Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com

Short communication

Survey on small ruminant bacterial mastitis in Italy, 2013–2014

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ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 11 July 2016
Accepted 12 July 2016
Available online xxx

Keywords:
Epidemiology
Mastitis
Sheep
Goats
Bacterial infection

ABSTRACT

Mastitis is the most important disease of dairy small ruminants affecting animal welfare, agricultural economy, and food safety. Only a few investigations on the bacterial epidemiology of udder infections have been performed. Aim of the study was to describe the Italian epidemiology of bacterial mastitis in small ruminant dairy herds. An *ad hoc* electronic data collection module was created by the National Reference Center for Sheep and Goat Mastitis (C.Re.N.M.O.C). Public health veterinary laboratories of the Experimental Zooprophylactic Institutes (EE.ZZ.II) (n = 10) were selected. Nine (90.0%) EE.ZZ.II. participated to the survey and 8 (87.5%) provided a full report. Bacteriological culture results from 30,232 sheep and goat milk samples collected in 1795 herds between 2013 and 2014 were analyzed. Coagulase-negative staphylococci (CNS) were the most frequently isolated bacteria in dairy sheep and goats, followed by *Staphylococcus aureus*; other bacterial species were *Pseudomonas* spp., *Streptococcus uberis*, *Enterobacteriaceae*, *Enterococcus* spp., *Streptococcus* spp. and *Coryneiforms*. Italian results confirm previous findings described in other countries; CNS are the most prevalent bacteria, probably due to subclinical symptoms, whereas *Staphyloccocus aureus* is the most prevalent clinical mastitis etiological agent. The present survey, based on the first, Italian standardized data electronic collection focused on small ruminant mastitis, may represent the backbone for future control and preventive strategies nationwide.

1. Introduction

Two third and one fourth of the global sheep and goat milk production are estimated to be located in the rural Mediterranean areas, where agriculture is mainly based on small ruminant dairy farming (Boyazoglua and Morand-Fehrb, 2001).

In Italy 6.8 million sheep and 862,000 goats produce milk, principally used for cheese-making activities (ISTAT, 2013). Mastitis, which is the most relevant ovine and caprine disease, affects animal welfare and causes economic loss owing to poor milk quality and yield, animal culling and replacement, and increased health-care interventions (Gonzalo et al., 2002; Leitner et al., 2007, 2008)

Subclinical mastitis prevalence ranges from 5 to 30% (Bergonier et al., 2003 Contreras, 2003); however, only few investigations describing the local epidemiology of bacterial mastitis in small ruminants have been performed and reported until now (Contreras et al., 2007). In Italy, data on the bacterial prevalence of small ruminant mastitis were reported by Cannas et al. (2013), whose findings showed only the Sardinian scenario.

Aim of the study was to retrospectively estimate the prevalence of the different bacteria causing mastitis in small ruminants in Italy, using reports from bacteriological results of milk samples routinely submitted to public veterinary diagnostic laboratories of the Experimental Zooprophylactic Institutes (EE.ZZ.II.).

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http://dx.doi.org/10.1016/j.smallrumres.2016.07.010
0921-4488/

2. Materials and methods

2.1. Study sample

EE.ZZ.II.s, whose 10 Head Offices and over 90 Diagnostic Sections are coordinated by the Italian Ministry of Health, are involved in country-wide surveillance activities and experimental studies focused on animal health and food safety. All Italian EE.ZZ.II.s were enrolled in the survey on bacterial mastitis to get more precise national estimates.

All consecutive bacteriological reports of individual or half-udder sheep and goat milk samples with suspected mastitis were retrieved from nine public veterinary laboratories of Italian EE.ZZ.II between January 2013 and December 2014; reports described only the isolated bacterial species and the geographical region where milk sampling was performed. The reports were collected from the centres without any selection criteria.

2.2. Bacteriological analysis

Bacteriological analysis was performed by ISO/IEC 17025 accredited laboratories of the EE.ZZ.II., following National Mastitis Council cultural standardized procedures (NMC, 2004) within 48 h of collection. Milk samples were kept at room temperature and, 10 μl of milk from each sample, shaked in a vortex mixer, was spread onto blood agar plate (5% sheep blood) or, if necessary, onto other culture media, such as MSA (mannitol salt agar) and MacConckey agar. All plates were incubated anaerobically at 37 °C for 24–48 h and, then, examined for any bacterial growths. Sample showing a single colony of suspected primary pathogen even in the presence of other colonies, was considered positive; in case of isolation of coagulase-negative staphylococci, samples with <5 (500 CFU/ml) colonies were considered negative. Samples with 2 or more types of non-primary pathogen colonies were considered negative. Pure culture isolation incubating the plates at the same condition was carried out if necessary.

2.3. Data collection and analysis

An *ad hoc* electronic data collection form was created in 2014 by the National Reference Center for Sheep and Goat Mastitis (C.Re.N.M.O.C) using GoogleDocs (Google Inc., Mountain View, CA) interface. Only EE.ZZ.II. laboratories were recruited. Two different questionnaires were prepared in order to independently collect sheep ad goat data. Each questionnaire showed two sections on milk samples and herds, respectively. Each section was characterized by two different parts: 1) general information (EZI, Italian region, reference year); 2) bacteriological analysis other than *Mycoplasma* spp. (CNS, *Staphyococcus aureus*, *Pseudomonas* spp., *Streptococcus uberis*, *Enterobacteriaceae*, non-fermenting gram-negative, other streptococci, *Coryneiforms*).

2.4. Statistical analysis

Data management and epidemiological analysis were performed using Excel (Microsoft Corporation, Redmond, WA, USA) and STATA®12 (StataCorp, College Station, TX, USA). Counts and percentages were computed.

3. Results

A total of 30,232 bacteriological reports of sheep and goat milk samples from 1795 herds were collected between 2013 and 2014. Ten Italian EE.ZZ.II. were invited to participate to the survey, 9 (90.0%) EE.ZZ.II. responded to the questionnaire, and 8 (88.9%) provided a full report. Information about sheep and goats were retrieved from 9/20 (45.0%) and 11/20 (55.0%) Italian regions, respectively. Regional sample differences mirrored the unequal distribution of the dairy caprine and ovine herds in Italy: 94% and 82% of all sheep and goats were located in Central and Southern Italy, including the largest Italian islands of Sardinia and Sicily.

3.1. Sheep

Bacteriological analyses were performed on 23,040 milk samples from 1571 herds (Table 1). Most of them (73.7%) were collected in Sardinia, followed by Tuscany(8.5%), Lazio(8.3%), and Sicily (7.5%). Overall, 45.3% milk samples and 82.4% dairy farms showed at least one positive culture. CNS were the most prevalent bacteria (39.9% positive milk samples/48.8% positive herds), followed by *Staphylococcus aureus* (13.5% positive milk samples/39.8% positive herds), *Pseudomonas* spp. (4.3% positive milk samples/9.8% positive herds), *Streptococcus uberis* (4.2% positive milk samples/11.8% positive herds), *Enterobacteriaceae* (3.8% positive milk samples/15.6% positive herds), other streptococci (3.6% positive milk samples/9.5% positive herds), non fermenting gram-negative bacteria (1.8% positive milk samples/12.7% positive herds), and *Coryneiforms* (1.0% positive milk samples/4.6% positive herds) (Table 1).

3.2. Goats

7192 milk samples collected from 358 herds were microbiologically tested (Table 1). A higher proportion of milk samples was

Table 1
Microbiological results for dairy herds and milk samples, 2013–2014.

Dairy Herds		
Variables	Sheep n (%)	Goats n (%)
Dairy herds examined	1571	358
Dairy herds with ≥1 bacterial isolation	1295/1571 (82.4)	253/358 (70.7)
Coagulase-negative staphylococci	632/1295 (48.8)	137/253 (54.2)
Staphylococcus aureus	515/1295 (39.8)	105/253 (41.5)
Pseudomonas spp.	127/1295 (9.8)	12/253 (4.7)
Streptococcus uberis	153/1295 (11.8)	15/253 (5.9)
Enterobacteriaceae	202/1295 (15.6)	41/253 (16.2)
Non fermenting gram-negatives	164/1295 (12.7)	9/253 (3.6)
Streptococcus spp.	123/1295 (9.5)	29/253 (11.5)
Coryneiforms	59/1295 (4.6)	13/253 (5.1)
Individual/half udder milk samples		
Variables	Sheep n (%)	Goats n (%)
Milk samples examined	23,040	7192
Milk samples bacteriologically positive	10,426/23,040 (45.3)	2115/7192 (29.4)
Coagulase-negative staphylococci	4162/10,426 (39.9)	1084/2115 (51.3)
Staphylococcus aureus	1404/10,426 (13.5)	501/2115 (23.7)
Pseudomonas spp.	446/10,426 (4.3)	43/2115 (2.0)
Streptococcus uberis	438/10,426 (4.3)	67/2115 (3.2)
Enterobacteriaceae	392/10,426 (3.8)	141/2115 (6.7)
Non fermenting gram-negatives	190/10,426 (1.8)	26/2215 (1.2)
Streptococcus spp.	375/10,426 (3.6)	150/2115 (7.1)
Coryneiforms	105/10,426 (1.0)	56/2115 (2.6)

collected in Lombardy (37.6%), Sardinia (18.5%), Lazio (20.0%), Trentino-Alto Adige (15.4%), and Tuscany (3.8%). Bacteria were isolated from 29.4% milk samples and 82.4% dairy herds. CNS was the most frequently isolated bacteria (51.3% positive milk samples/54.2% positive herds), followed by *Staphylococcus aureus* (23.7% positive milk samples/41.5% positive herds), other streptococci (7.1% positive milk samples/11.5% positive herds), and *Enterobacteriaceae* (6.7% positive milk samples/16.2% positive herds) (Table 1).

4. Discussion

This study represents the first multicenter cross-sectional study on the aetiology of bacterial mastitis in small ruminant in Italy and the first standardized data electronic collection nationwide.

It confirms previous findings reported by other countries, where CNS and *Staphylococcus aureus* are the main causes of subclinical and clinical IMI both in ewes and goats, respectively, as well as the lower prevalence of other pathogens, such as *Streptococcus* spp., *Pseudomonas* spp., *Corynebacterium* spp., and *Enterobacteriaceae* (Bergonier et al., 2003; Contreras, 2003; Contreras et al., 2007; Las Heras et al., 1999; Gonzalo et al., 2004)

The survey shows several shortcomings: sampling, clinical, and laboratory methods could be different in the Italian regions and laboratories; moreover, the statistical power associated with different sample sizes cannot provide a reliable estimate of the epidemiological bacterial burden, particularly for those less prevalent in some settings.

Proportional bacterial differences between sheep and goat herds were not relevant from an epidemiological perspective, with the only exception of *Pseudomonas* spp. and *Streptococcus uberis*, which were most frequently detected in sheep; environmental (*i.e.*, water quality for milking machine washing) and management factors (*i.e.*, good milking procedures and facility hygiene) could have affected the epidemiological scenario.

Environmental bacteria (*i.e.*, *Pseudomonas* spp., *Streptococcus uberis, Enterobacteriaceae*, Enterococci, and *Streptococcus* spp.) cannot be eradicated because of their ubiquity; poor hygiene activities (*i.e.*, use of non-potable water for milking machine washing, contaminated litter, pollution of milking and post-milking areas) can favour udder invasion/colonization and spread of bacterial strains.

This is the first, nationwide report, characterized by a standardized methodology and by a large sample size.

New prospective studies are needed in order to better assess temporal and geographical differences. Incidence studies, based on an active surveillance system and including more bacterial species, can better inform on control and preventive strategies to be implemented in different geographical settings. Serious public health issues, such as antimicrobial resistance, could be adequately monitored only after the implementation and scale-up of appropriate surveillance system, as well as the efficacy of the public health interventions.

An epidemiological and laboratory networking, based on quality-assured activities in national and international contexts, can address the most common public health threats in the ovine and caprine sectors.

The network of different laboratories could help homogenize the methodology and assess the efficacy of control programs nationwide. It requires a coordination of all the stakeholders, who should agree on a shared vision of public health aims. The implementation of qualitative and quantitative indicators and their temporal evaluation could favour an improved allocation of economic resources and the design of tailored strategies.

Conflicts of interest statement

None.

Uncited reference

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