Epidemiology of Mastitis Pathogens on Amish Dairy Farms in Missouri

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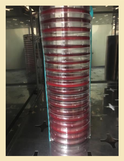
Background

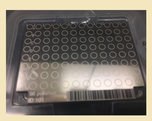
Often caused by an intramammary infection (IMI), mastitis is the most prevalent and costly disease of dairy cattle and results in milk with an elevated Somatic Cell Count (SCC). Federal milk quality standards require a SCC ≤ 750,000 cells/mL. Currently some Amish communities struggle to meet this requirement and thus have difficulty marketing their cows' milk. While the epidemiology of mastitis pathogens on modern, machine-milked dairies is reasonably well understood, our understanding of the major causes of mastitis on Amish dairy farms that milk by hand is lacking. The purpose of this study was to investigate the epidemiology of mastitis pathogens on Amish dairies in MO.

Materials and Methods

Mammary quarter foremilk samples were collected from all lactating cows on 6 farms (3-16 cows/farm) for bacterial culture and SCC determination.

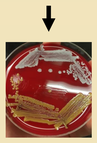


Samples were cultured on Columbia Blood Agar for 24-48 hours at 37℃.

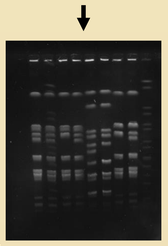


A representative of each colony morphology was isolated and speciated using Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) mass spectrometry. Data were analyzed to determine pathogen prevalence and a Kruskal-Wallis one-way ANOVA with post-hoc pairwise comparisons was used to determine differences in SCC between pathogen groups ($P < 0.05$).

Representative isolates were stored in phosphate buffered glycerol at -80°C.



Prevalent species were re-cultured from storage medium.



Isolates were strain typed using the restriction enzyme *SmaI* to digest the DNA and Pulsed Field Gel Electrophoresis (PFGE) to separate the fragments. Commercial software (Bionumerics®) was used to analyze the banding patterns.

Results

Overall, milk samples were collected from 56 cows present in herds 1 (n = 8), 2 (n = 16), 3 (n = 3), 4 (n = 14), 5 (n = 7), 6 (n = 8). With 12 quarters from 11 cows being blind, a total of 212 samples were collected. After removing 10 quarters with contaminated samples (≥ 3 colony types) and 30 quarters with a mixed IMI (2 colony types), 172 quarters remained to determine the association of pathogen type with SCC (Table 1).

Table 1. Prevalence of no growth and IMI by pathogen type and their associated milk SCC.

IMI Status	No. (%)	Median (Range) SCC x 10^3 cells/mL
No Growth (NG)	61 (35.5)	13 (0-650)
Staph. aureus (SA)	82 (47.7)	1,493 (10-7,352)
Non-aureus staphylococci (NAS)	24 (14.0)	221 (0-1,715)
Other	5 (2.4)	464 (81-1,884)

Given that SA was associated with the highest median SCC and was the most prevalent pathogen, all SA isolates were subjected to PFGE. Strain typing revealed 48 distinct strains. The prevalence of IMI caused by a given strain ranged from 1-13 mammary quarters. Herd 2 possessed the greatest number of different strains (n = 20) followed by herds 1 (n = 14), 5 (n = 6), 6 (n = 4), 3 (n = 3), and 4 (n = 3), respectively. Strains B and E were present in > 1 herd. After removing the mixed IMI, leaving only the single colony type IMI, strain type was compared to SCC (Table 2).

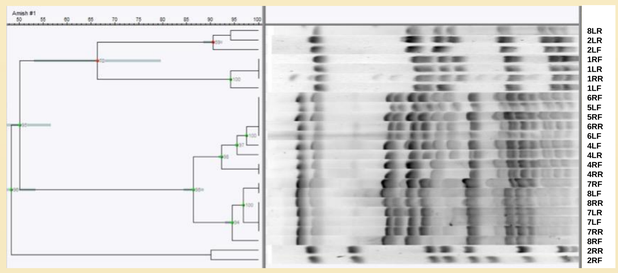
Table 2. Prevalence of different SA strain types causing IMI and their associated median milk SCC among 82 mammary quarters with a single colony type of SA isolated from milk.

Strain	No. of Single IMI	Cows	Herd	Median (range) SCC x 10^3 cells/mL
A	13	7	2	2,111 (200-4,851)
B	5	5	2,5	2,263 (10-5,199)
C	5	3	6	645 (371-1,794)
D	4	3	2	2,140 (1,131-6,860)
E	4	3	4,5	401 (269-4,451)
F	3	2	1	1,970 (325-5,199)
G	3	2	1	1,838 (492-2,599)
H	3	1	2	800 (746-1,838)
I	2	1	2	5,626 (4,851-6,400)
J	2	2	1	3,923 (985-6,860)
K	2	2	6	2,661 (1,202-4,120)
L	2	2	2	1,514 (429-2,599)
M	2	1	2	1,066 (919-1,213)
N	2	2	2	833 (746-919)
O	2	1	2	387 (246-528)
Strains represented by a single IMI (P – PP)	27	20	1-6	1,666 (10-7,352)

Strains identified in instances where SA was isolated from a mixed IMI (n = 20 IMI) included B (n = 1), C (n = 2), F (n = 2), G (n = 1), H (n = 1), K (n = 2), AA (n = 1), EE (n = 2), QQ (n = 1), RR (n = 1), SS (n = 3), TT (n = 1), UU (n = 1), and VV (n = 1).

Within herd, all SA isolates, both single colony and mixed IMI, were clustered based on PFGE banding pattern to determine relatedness of SA strains. Figure 1 shows an example dendrogram representing isolates from Herd #1.

Figure 1. Dendrogram showing the relatedness of SA isolates from Herd #1.



Conclusion

SA was the most prevalent mastitis pathogen on these 6 dairies and was associated with the highest median SCC. The high degree of diversity of SA strains within individual herds suggests these strains, with the exception of A, are not particularly contagious and that movement of strains between cows during milking was not widespread. Therefore, a point source with contagious transmission as generally seen on conventional dairy farms was not common. Hence, *S. aureus* IMI in these herds was more consistent with infection by non-host-adapted strains possibly acquired from the cows' environment. Further study into the origin of the isolated strains is needed.

Acknowledgements

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