ESTIMATION OF SOMATIC CELL COUNT, AS GOLD STANDARD TO DETECT SUBCLINICAL MASTITIS IN DROMEDARY CAMEL


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ABSTRACT

The objective of the present study was to estimate cut-off point of somatic cell count (SCC) to detect subclinical mastitis using mathematical modelling in dromedary camel. Camel milk samples were collected from individual quarters (n=243) of 25 milking camels. Approximately five minutes prior to milking, camel received oxytocin (2011U, IV), the test was washed and the camel calf was released to stimulate the dam. After 2 minutes, the suckling was interrupted and the teat was dried with tissue and sampling was conducted to perform CMT, and to collect milk for SCC. The range of SCC corresponding with CMT scores of 0, 1, 2 and 3 were 0-51000, 57000-108000, 116000-306000, 342000-835000 and 2129000-6135000, respectively. The threshold values for SCC to detect subclinical mastitis in camel were calculated by considering two different approaches: frequentist analysis (306000 cells/ml) and Bayesian analysis (590000 cells/ml). In conclusion, SCC values beyond 206000 cells/ml could be considered as subclinical mastitis in camel.

Key words: Dromedary camel, SCC, subclinical mastitis

Prevalence of mastitis in camel was assumed to be low due to the thin streak canal, covering udder to restrict suckling (Manefield and Tinson, 1996; Warnery and Kaaden, 2002), the least contact of udder to contaminated bed throughout rest period (personal observation), the low density of population due to scattered individuals throughout the pasture and finally the common practice of hand milking rather than machine milking. Although machine milking has been adopted for camel in very few countries (Yagi, 1982; Nagy et al., 2013), dairy camel industry still depends on hand milking in most countries worldwide. It is expected that by machine milking can predispose camels to mastitis, particularly in the subclinical form.

The prevalence of subclinical mastitis in this species varied among different studies (15-67.4%); Bhatt et al., 2004; Aher et al., 2010; Seifu and Tafebese, 2010; Alamini et al., 2013). Part of this variation could be due to the variety of methods used to identify subclinical mastitis including CMT, SCC, and bacteriological investigation in this species. Although, SCC has become the gold standard to measure milk quality, there is no cut-off point estimated for somatic cell count (SCC) to detect subclinical mastitis in dromedary camel. The objective of this study was to investigate the cut-off point of SCC, as a gold standard, to detect subclinical mastitis in dromedary camel.

Materials and Methods

The present study was conducted during 2012 and 2014 between months May and June, in Golestan Province the main region for dairy camel industry in I. R. Iran. Dromedary milking camels (n=95), 7-11 years of age, 2-4 months after calving, with the average daily milk production of 6 kg, were used in this study. They were milked manually trice daily (5:00, 16:00, 21:00) and maintained on pasture throughout the day.

California mastitis test was carried out according to the method described previously (Schalm and Noorlander, 1957) for cow. In brief, at camel-side, each quarter milk sample was placed in one clean well of a special plastic test paddle and mixed with an equal volume of commercial CMT

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solution (Kruuse, UK). As the plate was rotated gently, any changes in the colour and consistency were observed and interpreted: Scores were given within the range of 0-3: with 0 for no reaction, T for a trace (slight thickening that tends to disappear with continued movement of the 'paddle'), 1 for a weak positive (distinct thickening, but no tendency toward gel formation), 2 for a distinct positive (mixture thickness immediately) and 3 for a strong positive (adhered gel). Accordingly, scores 1 and 2 were considered as suspicious and scores 2 and 3 were considered as positive for subclinical mastitis.

Somatic cell count was done from milk samples collected into the tube with potassium dichromate (Frolan, Boches, Switzerland) and were counted using Fossomatic machine (Fossomatic 5000, Fossomatic Company, Denmark). Standard sample consisting of 389,000 cells was used to calibrate the machine before the SCC. Samples of 25 ml volume were assigned into special racks and allowed to be automatically homogenised and counted individually by the detector.

**Experimental design**

Camel milk samples were collected, at morning time, from individual quarters (n=243) of 95 milking camels, without any observable disease and clinical signs of mastitis. Five minutes prior to milking, camel received oxytocin (20 I.U. IM; Aburathian Pharmaceutical Co., Iran), the teat was washed and the camel calf was released to stimulate milk let down from the dam. After 2 minutes, the suckling was interrupted and CMT was performed. After discarding the first few squirts of milk, about 50 ml of milk were collected into sterile bottle. Samples were kept on ice block and transported to the laboratory and examined within 8 hrs after milk collection for SCC measurement.

**Statistical model**

A threshold from both the frequentist and the Bayesian perspectives was investigated. In frequentist analysis, a summary and quantile of the data were used to identify outliers and exhibit the form of the distribution. Then, Maximum Likelihood (ML) method was used to estimate the parameters of the model (Lehmann and Casella, 1998). Bayesian analysis of extreme events was used to estimate the Bayesian interval for threshold (Behrens et al, 2004). In this mixture model, a parametric form for the centre and a Generalised Pareto Distribution (GPD) for the tail of the distribution were used with all observations to infer about the unknown parameters from both distributions (Behrens et al, 2004). Then the algorithm based on Markov Chain Monte Carlo (MCMC) was used to make inferences about the posterior distribution. All of the computer programs and MCMC simulations were performed using R Statistical software.

**Results**

According to CMT results, out of 243 quarters of 95 dairy camels, 122 quarters (50.2%) were negative, 77 quarters (31.7%) were suspicious (CMT scores of T and 1) and 44 quarters (18.1%) were positive (CMT scores of 2 and 3) for subclinical mastitis (Table 1).

The range of SCCs for CMT 0, T, 1, 2 and 3 were 0-51, 57-108, 116-306, 342-8435 and 2129-8435 (x1000) cells/ml, respectively (Table 1). Distribution function of the data could be estimated by considering a distribution function that belongs to the family of extreme value theory. After fitting distribution to data, it was revealed that a Frechet distribution would be an appropriate choice. Selection criteria were based on rank of statistics in three goodness of fit tests, including Kolmogorov-Smirnov, Anderson Darling and Chi-Squared tests (Lehmann and Casella, 1998). The ML method estimates the parameters of the Frechet distribution. These data are well approximated with a distribution which belongs to the family of Generalised Extreme Value (GEV distributions). Accordingly, upper percentiles of the data between 50-97.5% were considered to elucidate a possible threshold value for somatic cell counts and to suggest that SCC greater than 306000 could be considered as abnormal.

In Bayesian analysis, after the outliers were excluded, 100 chains were simulated and in each chain 1000 data were generated. After chain convergence, the mean of threshold value and 83.75% of the Highest Density Region (HDR) were calculated. Accordingly, the lower bound of the HDR was considered as a Bayesian point estimate for the threshold value of 390000 cells/ml.

**Discussion**

Using two different mathematical models: frequentist and Bayesian approaches, the threshold value for SCC to detect subclinical mastitis in camel was suggested to be either 506000 or 390000.
cells/ml, respectively. Defining the cut-off point is one of the most important steps in controlling subclinical mastitis in camel, which is estimated using mathematical modeling in the present study.

CMT and SCC have been used as diagnostic tools to detect subclinical mastitis in camels (Abdurahman et al., 1998; Schepers et al., 1997; Almaw and Molla, 2000; Younan et al., 2001; O’Mahony et al., 2006). In the present study, the range of SCC in relation to CMT was presented for the first time in camel, which revealed that the relationship between the range of SCC and CMT in camel was different from that reported in cattle (Dohoo and Meek, 1982) and goat (Perrin et al., 1997). The range of SCC with the CMT scores of 0, 1 and 2 was between 0 and 360,000 cells/ml whereas, with the same CMT scores, the SCC has been reported to be ≥100,000 cells/ml in cattle (Dohoo and Meek, 1982) and ≥75,000 cells/ml in goat (Perrin et al., 1997). In this study, SCC of ≥32,000 cells/ml had CMT scores of 2 and 3. This has been indicated to be ≥100,000 and >75,000 cells/ml in cattle and sheep, respectively (Dohoo and Meek, 1982, Perrin et al., 1997). As a result, CMT scores (≤1 or ≥2) represents considerably lower values of SCC in camel compared to those in cattle and sheep. Any explanation for such biological difference would be the subject of further research.

If the cut-off point of 306,000 cells/ml is used as the gold standard to detect subclinical mastitis in camel, corresponding to CMT scores of 2 and 3, the apparent prevalence of subclinical mastitis in the present study was 9.46% (23/243 quarters). There has been considerable variation among studies in terms of the prevalence of subclinical mastitis in camel, partly due to the substantial variation in defining criteria and lack of a well-defined cut-off point to determine the prevalence of subclinical mastitis in camel. In this context, using CMT as a criteria to detect subclinical mastitis, the prevalence of subclinical mastitis has been reported to be 36.87% (59/160 camels; Suhir et al., 2005) and 15% (9/60 quarters; Alamin et al., 2013) in Sudan, 15.8% (80/505 quarters; Aberra et al., 2010), 20.7% (30/145 camels; Aberra et al., 2010), 22% (43/195 camels, Almaw and Molla, 2000), 67.4% (433/642 quarters; Seif and Tafesse, 2010), 39.4% (137/348 camels, Regassa et al., 2013) in Ethiopia, 11.67% (21/180 camels; Ibrahim et al., 2011) in Saudi Arabia, 38% (57/150 camels; Sibat et al., 2012) in Pakistan, and 41% (41/100 quarters) and 72% (18/25 camels) in India (Bhatt et al., 2004). Indeed, this variation perpetuate unless we define an appropriate cut-off point for determination of subclinical mastitis in camel. The prevalence of subclinical mastitis in sheep (Bergonier and Berthelot, 2003; Contreras et al., 2003; Contreras et al., 2007) and cow (Plozza et al., 2011) were 5-30 and 11-43%, respectively. Therefore, the prevalence of subclinical mastitis in camel is within the range of subclinical mastitis in other food animals.

The prevalence of mastitis was relatively low in the present study. It is well known that susceptibility to mastitis is determined by a combination of factors including bacterial virulence, environmental conditions (housing, management, feeding and milking technique) and animal-related factors (milk yield, genetics). These factors are interdependent to each other and their impact depends on the type of pathogen (Burvenich et al., 2003). The streak canal is relatively thin in camel which could play a role in low prevalence of mastitis in this species (Manefield and Tinson, 1996). Moreover, the cover used to prevent the calf from suckling has been suggested as a reason for low rate of mastitis in camel (Manefield and Tinson, 1996; Wernery and Kaaden, 2002). It is believed that the cover protects the animal from mechanical traumas. Yet it should be considered that the cover could be moistened with milk and become contaminated with bedding, and consequently predispose the animal to intra-mammary infections. Nevertheless, given no research has been conducted in this regard, any hypothesis requires to be tested by a well-designed controlled study. In addition, machine milking is uncommon in camel, which might have contributed as an additional reason for low prevalence of mastitis in this species. The other suggested factors for low prevalence of mastitis in camel are the type of rearing, few contact of mammary glands with the bedding, low density of animals in the pasture and the dryness of the bedding. Finally, one of the potential factors in this context is the antimicrobial components of camel milk (El-Hattam et al., 2007; Salami et al., 2010). Further studies are warranted to investigate the underlying mechanisms for low prevalence of mastitis in camel.

In conclusion, the present study revealed that there is low number of SCC for different scores of CMT in camel as compared with corresponding figures in other ruminants. Additionally, using frequentist and Bayesian approaches, we defined cut-off point for detection of subclinical mastitis in dromedary camel.

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