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Prevalence of intramammary infection pathogens in dairy herds of the Northern Apennine mountains, Italy

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Abstract

This Research Communication presents a cross-sectional study to compare the prevalence of contagious, environmental, opportunistic and other intramammary infection pathogens in mountain dairy herds. In the Italian Apennine mountains, areas where dairy farming is thriving are interspersed among areas where only a few dairy herds remain. The disappearance of some dairy farms relates to the reduction of agricultural and veterinary services in a process that can jeopardize dairy herd profitability. Sixteen herds were screened for intramammary infection (IMI) pathogens. Antimicrobial susceptibility testing of mastitis pathogens was performed, and data on antimicrobial use was collected from the herd treatment registry. The prevalence of contagious IMI pathogens was significantly higher in areas where dairy farming is reducing, whereas environmental and opportunistic infections were more abundant in herds in thriving areas where farmers had a more consistent relationship with veterinarians. Antimicrobial resistance levels were low throughout all areas and did not relate to antimicrobial use, although it was significantly higher in areas where dairy herds were thriving.

Intramammary infections (IMI) affect cow health and milk quality, and thus herd profitability (Cobirka *et al.*, 2020). IMI pathogens are usefully classified based on their epidemiology (Cobirka *et al.*, 2020; Riekerink *et al.*, 2020). Contagious pathogens (*Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis*, etc.) spread from the mammary glands of infected cows, acting as reservoirs, through milking equipment and udder manipulations (Cobirka *et al.*, 2020). Environmental pathogens, which include a wide range of species (*Aerococcus* spp., *Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., etc) reside in bedding material and colonize the udder when the teat is in contact with it (Cobirka *et al.*, 2020). Non-aureus staphylococcal and mammalicoccal species (NASM; *Staphylococcus* spp., *Macrococcus* spp. and *Mammalicoccus* spp.) act as opportunistic pathogens, since they inhabit the teat skin of healthy cows and infect the udder when the host's immune response is reduced (Riekerink *et al.*, 2020). Finally, other bacterial species (*Bacillus* spp., *Lactococcus* spp., etc.) can occasionally cause mastitis (Riekerink *et al.*, 2020). In addition, non-photosynthetic algae of the *Prototheca* genus (*P. zopfii* genotype 1 and 2, renamed *P. ciferri* and *P. bovis, P. wickerhamii, P. cutis, P. blaschkeae*, etc.) can cause mastitis as well as other diseases in humans and animals (Huilca-Ibarra *et al.*, 2022).

Each pathogen type has different epidemiology and risk factors, yet effective mastitis control requires a strategy appropriate to the pathogen (Roberson, 2003). Therefore, it is essential to identify the causative agent to avoid empirical and possibly ineffective treatment. In addition, antimicrobial susceptibility testing (AST) should guide the choice of the treatment to reduce antimicrobial misuse, and limit antimicrobial resistance. However, in certain areas of the Italian mountains, where the number of dairy herds has been in decline for decades (Bakudila, 2018), the access to agricultural services (specialized veterinarians, veterinary laboratories, diagnostic services, etc.) is limited or completely absent. Examples of such areas include the south-west-facing slope of the Apennines in the province of Lucca, Tuscany and the province of Alessandria, Piedmont region. Both have experienced among the most severe demographical abandonment in northern Italy, with dairy herds having almost entirely disappeared (veterinarians of the local veterinary services, personal communication). There, no designated product of origin (PDO) or other system for local dairy product protection exists to preserve the economic profitability of dairy herds in the face of low prices for milk and intense competition from large companies. A short distance away, in the mountains of the provinces of Modena, Parma, Reggio Emilia and Bologna, thanks to the establishment of the Mountain Parmigiano Reggiano PDO assignation, the number of active dairy herds has steadily increased (veterinarians of the local veterinary services, personal communication).

Mountain dairy farming provides ecosystem services and represents a sustainable food system (Montrasio *et al.*, 2020), but its low profitability leads to the rarefaction of dairy herds which is connected to the reduction of agricultural services in a vicious cycle, and no effective countermeasures have been implemented yet (McDonald *et al.*, 2000). This cross-sectional study aimed to assess whether the different productive context affects the prevalence of IMI pathogen types. We hypothesized that the lack of agricultural services places the profitability of dairy herds in jeopardy as health issues go unmonitored and unresolved. Additional information on antimicrobial use and antimicrobial resistance was collected.

Materials & methods

Ethics committee's approval was not required since animal handling and sample collection were carried out as for routine milking.

Study area

Three study areas were selected to represent different geographic areas of the Northern Apennines, and local veterinary services helped in contacting 18 dairy herds (six per area) situated at a height of (mean ± standard deviation) 555 ± 181 m. In the valleys within Erro and Borbera rivers in the province of Alessandria (Piedmont region, AL), four out of the six herds agreed to participate in the study (situated at 643 ± 286 m). In the high valley of the Serchio river, also known as Garfagnana, in Lucca province (Tuscany region, LU) all the six herds were sampled (situated at 435 ± 95 m). Around Castelnovo 'ne Monti, high valleys of Secchia and Enza rivers, in the Reggio Emilia province (Emilia Romagna region, RE) six herds were selected based on sampling convenience (657 ± 66 m). Samplings were performed between April 23rd and September 1st, 2021.

Intramammary infection

Based on herd size, up to 23 lactating cows per herd were randomly selected, excluding those undergoing antibacterial treatment, and composite milk samples were collected using aseptic technique (online Supplementary File, milk sampling procedure). Bacteriological and mycological examination were conducted, aiming to discriminate between contagious, environmental, opportunistic, *Prototheca* and other IMI (online Supplementary Table S1). IMI was diagnosed as described by National Mastitis Council guidelines (online Supplementary Table S2). The odds of different types of IMI were estimated through a generalized estimating equation, adjusting for individual cow risk factors (days in milk and lactation number) and accounting for repeated measures within herds (online Supplementary File, intramammary infection risk estimation). Marginal IMI prevalence was estimated for each area, and relative risk (RR) was calculated comparing AL and LU to RE.

Antimicrobial resistance

Minimum inhibitory concentration of 14 antibacterials for *S. aureus* and *Streptococcus* spp. isolates was tested following the Clinical and Laboratory Standards Institute guidelines (Clinical Laboratory Standard Institute (CLSI), 2018). Based on clinical breakpoints, isolates were interpreted as susceptible (S), susceptible to increased dosage/intermediate (I) or resistant (R). For the analysis, 'I' results were counted as 'S'. Isolates resistant to three or more antimicrobial classes were considered as multi-drug resistant (MDR: online Supplementary Table S3 and S4).

Antimicrobial use

Information on antibacterial use (treatment date, reason for treatment, number of treated cows and prescribed drug) was collected from each herd's treatment registry (online Supplementary Table S5). Treatment frequency was estimated calculating antimicrobial treatment incidence rate (ATI), modifying the formula proposed by Stevens *et al.* (2016). The areas were compared through antimicrobial treatment incidence rate ratio with RE as reference (online Supplementary Material, antimicrobial treatment incidence rate estimation).

Results

Herds included cows of multiple breeds, mainly Friesian Holstein, Brown Swiss and Italian red spotted, but also Reggiana and Piedmontese. Twelve farmers raised cows tie-stall and milked with pipeline (AL = 2/4, LU = 5/6, RE = 5/6), three free-stall with a proper milking parlour (AL = 2/4, RE = 1/6), and one used the milking cart.

Intramammary infection

Overall, 246 composite milk samples were analysed (online Supplementary Table S6). The adjusted IMI prevalence was 71.3% (CI: 60.7-81.9) and did not differ between RE and AL (RR = 0.92, CI: 0.42-1.05) or LU (RR = 0.87, CI: 0.34-1.04). Also, the total CFU did not differ among areas. From 179 positive cows, 254 isolates were obtained (Fig. 1). A single bacterial species was isolated from 117 cows, two species from 49, three or more species from 16. The highest prevalence was observed for opportunistic (42.7%, CI: 30.5-54.9), followed by environmental (21.1%, CI: 12.5-29.7), contagious (19.4%, CI: 7.0-31.8), and other bacteria (8.5%, CI: 3.7-13.2) (Fig. 1). The contagious IMI prevalence was significantly higher in AL (RR = 11.41, CI: 1.75-12.32) and LU (RR = 8.38, CI: 2.27-10.77) compared to RE (online Supplementary Table S7), with *S. aureus* being the most frequently isolated species (73.2%), followed by *S. agalactiae* (22.0%), and *C. bovis* (9.8%). Compared with RE, the prevalence IMI by environmental or other bacteria were not significantly lower in AL or LU. On the contrary, the prevalence of opportunistic IMI was lower in AL (RR = 0.40, CI: 0.08-0.98) and LU (RR = 0.49, CI = 0.23-0.83) than in RE. Three milk samples from the same LU herd were positive for *Prototheca zopfii*.

Antimicrobial resistance

Of the 28 *S. aureus* isolates, all were resistant to trimethoprim/sulfamethoxazole, whereas none was resistant to 1st, 3rd or 4th generation cephalosporins, quinolones, lincosamides, or rifamycins (online Supplementary Table S8). Three *S. aureus* isolates resistant to erythromycin came from a RE herd where macrolides constituted the third most frequent treatment. One *S. aureus* isolate resistant to penicillins without beta-lactamase inhibitors was isolated from a LU herd where these antibacterials were not among the most frequent treatments. Similarly, a *S. aureus* resistant to kanamycin was collected from an AL herd where no aminoglycosides use was recorded. Only one MDR *S. aureus* was isolated from

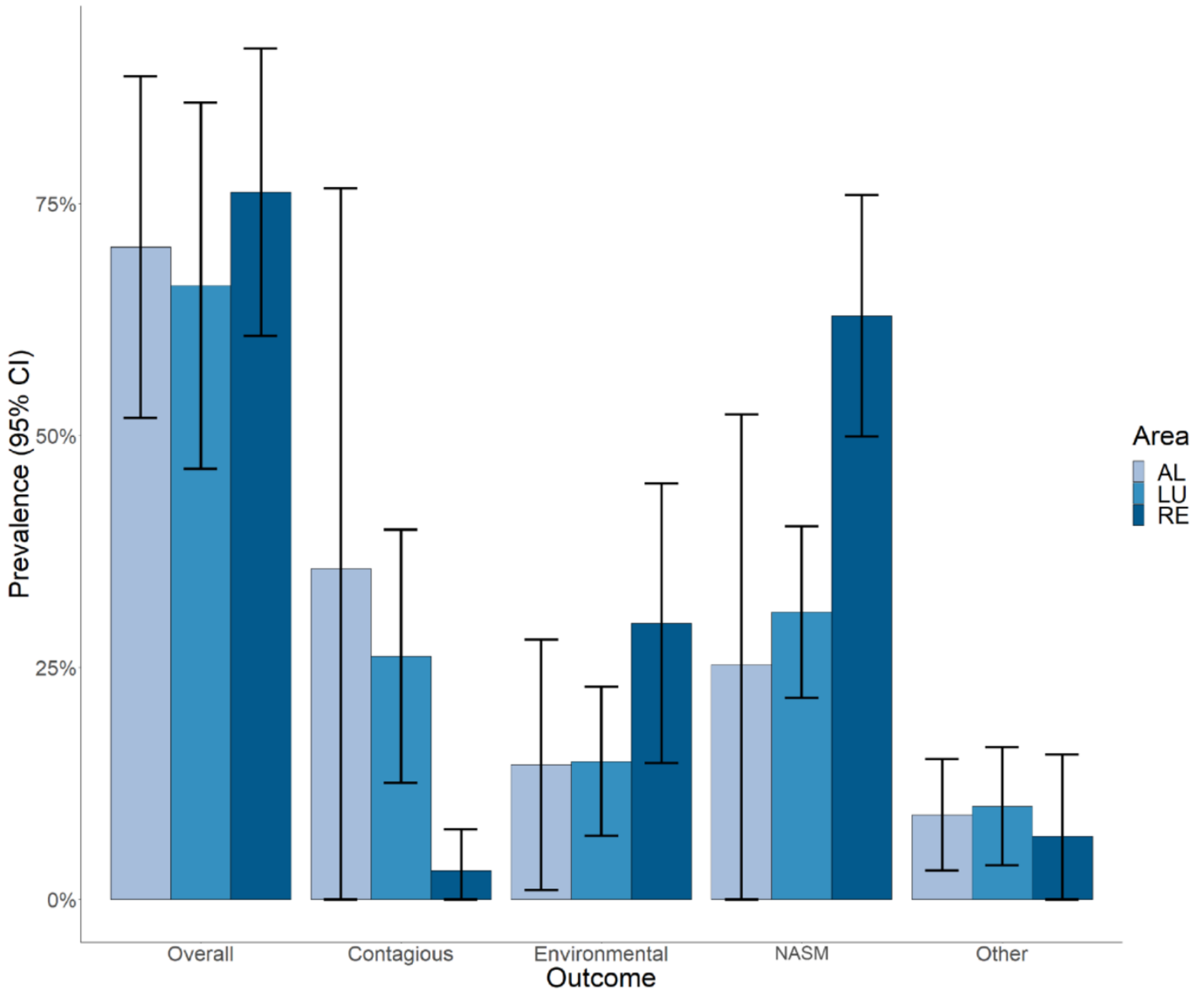

Figure 1. The height of the bars reflects the prevalence of overall, contagious, environmental, NASM, and other agents, by area. Whiskers span from the lower to the upper limits of the 95% confidence interval. Prevalence and 95% confidence intervals were adjusted by days in milk and number of lactations.

a LU herd. Of the 27 *Streptococcus* spp. isolates (*S. uberis* = 14, *S. agalactiae* = 8, *S. dysgalactiae* = 3, *S. pluranimalium* = 1, *S. suis* = 1), all were susceptible to cephalosporins, quinolones, rifamycins, and sulphonamides with diaminopyrimidines. Twenty isolates were resistant to oxacillin, and twelve were resistant to lincosamides. All *S. agalactiae* isolates were susceptible to all antibacterials, apart from two isolates from LU herds both resistant to erythromycin, and one also to pirlimycin (online Supplementary Table S9). Nine MDR *S. uberis* were isolated from nine cows of a RE herd, and a MDR *S. agalactiae* was isolated from a LU herd.

Antimicrobial use

Overall, IMI was the main reason for therapeutic treatment (50%), followed by periodic antiparasitic therapies, which accounted for 22% but were implemented only by two RE farmers. Other reasons for treatment are displayed in Fig. 2. Up to 65% of the recorded treatments were antibacterials. This proportion varied significantly among areas (AL = 73%, LU = 85%, RE = 56%; p = 0.023). The overall ATI was 0.021 antimicrobial treatments per 100 cow-days (95%CI: 0.015-0.028) (online Supplementary Table S10). The antibacterial ATI of AL herds was significantly lower than in LU and RE herds (Table 1).

The median number of cows treated with antibacterials at a time varied by area ($P < 0.001$; AL = 2, LU = 3, RE = 1). IMI was by far the main reason for antibacterial treatment (78%), other reasons are displayed in Fig. 2. Overall, 28% of the treatments were a combination of multiple antibacterials (AL = 16%, LU = 22%, RE = 40%). The most administered antibacterial classes were 1st generation cephalosporins (36%), followed by rifamycins (20%) and penicillins (18%), macrolides (including lincosamides, 8%), sulfonamides (8%), aminoglycosides (4%), 3rd and 4th gen. cephalosporins (3%), and tetracyclines (2%).

Discussion

In AL, small family herds owned by elderly farmers coexisted with medium-large herds managed as a family business. The first type resembled the LU herds. AL herds were located within a natural reserve and surrounded by uncultivated woods, while LU herds neighboured medium-sized municipalities, but the steepness of the slopes reduced the availability of arable land, and the lack of maintenance favoured the encroachment of the forest into agricultural land. The second type of AL herd was similar to one RE herd. They share the large herd size, the presence of a milking parlour and the free-stall housing method. The remaining five RE herds

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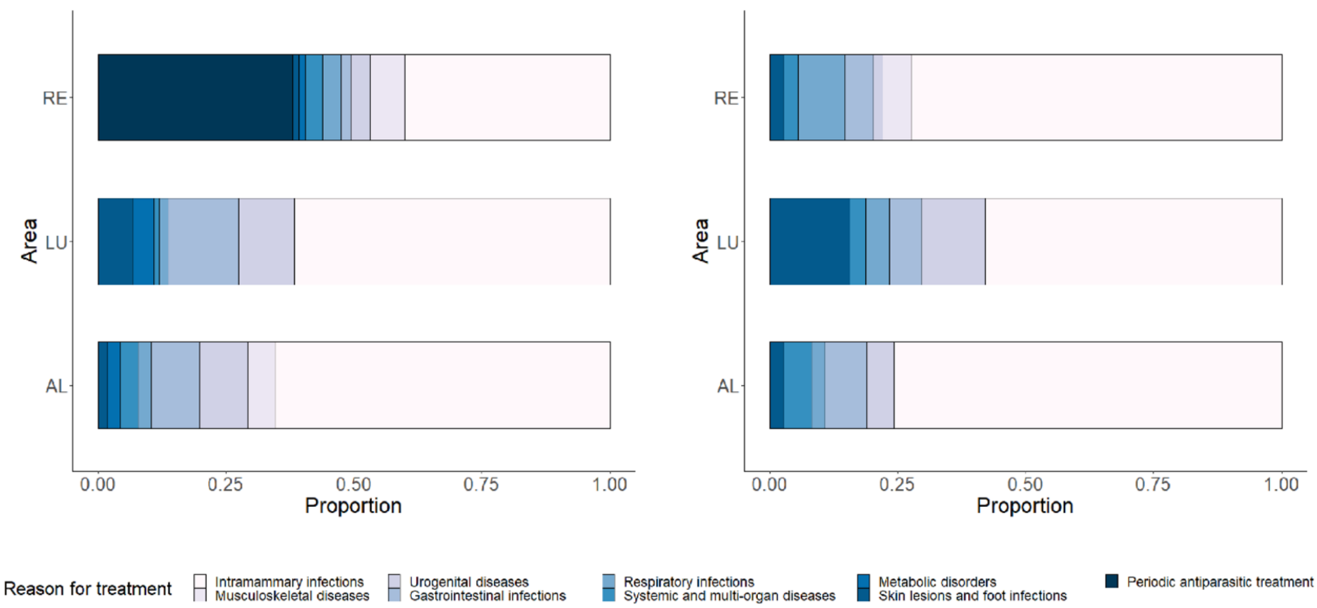

Figure 2. Proportion of each reason for treatment (left) and for antibacterial treatment (right) by study area.

Table 1. Total number of treatments and antibacterial treatments per month for dairy farms in three Apennine regions

Area	Treatment per month	Atb per month	ATI/100 cowdays	ATIR
AL	0.68 (0.59 – 0.76)	0.45 (0.39 – 0.52)	0.009 (0.000 – 0.019*)	0.165 (0.05 – 0.61*)
LU	0.50 (0.36 – 1.41)	0.38 (0.30 – 0.47)	0.012 (0.005 – 0.020*)	0.232 (0.08 – 0.64*)
RE	2.38 (1.62 – 6.95)	1.75 (1.12 – 3.62)	0.027 (0.020 – 0.034*)	1 (ref.)

Values are medians with (min-max) values or (*) 95% CI
AL: Alessandria, Piedmont region
LU: Lucca, Tuscany region
RE: Reggio Emilia, Emilia Romagna region
Atb: anti-bacterial treatments
ATI: antimicrobial treatment incidence rate
ATIR: antimicrobial treatment incidence ratio, reference is RE

looked similar: medium-sized, tie-stall and delivering milk to the cooperative dairy. The gentler topology of the Emilian side of the Apennines favours the cultivation of most of the territory and the presence of many dairy cattle herds. Also, human settlements are not concentrated on the valley floor but dispersed in a network of connected villages. The five RE herds had lower contagious IMI, but higher environmental and opportunistic IMI suggesting that the consistent and collaborative relationship between RE farmers and the veterinarian, demonstrated by the treatment registry, contributed to an effective mastitis control strategy. Indeed, an accurate diagnosis and systematic collaboration between farmer and veterinarian is essential for the control plan to be effective (Roberson, 2003). The lack of routine laboratory testing could be among the reasons for the higher prevalence of contagious IMI in AL and LU herds, where agricultural services were fewer and more difficult to access. On the other hand, increased opportunistic IMI prevalence in RE herds suggested that the pathogens compete for the same biological niche.

We did not observe any difference in antimicrobial resistance among areas, and most isolates were susceptible to nearly all antibacterials. MDR isolates were identified only from three herds, and among them, nine *S. uberis* with identical resistance patterns were isolated from a single herd. This was insufficient to evidence the spread of a single strain, but it could support the hypothesis that *S. uberis* can, under certain circumstances, act as a contagious pathogen (Zadoks *et al.*, 2003). Comparing AST results with antimicrobial use, we observed that resistances could not be explained by the treatment records. The incidence of antimicrobial treatments was lower than recently reported in dairy cattle herds in other areas (Stevens *et al.*, 2016). Also, 3rd and 4th generation cephalosporins were seldom used, in contrast with what is reported elsewhere (Stevens *et al.*, 2016), but in accordance with EU regulation that prevents the use of these antibacterials reserved for human medicine. Although antimicrobial classes did not differ among areas, we observed a significantly lower ATI in AL and LU than in RE.

In conclusion, we observed that contagious IMI prevalence was higher in Apennine areas undergoing depopulation and agricultural abandonment, suggesting the need for eradication plans and increased surveillance. Measuring udder health is essential for understanding the health status of the dairy herd, yet it is only a starting point. Indeed, a census of the remaining herds would help identify their peculiarities and might counteract the rarefaction of mountain herds.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029925000676.

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