Prevalence of intramammary infections and relationship with somatic cell counts in Italian buffaloes

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INTRODUCTION

World milk production has doubled in the last decades and it is noteworthy that in the last years, buffaloes have been supplying about 12% of the world milk production. India and Pakistan have been producing 60 and 30%, respectively, of the world's buffalo milk. In both these countries buffalo milk contributes to 55 and 75% of the total milk produced (FAO, 2004).

Dairy buffalo production has been a tradition in many parts of the world like the Caucasian countries, Asia, and Egypt where fresh buffalo milk, dahi (cultured sour milk), ghee (butter oil), and yoghurt are popular. In Italy the dairy buffalo industry is flourishing on account of the popularity of buffalo mozzarella cheese. that is the first buffalo milk producer in Europe. Italian River Buffalo (*Bubalus bubalis*) rearing is historically proper of middle and southern regions. In recent years there has been a trend to install this kind of rearing in the north of Italy, trying to encounter consumers interest and, above all, avoiding restriction imposed by the European Community quotas policy to bovine farmers. As well as cows also for dairy buffaloes the most frequent disease and the main cause of loss is mastitis, although their apparent rusticity. The loss in milk production is even more evident and remarkable as buffaloes yield every day only about 8 litres. Elevate somatic cell count (SCC) is associated with a reduced milk yield and a clear alteration in milk components concentration (Harmon, 1994; Piccinini et al., 2006). Until now few studies have dealt with SCC in this species, trying to identify a threshold able to represent an udder inflammation marker, as well as 200.000 cell/ml is universally recognized in dairy cows (Dhakal, 2006). In general SCC is lower than in dairy cow but there's the caveat that a low value could not signify the absence of infection (Carvalho et al., 2008).

In this survey two Italian herds of dairy buffaloes, different in management, were followed at a quarter level during an entire lactation. The aim is to determine fluctuation of intramammary infections (IMI), Somatic Cell Score (SCS), milk production and their mutual phenotypic relationships.

MATERIALS AND METHODS

Two dairy herds placed in Northern Italy were monitored across a whole lactation. They were so characterized: the first herd consisted of 28 buffaloes with seasonal calving (from January to March), harboured in free-stalls, in a paddock with concrete floor cleaned by flushing; the second one practised seasonal calving too (between April and May) and the selected 20 buffaloes lived in free-stall on a dirt permanent bedding. In 10 distinct test days throughout the lactation, for each lactating buffalo milk yield was registered and quarter milk samples were aseptically collected into sterile tubes. A total of 1760 observations were considered. Each sample underwent microbiological analyses to access infected/uninfected status of the udders and the responsible bacteria according to recommendations of National Mastitis Council (NMC, 1999). Somatic Cell Score (SCS) was determined by an automated fluorescent somatic cell counter (Bentley Somacount 150, Bentley Instrument, Italy). Fat and protein rates were assayed on composite milk by an automated infrared absorption spectrophotometric analyser (Milkoscan, Foss, Hillerød, Denmark). Statistical analyses focused on the relationships between infection and SCC and the other registered parameters: parity, day in milk, milk yield, position of the quarter, fat and protein percentages and random factors depending on single animal and quarter.

RESULTS

Mean values obtained from a basic statistics are shown in Table 1. The concentrations of milk components are consistent with previous data and it is relevant that the Somatic Cell Score (LS) is lower in buffaloes than in cows and that the prevalence of infection, both at animal and quarter level, is high in both farms.

Table 1: basic descriptive statistics.

Trait	Mean
Milk (kg)	7.17
Fat (%)	8.88
Protein (%)	4.88
SCS (LS)	2.70
Infection status (%)	61
Infected cows (%)	100
Infected quarters (%)	99

Bacteriological culturing of milk samples gives an idea of the distribution of pathogens among these buffaloes herds. Quarters with negative bacterial cultures represent 39%, even if no quarter remained negative for all the trial. Considering samples yielding bacterial growth, the most common isolates were Coagulase Negative Staphylococcus (CNS) with a frequency of 78%, followed by 16% of *Streptococcus* spp. among which *Streptococcus uberis* accounted for the 80%. There was then a 3% of environmental microrganisms (*Bacillus* spp., yeasts, *Proteus* spp.). *Staphylococcus aureus* was not isolated in this survey.

Proportion of quarters infection resulted the least at the beginning of lactation and then raised up to the peak keeping an high prevalence between 60% and 70% during the middle of lactation. This trend is also traced by the new intramammary infections. Towards the end of lactation (255-270 days) infected quarters reached the maximal percentage, in concomitance with the highest risk of new infections and their consequent increase (Figure 1). The trend of mean SCS is substantially consistent with the infectious status: from a minimum at the onset of lactation, it raised across the days in milk to SCS 4 in the proximity of the milk off (Figure 2).

Figure 1: proportion of quarters infected (■) and new infections (▲) across lactation

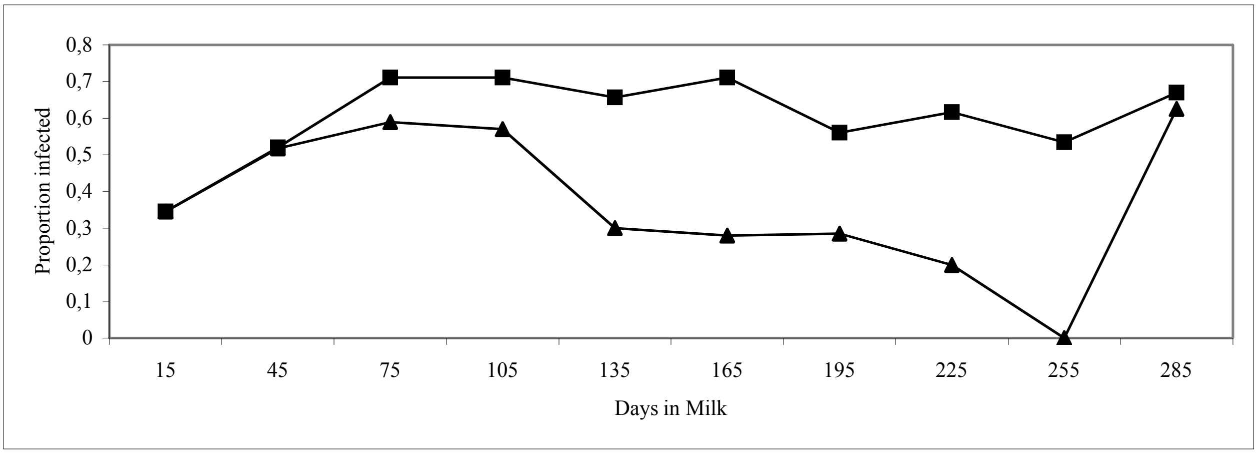

Figura SCS and the considered factors were confirmed by statistical analyses. The trend of the somatic cell score tends to increase within days in lactation and to be greater in winter and spring months (P <0.0001). It has a negative correlation with milk production (P <0.02) and a positive correlation with parity (P <0.0004), lowest in the 1st parity and highest in the 2nd one. SCS is greater in fore quarters (P <0.0002) maybe for a diluting effect in rear quarters that generally produce more milk. The infectious status of the udder affects the flow of the somatic cell score (P <0.01). Finally the elevation of the score depends on the infecting bacterial species (P <0.004): the greatest score is observed in quarters infected by *Streptococcus* spp. (above all *Streptococcus uberis)*, followed by environmental bacteria and the least one in CNS infections.

Milk yield followed a trend similar to cow lactation, with a peak around 45th day in milk and then a gradual decline. It had positive correlation with probability of IMI and a negatively influenced by high SCS. Rates of fat and protein didn't demonstrate a relationship with SCS: fat concentration raised across lactation as a consequence of diminished yield while protein percentage registered no significant variation.

DISCUSSION

Buffaloes are reported to have anatomic, histological and physiological features both of udders and teats that make them more resistant to mastitis (Carvalho et al., 2007). It agrees with the finding in this study of a low counting in somatic cell score also in affected quarters (SCS mean = 2.70) which is much less than is found in dairy cattle. Nevertheless, in this study intramammary infections revealed a remarkable frequency that is consistent with other reports: subclinical mastitis in Nepal are declared with a prevalence of 21,7 % justified by Dhakal (2006) with the predilection of buffaloes for wet, unhygienic and dirty environment and with close contact between healthy and infected animals. In another survey (Dhakal et al., 2007) the trend of clinical mastitis is explained not only through these poor hygienic conditions, but also with their association with temperature and humidity. It could explain the rising found in the survey of mastitis found in spring (increasing in temperature) and in winter (increasing in humidity).

Since the probability of infectious status raises with parity and all infected quarters have SCC > 200,000 cell/ml, it is possible to infer that the damage caused by subsequent/persistent infection increases across lactations and impairs udder defensive mechanisms. This point is confirmed by the observation that SCC slightly changes with increasing age if the quarter is uninfected (Harmon, 1994). Pal and Verma (1988) reported an increasing in clinical mastitis through lactations, with the highest incidence of them at 3rd calving. Other studies found a decreasing trend in clinical mastitis within lactations, with the greatest number of clinical mastitis at their 1st calving (Dhakal et al., 2007), . These differences among reports may be due to factors such as season and herd management. The positive correlation seen

between the somatic cell score and parity is also present between SCS and days in milk. The same trend is also seen in new intramammary infections while other studies have found higher incidence of clinical mastitis at the beginning of lactation (Dhakal et al., 2007; Yas et al., 1983) and declined in subsequent months. The reason may be explained with a decreased host defence capability during the post parturient period as observed widely in dairy cows (Nagahata et al., 1992). Concerning isolated bacteria, we registered the confirmation of CNS as the main responsible for IMI in lactating buffaloes (Dhakal et al., 2007) followed by *Streptococcus uberis* that causes the major raise in somatic cell content. In contrast with other survey (Dhakal, 2006; we didn't isolate *Staphylococcus aureus*, *E. coli, Corynebacterium* spp., *Micrococcus* spp.
Inflammation and its mediators alter the mammary epithelium physiology reducing synthetic activity that reflect on milk composition. The oxidative stress, with the increase of lipid peroxidation of cellular membrane, could have a role in this involution (Harmon, 1994; Kumar et al., 2007). Actually milk yield is negatively correlated with high SCS, in accordance with previous work (Piccinini et. al, 2006).
What joins dairy cows and buffaloes is the reduction in productivity and milk quality impaired by mastitis, parameters so important for farmers and transformation industry (Dhakal et al., 2007; Carvalho et al, 2007). In India it was recently estimated that the 72% of loss in milk production is due to subclinical mastitis (Kumar et al., 2007). Also in previous survey, mastitis appeared as the first item in incidents requiring treatment, followed by abortion and other obstetrical disorders (Cady et al., 1983).
Carvalho et al. (2007) described a lower somatic cell count than in cows and described California mastitis test as a bad indicator of infections with a higher frequency of test negative with a microbiological isolation than an isolation accompanied by a positive test. In our work somatic cell content resulted a good marker in recognizing all infected quarters through SCC >200,000 and confirming healthy quarters with SCC below this threshold.

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