Preventive Veterinary Medicine 120 (2015) 306–312

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Pathogen group specific risk factors for clinical mastitis, intramammary infection and blind quarters at the herd, cow and quarter level in smallholder dairy farms in Jimma, Ethiopia

T. Tolosa[a,b], J. Verbeke[b], Z. Ayana[a], S. Piepers[b], K. Supré[c], S. De Vliegher[b,*]

[a] Department of Microbiology and Veterinary Public Health, College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia
[b] M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics, and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[c] Milk Control Centre Flanders, Hagenbroeksesteenweg 167, 2500 Lier, Belgium

ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 6 May 2015
Accepted 7 May 2015

Keywords:
Clinical mastitis
Intramammary infection
Prevalence
Risk factor

ABSTRACT

A cross-sectional study on clinical mastitis, intramammary infection (IMI) and blind quarters was conducted on 50 smallholder dairy farms in Jimma, Ethiopia. A questionnaire was performed, and quarters of 211 cows were sampled and bacteriologically cultured. Risk factors at the herd, cow, and quarter level for clinical mastitis and (pathogen-specific) intramammary infection were studied using multilevel modeling. As well, factors associated with quarters being blind were studied. Eleven percent of the cows and 4% of the quarters had clinical mastitis whereas 85% of the cows and 51% of the quarters were infected. Eighteen percent of the cows had one or more blind quarter(s), whereas 6% of the quarters was blind. Non-*aureus* staphylococci were the most frequently isolated pathogens in both clinical mastitis cases and IMI. The odds of clinical mastitis was lower in herds where heifers were purchased in the last year [odds ratio (OR) with 95% confidence interval: 0.11 (0.01–0.90)], old cows (>4 years) [OR: 0.45 (0.18–1.14)], and quarters not showing teat injury [OR: 0.23 (0.07–0.77)]. The odds of IMI caused by any pathogen was higher in herds not practicing teat drying before milking (opposed to drying teats with 1 towel per cow) [OR: 1.68 (1.05–2.69)], cows in later lactation (>180 DIM opposed to ≤90 DIM) [OR: 1.81 (1.14–2.88)], cows with a high (>3) body condition score (BCS) [OR: 1.57 (1.06–2.31)], right quarters (opposed to a left quarter position) [OR: 1.47 (1.10–1.98)], and quarters showing teat injury [OR: 2.30 (0.97–5.43)]. Quarters of cows in herds practicing bucket-fed calf feeding (opposed to suckling) had higher odds of IMI caused by *Staphylococcus aureus* [OR: 6.05 (1.31–27.90)]. Except for BCS, IMI caused by non-*aureus* staphylococci was associated with the same risk factors as IMI caused by any pathogen. No access to feed and water immediately after milking [OR: 2.41 (1.26–4.60)], higher parity [OR: 3.60 (1.20–10.82)] and tick infestation [OR: 2.42 (1.02–5.71)] were risk factors for quarters being blind. In conclusion, replacement of old cows, prevention of teat injuries/lesions, drying teats with 1 towel per cow before milking, improving fertility in order to shorten the lactation period, allowing (restricted) suckling, access to feed and water immediately after milking, and improving tick control could improve udder health in Jimma.

1. Introduction

Population growth and urbanization cause an increase in the demand for milk in cities of developing countries (Narrod et al., 2011). In response, smallholder dairy farms were established in Jimma and other Ethiopian towns to fulfill the increasing demand for dairy products (Mekonnen et al., 2006; Tolosa et al., 2013). Zebu cattle are crossbred with exotic dairy breeds in these urban dairy farms. Crossbreeds have a higher genetic merit for milk production but seem to be more susceptible to mastitis (Almaw et al., 2008).

Recently, we reported a high prevalence of subclinical mastitis in crossbreeds in Jimma, Ethiopia using the California mastitis test (CMT). Sixty-two percent of the cows and 51% of the quarters were diagnosed to be subclinically infected (Tolosa et al., 2013) and 2 risk factors for subclinical mastitis were identified. Overall, quarters from cows in later stage of lactation (>180 DIM) [opposed to early lactation (<90 DIM)] and quarters from cows milked by stripping (as opposed to squeezing) had higher odds of subclinical mastitis, as reflected by the CMT test. However, the association of milking technique and subclinical mastitis was

* Corresponding author. Tel.: +32 9 264 75 45; fax: +32 9 264 75 34.
E-mail address: Sarne.Devliegher@UGent.be (S. De Vliegher).

http://dx.doi.org/10.1016/j.prevetmed.2015.05.001
0167-5877/

modified by tick infestation of the udder; milking technique did not influence the odds of mastitis in cows with tick-infested udders.

Using CMT, the prevalence of subclinical mastitis in smallholder dairy farms in Jimma was shown to be high (Tolosa et al., 2013) but little is known on the causative pathogens, and of the prevalence of clinical mastitis. Hence, the first objective of this study was to determine the prevalence of both clinical mastitis and IMI in Jimma through a cross-sectional study, and to identify the associated pathogens. Second, to identify potential control measures, risk factors at the herd, cow, and quarter level for clinical mastitis and IMI were screened. Because of their high prevalence, pathogen-group specific risk factors for IMI caused by *Staphylococcus aureus* and non-*aureus* staphylococci were studied in detail. As mastitis can lead to loss of quarter (Wenz et al., 2005) and many cows in Jimma have one or more blind (non-functional) quarter(s) (Tolosa et al., 2013), factors associated with quarters being blind were also analyzed.

2. Materials and methods

2.1. Description of the study area

Jimma town is located in Oromiya Regional State, 352 km South-West of Ethiopia's capital, Addis Ababa. The region has a tropical climate with annual rainfall ranging between 1400 and 1900 mm. The mean maximum and minimum temperature are 25–30 °C and 7–12 °C, respectively (Alemu et al., 2011). The area is mainly known for its coffee production but crop and livestock production are also important.

2.2. Herds and animals

Fifty out of 66 active dairy herds in Jimma were visited once by the first author between June 2012 and March 2013. Three herds were not visited because of absence of lactating cows, 7 farmers could not be contacted and 6 farmers refused to cooperate. Herd size ranged from 2 to 62 animals, young stock included. All lactating cows ($n = 211$) were sampled. Cows were housed in tie stalls with limited or no access to pasture and hand-milked twice daily. The average lactation stage and parity were 160 DIM (range of 2–730) and 2.6 (range of 1–8), respectively.

2.3. Data collection

Herd, cow and quarter characteristics potentially associated with clinical mastitis, (pathogen-specific) IMI and blind quarters were recorded using a questionnaire and through observation.

Herd-level information – Herd characteristics were subdivided in 4 categories. First, general farm management characteristics including farmer experience (3 categories: ≤13, 13–26, >26 years), herd size (2 categories: ≤10 versus >10 animals, youngstock included), calf feeding (2 categories: bucket-fed versus suckling), and grazing type (2 categories: limited access to pasture versus zero-grazing) were recorded. Secondly, milking procedures and hygiene were studied in more detail; questions on the number of milkers (3 categories: 1, 2, more than 2 milkers), washing of udder before milking (3 categories: no washing, yes teats only, yes whole udder), teat drying before milking (3 categories: no drying, yes by using 1 towel for multiple cows, yes by using 1 towel per cow), pre-stripping of foremilk before milking (yes versus no), access to feed and water immediately after milking (yes versus no), milking cows with clinical mastitis as last (yes versus no), and milking technique (stripping versus squeezing) were asked. Thirdly, information on cow comfort and hygiene was noted including subjects as stable floor type (concrete versus wood or soil), straw or saw dust bedding in use (yes versus no), changing frequency of bedding per day (4 categories: no bedding, 1 time, 2 times, 3 times), frequency of manure removal per day (4 categories: 1 time, 2 time, 3 times, variable), and presence of a calving pen (yes versus no). Finally, data on herd biosecurity and prevention was recorded; purchase of heifers or cows in the last year (yes versus no) and purchase of prepartum heifers in the last year (yes versus no) were noted.

Cow-level information – Age in years (2 categories: >4 versus ≤4), parity (2 categories: multiparous versus primiparous), and lactation stage (3 categories: ≤90 DIM, 90–180 DIM, >180 DIM) was recorded for every cow. Body condition score (5 scores: 1–5 scores) of all sampled cows was measured as described by Edmonson et al. (1989) and categorized as low (≤3) or high BCS (>3). Udder and leg hygiene was separately scored (4 categories) as described by Schreiner and Ruegg (2002). Flank hygiene was scored using the same definitions. Cows were clinically examined for presence of tick infestation on the udder (yes versus no).

Quarter-level information – For each lactating quarter, position (2×2 categories: right versus left and hind versus front) and presence of teat injuries/lesions (2 categories: yes versus no) was noted.

2.4. Milk sample collection

Quarter milk samples were collected aseptically from all lactating cows following National Mastitis Council guidelines (NMC, 1999). Quarters were palpated and first streams of milk were inspected to detect abnormalities (see further). After collection, milk samples were kept in a cool box during transportation to the laboratory.

2.5. Bacteriological culture

Bacteriological culture was performed according to NMC guidelines (NMC, 1999). From each sample, 10 μL of milk was plated on Colombia blood (5% sheep blood) and MacConkey agar (Oxoid, Hampshire, UK) and incubated aerobically for 24 h or 48 h at 37 °C. A mammary quarter was considered culture-positive when the growth of at least one colony was detected on the streaks (≥100CFU/mL). Samples yielding 2 different bacterial species were grouped as "mixed culture" whereas samples yielding more than 2 different bacterial species were considered to be contaminated and removed from the statistical analysis ($n = 7$, 0.8%). Bacteria were identified based on colony morphology, gram-staining, and conventional biochemical tests. For gram-positive cocci, catalase tests with hydrogen peroxide (3%) were used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Morphology, haemolysis patterns, and DNase, coagulase, and polymyxin susceptibility testing were used to distinguish *S. aureus* from non-*aureus* staphylococci, referred to as *Staphylococcus* spp. throughout this paper. Catalase-negative cocci were cultured on Edward aesculin media to differentiate aesculin-positive cocci and aesculin-negative streptococci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*). Christie, Atkins, Munch–Petersen (CAMP) tests were used to distinguish *S. agalactiae* and *S. dysgalactiae*. Gram-negative bacteria were identified using colony morphology, oxidase test and lactose fermentation on MacConkey agar.

2.6. Outcome variables

Five outcome variables were studied: (1) clinical mastitis, (2) IMI by any pathogen (IMI any pathogen), (3) IMI by *S. aureus* only (*S. aureus* IMI), (4) IMI by non-*aureus* staphylococci only (*Staphylococcus* spp. IMI), and (5) blind quarters. A quarter was considered having clinical mastitis if it was swollen and/or painful and/or if the