical infection or can be the result of contamination of the sample. Because at all sampling moments samples have been found with no growth of bacteria at all or pure cultures of bacteria, it is not very likely that the found unspecified bacteria are simply the result of contamination. If the other bacteria found in the milk samples are the result of a subclinical infection, then their presence can potentially alter the chance that the inoculation with *S. aureus* leads to an infection (Schukken et al., 1999). However because none of the other bacteria have been specified, no conclusions can be drawn from their presence.

White blood cell counts

The first line of defence of the udder is formed by neutrophils that rapidly migrate to the udder in response to an inflammation. (Paape & Capuco, 1997). When neutrophils leave the blood it is expected that their number in the blood temporary declines. No differences were found in this study between the number of white blood cells of any type between the infected and the control animals. This implicates that no or very few immune cells have migrated from the blood to the mammary gland. This is in agreement with the findings in the clinical results. If immune cells and especially neutrophils would have been involved in the inflammation mammary tissue damage would have been expected which would have become visible in clinical abnormalities.

General health examination

These goats were only followed during the first 48 hours after inoculation. The infected goats did not differ significantly during these 48 hours in their clinical examination from the control animals. This could be expected because of the low dose that was used. Other studies with higher doses of *S. aureus* did all find a clinical response on the presence of the bacteria within 24 hours after the challenge (Ma et al., 2007; Rainard, 2006; Reinoso et al., 2002; Castro-Alonso et al., 2009). Thus it is not probable that the trial was too short to be able to notice any symptoms because the goats would have developed them in the first 48 hours after the challenge.

Although the clinical values of the infected goats did not significantly differ from these of the control animals, they did exceed the reference values at times in the trial. Because there were no significant differences in the values between the two groups this exceeding can probably be attributed to environmental factors. These environmental factors can be for example the outdoor temperature that was very high at the day of inoculation (29,3°C) and that had risen very abruptly from 24,4°C the day before (Australian Government, Bureau of Meteorology). This in combination with the stress of the examination could explain the higher values. Furthermore could be expected that the infected animals were more excited and therefore showed higher temperature, respiration and heart rates, because they were also separated from their kids during the trial. Consequently the highest values for heart rate and temperature were measured in the infected goats. However the highest respiration rate was measured in goat 1000 which was not infected. This could however be the result of individual variances. Goat 1000 was a very stressed animal and hard to handle in comparison to the other goats.

Statistical analysis

The usage of t-tests could be questioned because of the small numbers of animals used in this study. To obtain a larger number of data in the t-tests data the collection of data collected after inoculation was used to compare the two groups (inoculated and not inoculated). However with doing this the correlation of data that have been collected from one goat has not been taken into account. Future research can overcome this problems by using a bigger number of animals in their trial.

According to the results of the bacterial counts the inoculation of the right udder halves of goat 159 and 94 has lead to an infection of this udder halves while the control animals did not show an intra mammary infection. Furthermore none of the animals (inoculated or not inoculated) showed any clinical signs nor differences in blood values during the trial. A subclinical mastitis is the infection of the udder without clinical symptoms. Based on these results can be concluded that this pilot study has successfully induced a subclinical mastitis.

Further research could focus on set ups with more animals involved to be able to map the different reactions of animals on a certain infection dose. Furthermore it is important to study mastitis in dairy goats in general, in order to find good and practical diagnostic tools. Milk from lactating goats may contain certain proteins, involved in the immune response, that show a change in quantity during infection of the udder. These could be used to detect an intra mammary infection and are therefore referred to as biomarkers. These biomarkers could also allow valuable insights in the processes occurring in the udder of a goat during infection. To identify biomarkers in goat milk new techniques are now available. Two dimensional gel electrophoresis and tandem mass spectroscopy can be used to differentiate and determine the properties of the proteins in milk (Smolenski et al., 2007). Later these biomarkers could be compared to biomarkers found in milk originating from cattle with *S. aureus* subclinical mastitis. Dairy goats could serve as animal model for subclinical mastitis in cattle if the found similarities would be significant.

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APPENDIX 1
Example of a form used for administration of general health parameters.

Goat Health Monitoring Protocol in Mastitis Biomarkers Research Project

Identification

Ear number goat	Name examiner	Date	Time
188	Nannie	28-10-09	15.15

General impression

	State/ findings	Comments
Demeanour	Stupor - Lethargic - Alert but relaxed – exited	
Stance	Stretched back - Normal on all fours - arched back - prancing	
Position	Standing - laying down in cost-sternal position - laying down on flank	
Food/water intake	Reduced – normal - increased ?	
Vocalisation	No – normal – teeth grinding – screaming	

General Health Check

	Findings	Comments
Respiration rate	< 15-20/min (normal)>	44
Hart rate	< 70-95/min >	120
Temperature	< 38.6-40.6°C >	39,2
Rumination	< 1-1.5/min >	
Comments		

Udder examination

	Findings	Comments
Temperature	Cool to the touch – hot to the touch	warm
Swelling	No – little/local – mediate – generalised- extended	because of pressure
Colour	Pale white - orange/pink - red black	of milk
Pain	Not painful - slightly painful – severely painful to the touch	
Lumps	No – some – mediate - many	
Injuries to teats/udder	Absent - present	
Milk extraction	Easy - difficult - painful	
Milk colour	White/bleu-ish – yellow (colostrum/pus) – red (blood) - watery	
Milk clots	Absent - present	
Comments		

Action

Good nursing: rug, heat and human company Medication: Antibiotic + NSAID type/dose/sequence

Dairy goats - a rare species or a suitable animal model to study intra mammary infections in dairy cattle?

APPENDIX 2

In table below all results are shown from blood samples of all goats at different times during the study.

Sample moment WBC	Goat 159	Goat 94	Goat1000	Goat188
D20	15.5 x 10^9/L	11.4 x 10^9/L	9.6 x 10^9/L	11.62 x 10^9/L
Prior to Inoculation	11.3 x 10^9/L	6.92 x 10^9/L	11.7 x 10^9/L	10.7 x 10^9/L
Inoculation + 24h	10.8 x 10^9/L	3.88 x 10^9/L	9.96 x 10^9/L	11.6 x 10^9/L
Inoculation + 7d	14.2 x 10^9/L	8.25 x 10^9/L	7.04 x 10^9/L	10.6 x 10^9/L
NEU				
D20	11.4 x 10^9/L	6.04 x 10^9/L	4.42 x 10^9/L	7.90 x 10^9/L
Prior to Inoculation	5.92 x 10^9/L	4.02 x 10^9/L	5.21 x 10^9/L	6.34 x 10^9/L
Inoculation + 24h	5.06 x 10^9/L	1.12 x 10^9/L	5.18 x 10^9/L	7.35 x 10^9/L
Inoculation + 7d	7.63 x 10^9/L	5.09 x 10^9/L	3.45 x 10^9/L	6.06 x 10^9/L
LYM				
D20	4.4 x 10^9/L	5.24 x 10^9/L	4.80 x 10^9/L	3.72 x 10^9/L
Prior to Inoculation	4.07 x 10^9/L	1.33 x 10^9/L	6.08 x 10^9/L	3.28 x 10^9/L
Inoculation + 24h	4.84 x 10^9/L	1.72 x 10^9/L	4.42 x 10^9/L	3.61 x 10^9/L
Inoculation + 7d	4.69 x 10^9/L	1.80 x 10^9/L	3.31 x 10^9/L	3.98 x 10^9/L
MONO				
D20	0.15 x 10^9/L	0.00 x 10^9/L	0.29 x 10^9/L	0.00 x 10^9/L
Prior to Inoculation	0.759 x 10^9/L	1.04 x 10^9/L	0.291 x 10^9/L	0.741 x 10^9/L
Inoculation + 24h	0.526 x 10^9/L	0.696 x 10^9/L	0.226 x 10^9/L	0.403 x 10^9/L
Inoculation + 7d	0.482 x 10^9/L	1.00 x 10^9/L	0.216 x 10^9/L	0.238 x 10^9/L
EOS				
D20	0.00 x 10^9/L	0.11 x 10^9/L	0.096 x 10^9/L	0.00 x 10^9/L
Prior to Inoculation	0.363 x 10^9/L	0.315 x 10^9/L	0.093 x 10^9/L	0.163 x 10^9/L
Inoculation + 24h	0.340 x 10^9/L	0.250 x 10^9/L	0.123 x 10^9/L	0.123 x 10^9/L
Inoculation + 7d	1.39 x 10^9/L	0.267 x 10^9/L	0.060 x 10^9/L	0.355 x 10^9/L
BASO				
D20	0.00 x 10^9/L	0.00 x 10^9/L	0.00 x 10^9/L	0.00 x 10^9/L
Prior to Inoculation	0.142 x 10^9/L	0.221 x 10^9/L	0.011 x 10^9/L	0.143 x 10^9/L
Inoculation + 24h	0.047 x 10^9/L	0.096 x 10^9/L	0.009 x 10^9/L	0.100 x 10^9/L
Inoculation + 7d	0.00 x 10^9/L	0.085 x 10^9/L	0.00 x 10^9/L	0.00 x 10^9/L

Results from analysis of blood samples. WBC = White Blood Cells, NEU = Neutrofils, LYM = Lymfocytes, MONO = Monocytes, EOS = Eosinofils, BASO = Basofils. D20 = 20 days after parturition.