Management practices associated with presence of *Staphylococcus aureus* in bulk tank milk from Ohio dairy herds

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ABSTRACT

Staphylococcus aureus is the most common contagious mastitis pathogen affecting cows worldwide. Practices to control this organism have been advocated for decades, and identification of risk factors in individual herds is crucial in prevention and control of *Staph. aureus*. The objectives of this paper were to estimate prevalence of *Staph. aureus* in Ohio dairies and to determine a potential association of herd characteristics and management practices with isolation of *Staph. aureus* in bulk tank milk. A questionnaire about herd characteristics, milking procedures, udder health, mastitis control, and biosecurity practices was mailed to 780 dairy producers; the response rate for the survey was 49%. *Staphylococcus aureus* prevalence was 48, 64, and 69% when 1, 2, or 3 samples of bulk tank milk from each herd were considered, respectively. Herds practicing prestrip, pre- and postmilking teat dip, and using a single towel per cow as part of the milking routine as well as herds where owners were involved in milking were at significantly reduced odds for detection of *Staph. aureus* in their bulk tank milk.

Key words: *Staphylococcus aureus*, bulk tank milk, management practice, questionnaire, mastitis

INTRODUCTION

Staphylococcus aureus is the most common contagious mastitis pathogen affecting dairy cows in North America, with herd prevalence estimated between 43 and 74% (USDA, 2008; Olde Riekerink et al., 2010). Importance of management practices in controlling mastitis is unquestionable, and the first step for a successful control program is knowledge about the organism's presence and prevalence in a herd as well as understanding of the prevailing risk factors.

Staphylococcus aureus resides primarily in the udder (Roberson, 1999); hence, its presence in bulk tank milk (BTM) provides evidence about infection status of cows that were milked into the tank before sample collection (Jayarao et al., 2004). Even though culture of quarter milk samples from all milking cows provides the most accurate estimation of within-herd prevalence, this method has the disadvantage of being costly and laborious. Culturing of BTM samples is a useful tool for evaluating milk quality and it has been used to estimate herd-level prevalence of *Staph. aureus* and to monitor udder health status (Farnsworth, 1993; Jayarao and Wolfgang, 2003; Jayarao et al., 2004). In a Canadian study in the 1990s, 58 out of 59 farms had at least 1 BTM sample positive for *Staph. aureus* (Kelton et al., 1999); more recently, prevalence of *Staph. aureus* was estimated at 74% in herds where at least 1 of 4 BTM samples cultured was *Staph. aureus* positive (Olde Riekerink et al., 2010). In US herds, presence of *Staph. aureus* was estimated to be 62% in organic herds, 42% in conventional nongrazing herds, and 43% in conventional grazing herds when a single sample of BTM was cultured (Cicconi-Hogan et al., 2013).

Although *Staph. aureus* can be isolated from environmental sources (e.g., bedding contaminated by milk leakage from infected cows; Capurro et al., 2010), the major mode of transmission for *Staph. aureus* is from cow-to-cow via fomites, such as milking unit liners, at the time of milking (Zadoks et al., 2002). Proper milking procedures, good milking hygiene, milking system maintenance, employee training, and fly control have been cited as components for prevention of new mastitis cases (Rodrigues et al., 2005; Anderson et al., 2012; Keefe, 2012).

Considerable changes due to selection of cows for milk yield and in housing and management of cows have occurred in the past decades in the US dairy industry. Milk production per cow has nearly doubled, and the average herd size increased 6 fold, from approximately 19 to 120 cows in the United States from 1970 to the early 2000s (ERS, 2000). Between 2001 and 2009 the number of dairy farms in the United States declined 33%, but this was counterbalanced by an increased number of larger herds (NASS, 2010). The

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Table 1. Summary of selected management practices included in the questionnaire about herd characteristics, udder health, and biosecurity submitted to Ohio dairy farms

Herd characteristics	Udder health, mastitis control, milking procedures	Biosecurity practices
Size	Person responsible for milking the cows (owner, hired)	Closed herd (raise heifers on farm and do not buy outside animals) or open herd (purchase animals, raise replacements outside home farm or take animals to shows bringing back to original farm)
Breed	Culture milk for clinical mastitis or culture BTM	
Average milk production		
Type of herd (conventional, organic)	Milking routine (prestrip, pre- and postdip, single towels, milking infected cows last or separately, knowledge about the presence of contagious pathogens in the herd)	Practice quarantine
Average SCC		Test purchased animals before commingling with the home herd
Type of housing for milking and dry cows		
Frequency of milking		
Type of parlor		
Type of bedding		
Access to pasture		

dairy industry in Ohio has undergone similar changes, and published data available from Ohio herds regarding management practices or prevalence of contagious mastitis organisms may no longer be current (Sischo et al., 1993; Khaitisa et al., 2000). Thus, the objectives of the present study were (1) to estimate prevalence of *Staph. aureus* in Ohio dairy bulk tank milk samples, (2) to assess herd characteristics and management practices associated with presence of *Staph. aureus* in BTM, and (3) to describe topics of importance to dairy producers in Ohio.

MATERIALS AND METHODS

Target Population and Questionnaire Instrument

A questionnaire was developed to survey dairy producers regarding herd characteristics and management practices, as well as to inquire about educational needs and interests of dairy producers in Ohio. According to Ohio Department of Agriculture, there were 2,750 dairy farms in Ohio in the middle of 2015 (www.agri.ohio.gov). In the current study, questionnaires were mailed with a paid return envelope to 780 randomly selected dairy producers located across the state of Ohio. This was done in collaboration with the milk testing laboratory responsible for raw milk testing for 95% of milk producers in the state. Information about general management practices in dairy farms was collected through an 8-page survey containing 42 questions about herd characteristics, milking procedures, mastitis control, and biosecurity practices (Table 1). Additionally, producers' interest level in receiving further education on

21 topics selected by The Ohio State University Veterinary Extension program, ranging from manure nutrient management to dairy facilities, cow comfort, and record-keeping systems, was assayed with responses categorized as high, moderate, low, or no importance to producers. In the questionnaire, permission to test their BTM was also requested.

The questionnaire followed Dillman's survey method with some adjustments (Dillman, 2000). Briefly, an introductory letter was sent to producers a week before the actual questionnaire, describing the goals of the study. Before mailing to dairy producers, the questionnaire was pilot-tested with a group of dairy veterinarians, dairy producers, and faculty and graduate students with dairy interest; the wording was modified as needed based on received feedback. It was designed with closed or semiclosed questions. Two reminders, a month apart, were sent via mail to those producers who had not yet responded. In addition to the answers provided by the producers through the questionnaire, their milk-testing laboratory provided the investigators data on SCC in the herds from the previous 12 mo. Identity of the producers was unknown to the investigators throughout the entire study. Integrity of data was ensured through inspecting data entry from randomly selected questionnaires twice and identifying illogical values by checking for outliers and unlikely values and correcting as needed and appropriate.

Sample Collection and Laboratory Processing

Bulk tank milk samples were collected by the milk-testing laboratory personnel at 3 different time points

in a monthly interval. Despite of the initial intention of sampling and culturing 3 BTM samples from each herd, due to logistic challenges, a full set of 3 samples was not received from all herds.

Milk samples were frozen at arrival and kept at -20°C until further processing. One hundred microliters of milk was plated on *Staph. aureus*-selective Baird Parker medium (Remel Inc., Lenexa, KS). The plates were incubated at 37°C , examined for growth at 24 and 48 h, and suspect colonies (black colonies with or without halo) were further subcultured on blood agar plates (Remel Inc.). Identification of *Staph. aureus* was based on colony morphology and hemolysis on blood agar plates, Gram stain, positive catalase test, and a positive tube coagulase rabbit plasma test read at 4 and 24 h.

Statistical Analysis

Descriptive statistics on herd characteristics and different management practices implemented in the herds were summarized and compiled using PROC FREQ procedure of SAS (v. 9.2, SAS Institute Inc. Cary, NC). A herd was considered positive for *Staph. aureus* when at least 1 BTM sample was positive on culture. Prevalence was calculated 2 ways. First, data from all herds were used, regardless of how many samples were cultured from each herd. Second, prevalence was calculated by using a subset of the data with information only from herds that provided 3 BTM samples for culture. Initial univariate screening to identify management practices associated with presence of *Staph. aureus* in BTM samples (yes/no) as the outcome was performed running logistic regression (PROC GLIMMIX of SAS, with binomial distribution and logit link). Variables associated with *Staph. aureus* in the initial screening with $P < 0.25$ were considered for a full model. To avoid potential collinearity problems during the modeling, variables were first checked for correlation and those found to be correlated with each other were entered into a full model separately, only one at a time, or they were combined into a single variable if feasible (e.g., individual milking procedures). The variable with the smallest P -value and strongest association with the outcome was chosen and included in the final model. Nonsignificant variables were eliminated from the full model one at a time, starting with the least significant, until all variables remaining in the model had $P < 0.1$. Additionally, using annual average SCC of the herd as a continuous outcome, a similar modeling procedure as described above was followed using a linear regression approach (PROC MIXED of SAS). Least squares means of SCC were compared

between herds with 0, 1, 2, or 3 *Staph. aureus*-positive BTM samples using Tukey-Kramer adjustment. All analyses were performed using all herds and a subset of herds that contributed all 3 samples of BTM. To explore associations or trends between interest level in educational topics and presence of *Staph. aureus* in BTM samples or BTM SCC, Cochran-Armitage trend test (PROC FREQ of SAS) was run. For the analyses, low and no interest levels were collapsed into one category and BTM SCC was dichotomized into $\leq 150,000$ and $>150,000$ cells/mL.

RESULTS

Prevalence of *Staphylococcus aureus*

A total of 384 surveys were returned, resulting in a response rate of 49%. However, 4 questionnaires could not be included in the analysis (1 producer had exited the dairy business and 3 questionnaires were returned empty), giving us a total of 380 responses. Some questions in the survey were not answered by all responders, and for those questions the denominator will be provided when responses (as a percentage) to these questions are shown. Of the 380 herds included in the analysis, 307 provided milk samples for culture. However, herds contributed a different number of BTM samples: one sample was received from 39 (13%) herds, 2 samples from 78 (25%) herds, and 3 BTM samples were received from 190 (62%) herds, resulting in 765 BTM cultured. In total, 73 of the responding herds did not agree to have their BTM sampled.

When all herds were included in the analysis, regardless of the number of samples collected and if considering only the first BTM sample per herd, *Staph. aureus* prevalence was 48% (146/307, 95% CI = 42–53%). If the first 2 samples were interpreted in parallel (in other words, a herd was considered positive if either one of the samples was positive), 198 of the 307 herds were found to be positive (64% prevalence, 95% CI = 59–70%). If all 3 samples were considered, a total of 213 herds (69%, 95% CI = 64–74%) were found positive for *Staph. aureus* in at least 1 of the samples.

If only herds with all 3 samples ($n = 190$) were included in the analysis and only the first sample from each herd was considered, *Staph. aureus* prevalence was 47% (90/190, 95% CI = 40–55%). If the first 2 samples were considered and interpreted in parallel, 122 herds were positive (122/190 = 64%, 95% CI = 57–71%). When all 3 samples were considered and interpreted in parallel, 137 out of 190 (72%, 95% CI = 65–78%) were positive. In other words, 28% (53 of these 190 herds) were negative in all 3 samplings.

Herd Characteristics

Of all the responding producers, 28% had <50 cows, 61% had between 50 to 199 animals, and the remaining 11% had ≥ 200 cows (with 4% of herds with >500 cows). A majority of the producers (80%) reported milking Holstein cows, 9% reported Jerseys, and the remaining producers had other, more than one breed, or cross-breeds in their herds. A small percentage (4%) of the responders indicated to operate an organic dairy farm whereas 96% had a conventional farm. Sixty-two percent reported being part of DHIA. The smallest herds were least likely to belong to DHIA: 52% of herds with <50 cows, 64% of herds with 50 to 199 cows, 72% of herds with 200 to 499 cows, and 73% with ≥ 500 cows reported being part of DHIA and testing on a regular basis.

Most farms (91%) reported milking cows twice per day, the remaining 9% milked more frequently than twice a day. Herringbone was the predominant parlor type, used in 52% of the herds. In 75% of the responding herds milking cows were housed in freestall barns, and in 10% of the herds they were in tiestall barns. All tiestall herds had <200 cows: 75% of them had <50 cows and 25% had 50 to 199 cows. In the remaining 15% of the herds, other housing arrangements (e.g., loose housing or manure pack, combination of different facility solutions) were available for milking cows. Dry cows were kept in loose housing or manure pack in 55% of the herds, in 40% of the herds dry cows were in freestalls, and in 5% of the herds cows stayed in tiestalls through the dry period.

Straw was used as the only type of bedding in 40% of herds, whereas sand only was used in 16% of the herds. Sawdust only was used in 9% of the responding herds. In the remaining 35% of the herds, combinations of these bedding materials or other types of bedding were used. In 67% of the herds producers let cows graze on pasture when it was available (46% reported allowing all cows to pasture, 15% only dry cows, and 6% only milking cows). In 10% of the herds cows had access only to an outside exercise lot and 8% of the herds were provided access both to pasture and exercise lot. In 15% of the herds, cows had access to neither pasture nor exercise lot at any time.

In 44% of the responding herds, average milk production was reported to be between 6,804 and 9,072 kg per year (15,001–20,000 lbs per year), followed by 43% of herds which produced between 9,072 and 13,608 kg per year (20,001–30,000 lbs per year). In 11% of the responding herds, reported herd-average production was less than 6,804 kg per year (15,000 lbs), and in 2% production was over 13,608 kg (30,001 lbs).

None of the previously mentioned herd characteristics were associated ($P > 0.25$) with the presence of *Staph. aureus* in BTM, except for use of sand as bedding ($P = 0.12$) and outside access to pasture or exercise lot ($P = 0.11$; Table 2). All variables associated with *Staph. aureus* detection in the univariate screening with $P < 0.25$ using data from all herds are presented in Table 2.

Udder Health, Milking Procedures, and Biosecurity Practices

Adoption of the recommended milking routine or mastitis-management procedures in the responding herds can be summarized as follows (Table 2): 57% of the herds reported checking foremilk (prestrip), 82% used premilking teat disinfection (predip), 97% used post-milking teat disinfection (postdip), 92% used a single towel per cow, and 38% segregated known infected cows or milked those cows last. A lower percentage of the 380 herds, however, performed all these procedures at the same time; for instance, 49% of the herds practiced prestrip, predip, postdip, and used individual towels for each cow (BTM was positive for *Staph. aureus* in 64% of these herds). Only 16% reported milking cows known to be *Staph. aureus*-positive last in addition to doing prestrip, predip, postdip, and using single washable towels.

Of the 372 responding herds, 37% reported to have never cultured milk from clinical mastitis cases, 51% cultured occasionally, and 12% were equally divided among those that cultured from all cases or only from chronic cases. In 45% of the 367 responding herds BTM was not cultured to monitor udder health in the herd, whereas 38% reported culturing monthly and 17% occasionally.

Forty-nine percent (159/327) of the responders did not recall *Staph. aureus* having been cultured from their cows earlier, whereas 51% (168/327) did. The difference in proportions of herds that had *Staph. aureus* detected in BTM was not different between these groups (Table 2).

Based on producer reporting, 62% of the responding herds had average SCC below 300,000 cells/mL during the 3 mo before answering the survey. This agreed well with the actual average SCC from the previous year provided by the milk testing laboratory: 10% of the herds had average SCC <150,000 cells/mL and 51% of herds had average SCC between 150,001 and 300,000 cells/mL (Table 2).

Thirty-eight percent (141/372) of the producers considered themselves to have an open herd and reported purchasing animals from outside, raising heifers outside the home farm, or taking animals to fairs and

Table 2. Proportions of Ohio dairy farms implementing certain management practices and their association with being positive for *Staphylococcus aureus*¹

Management practice	Proportion of farms (%)	<i>Staph. aureus</i> (%)		Odds ratio	<i>P</i> -value
		Positive	Negative		
Milking procedures (n = 380)					
Practice prestrip	57	66	44	0.55	0.18
No prestrip	43	73	27	—	
Use predip	82	67	33	0.51	0.06
No predip	18	80	20	—	
Use prestrip, pre- and postdip	53	64	36	0.63	0.05
Do not use	47	74	26	—	
Use prestrip, pre- and postdip, and single towel	49	64	36	0.60	0.04
Do not use	51	75	25	—	
Responsible for milking (n = 380)					
Only hired employees	10	80	20	1.98	0.14
Owner and hired	90	68	32	—	
SCC (cells/mL n = 380)					<0.01
>300,001	39	74	26	4.26	
150,001–300,000	51	71	29	3.67	
≤150,000	10	40	60	Ref.	
<i>Staph. aureus</i> has been cultured before in the herd (n = 254)					
Yes	51	71	29	1.39	0.21
No	49	64	36	—	
Practice quarantine (n = 141)					
Yes	20	54	46	0.46	0.06
No	80	72	28	—	
Access to pasture or exercise lot (n = 380)					
Outside access	85	71	29	1.63	0.11
No outside access	15	60	40	—	
Bedding (n = 380)					
Sand	32	63	37	0.66	0.12
No sand	68	72	28	—	

¹A total of 307 herds that responded to a questionnaire and provided up to 3 bulk tank milk (BTM) samples for culture were included in the analysis. A herd was considered positive for *Staph. aureus* if at least 1 BTM sample was positive.

shows (BTM from 67% of these 141 herds was positive for *Staph. aureus*). The remaining 62% (229/372) considered themselves as closed herds (they raise all replacements at farm and do not buy animals or take their animals to fairs) and 70% were positive for *Staph. aureus*. Of the open herds bringing in new animals from outside, 80% did not quarantine incoming animals before introducing them to the existing herd, and out of those herds that also provided milk samples for culture 72% (76/105) were BTM-positive for *Staph. aureus*. Of the herds that did quarantine (20%) newly purchased animals before introducing them to the existing herd and also provided milk for testing, 54% (14/26) were BTM-positive for *Staph. aureus* ($P = 0.06$; Table 2). Owners were solely responsible for milking cows in 64% of the farms (69% of these herds were positive for *Staph. aureus*), whereas the remaining 36% of herds had hired employees to assist with milking (BTM from 68% of these herds were positive for *Staph. aureus*). In only 10% of the farms, hired workers were reported to be solely responsible for milking of cows (BTM from 80% of these herds were positive for *Staph. aureus*; Table 2).

The final model (using the full data set of 307 herds) with variables that were associated with detection of

Staph. aureus in BTM is presented in Table 3. Detection of *Staph. aureus* in BTM from herds that reported not practicing prestrip, pre- and postdip, and not using single towels per cow was significantly higher, and those herds had almost 2 times higher odds to be found positive when compared with responding herds where all of those practices were part of the milking routine [odds ratio (OR) = 1.85, 95% CI = 1.1–3.1]. Herds where hired employees only were responsible for milking had 2.4 times higher odds of having *Staph. aureus* detected in their BTM compared with herds where owners participated in the milking routine (OR = 2.41, 95% CI = 0.9–6.3). The odds of detecting *Staph. aureus* also increased with increasing BTM SCC. Herds with SCC higher than 300,000 cells/mL had almost 5 times the odds (OR = 4.58, 95% CI = 1.9–10.8) and herds with SCC between 150,000 and 300,000 cells/mL had almost 4 times the odds (OR = 3.80, 95% CI = 1.7–8.7) of having *Staph. aureus* in BTM compared with herds with SCC less or equal to 150,000 cells/mL (Table 3).

When analyzing the subset of data from herds with all 3 samples, the results were similar to those with all herds in the analysis. Detection of *Staph. aureus* increased with increasing SCC. In herds with SCC

higher than 300,000 cells/mL the odds of *Staph aureus* detection were over 7 times higher (OR = 7.25, 95% CI = 2.5–20.7) than in herds with SCC lower than 150,000 cells/mL. When herds with SCC between 150,000 and 300,000 cells/mL were compared with herds with SCC lower than 150,000 cells/mL the odds of *Staph aureus* detection was almost 4 times higher (OR = 3.64, 95% CI = 1.4–9.0). Detection of *Staph. aureus* in BTM from herds that reported not to practice prestrip, pre- and postdip, and not to use single towels per cow were significantly higher when compared with those herds where all of mentioned practices were part of the milking routine (OR = 1.99, 95% CI = 1.0–3.94).

When assessing the association between BTM SCC and different management practices and herd characteristics, increasing BTM SCC, as expected, was statistically significantly associated with an increased likelihood of a herd being positive for *Staph. aureus* ($P = 0.02$ and $P < 0.01$ using all herds and a subset, respectively). No other tested variables were significantly associated with SCC except that herds with more than 200 cows had lower BTM SCC (on average lower by 30,000 cells/mL) than smaller herds ($P = 0.01$). The results were similar regardless whether all 307 herds (with 1 to 3 samples cultured) or only 190 herds (all with 3 BTM samples tested) were used in the analysis. A positive relationship was observed between BTM SCC and number of times BTM was detected positive for *Staph. aureus*. Higher BTM SCC (300,000 cells/mL) was found when all 3 BTM were positive compared with when 1 or 2 BTM samples were positive (286,000 and 279,000 cells/mL, respectively). When BTM was negative for *Staph. aureus*, the average BTM SCC in these herds was 257,000 cells/mL; differences were not statistically significant, though.

When comparing responding herds that had provided milk for testing with those that did not agree to have their milk cultured, no significant differences in herd characteristics or management practices were observed between these groups.

Topics of Importance to Dairy Producers

Topics of importance among Ohio dairy producers for obtaining further training are listed in Table 4. Mastitis control and troubleshooting high SCC was rated first in importance, with 74% of the responding producers mentioning it as highly important theme, followed by nutrition with 54%, foot health (lameness) with 53%, and poor reproductive performance with 53% of the producers indicating high interest in those topics. In general, detection of *Staph. aureus* in BTM was more common in herds where producers expressed low or no interest in continuing education. A total of 74% of the responders considered mastitis control and SCC troubleshooting highly important, and 68% of these herds had *Staph. aureus* detected in their BTM. Of those 22% responding herds that found mastitis control education of moderate importance, 78% were positive in their BTM for *Staph. aureus*. The remaining 4% of responders reported having little or no interest on this topic and 86% of these herds were positive for *Staph. aureus*. Although an increasing proportion of *Staph. aureus* positive BTM was found with decreasing interest in mastitis control education, this trend was not significant ($P = 0.17$). A similar trend was observed regarding educational interest in dry-off therapy ($P = 0.02$), transition cow management ($P = 0.10$), cow comfort ($P = 0.12$), and nutrition ($P = 0.14$).

Table 3. Results from the final model with presence of *Staphylococcus aureus* in bulk tank milk as the outcome, including all variables associated with *Staph. aureus* presence with $P < 0.1$ ¹

Item	Parameter estimate	SE	P-value	Odds ratio	95% CI
SCC					
>300,000 cells/mL	1.52	0.41	<0.01	4.58	1.9–10.8
150–300,000 cells/mL	1.38	0.43	<0.01	3.80	1.7–8.7
<150,000 cells/mL	Ref.	—	—	—	—
Milking procedures ²					
Do not practice	0.66	0.26	0.01	1.85	1.1–3.1
Practice	Ref.	—	—	—	—
Responsible for milking					
Hired employee only	0.84	0.49	0.07	2.41	0.91–6.34
Owner and hired	Ref.	—	—	—	—

¹The full data set included data from 307 herds that responded to a mailed questionnaire and provided at least 1 bulk tank milk sample for culture. A herd was considered positive for *Staph. aureus* if at least 1 bulk tank milk sample was positive.

²Milking procedures consist of prestrip, pre- and postmilking teat dip, and use of single towel implemented concomitantly.

Receiving further training about on-farm biosecurity protocols was of no or little importance for more than one-third (36%) of the responders, whereas 46% found biosecurity to be of moderate importance and the remaining 18% to be of high importance. Of the responders who reported having a closed herd, 62% found biosecurity a highly or moderately important topic and 38% had no or little interest in the topic; the respective percentages were similar (67 and 33%, respectively) for open herds. *Staphylococcus aureus* was found more frequently (80% positive) in herds with no interest in biosecurity compared with herds with moderate or high level of interest in the topic (69 and 74%, respectively), even though the trend was not significant.

DISCUSSION

The herd prevalence of *Staph. aureus* based on BTM samples in the current study (69%) was higher than in an earlier Ohio study conducted more than 2 decades ago that found BTM *Staph. aureus* prevalence of 45% (Sischo et al., 1993). More recent US and Canadian studies have reported *Staph. aureus* prevalence in BTM to range from 43 to 74%, respectively (USDA, 2008; Olde Riekerink et al., 2010). In the USDA (2008) study a single BTM sample was used, whereas the Canadian study used 4 samples per herd collected at different time points (Olde Riekerink et al., 2010). A limitation of the current study was that 3 samples were not obtained from all herds; thus, 69% is likely an underesti-

mation of true *Staph. aureus* prevalence in Ohio dairy herds. This is corroborated by the observation in the current study that herd prevalence was estimated at 72% when a subset of data only from herds with 3 bulk tank samples was used to calculate the portion of herds positive for *Staph. aureus* in BTM. This agrees with earlier studies that reported that the sensitivity of a single BTM sample for detection of *Staph. aureus* is low (between 21 and 42%) and probability of detecting *Staph. aureus* from BTM is likely dependent on within-herd prevalence of *Staph. aureus* IMI (Godkin and Leslie, 1993; Olde Riekerink et al., 2010) with an increased likelihood of detection in high prevalence herds. Determining within-herd prevalence involves culturing quarter or composite milk samples from all cows, which is associated with higher cost and more labor compared with BTM culturing. In the current study sensitivity was increased by culturing up to 3 BTM samples per herd and interpreting results in parallel and by using 100 µL of inoculum on selective media, which have been reported to have sensitivity of 92% and specificity of 93% when culturing 100 µL of composite milk for *Staph. aureus* (Zanardi et al., 2014).

Staphylococcus aureus is considered a major pathogen because of its effect on milk quality and SCC (Keefe, 2012). Findings of the current study demonstrated that the odds of detecting *Staph. aureus* in the BTM increased with increasing BTM SCC. However, a decreased probability of detecting *Staph. aureus* in BTM was found in herds that reported implementing 4 of

Table 4. Summary of responses indicating the level of importance (%) of educational needs among dairy producers in Ohio

Topic	Importance (%)			Number of responders (n)
	High	Moderate	Little/No	
Mastitis control and SCC	74	22	4	293
Troubleshooting nutrition problems	54	37	9	286
Troubleshooting poor reproduction	53	36	11	289
Foot health care and lameness	53	34	13	288
Effective reproductive strategies	51	37	12	289
Herd health and vaccination	48	39	13	292
Facilities and cow comfort	46	40	14	284
Identification animals of medical attention	42	33	25	285
Transition cow management	40	41	19	286
Best management and welfare	39	41	20	286
Management of calves and heifers	37	49	14	291
Economics of dairy business	36	43	21	283
Dry-off therapy	33	49	18	289
Calving management	29	51	20	287
Record keeping	29	51	20	285
Economics of alternative feeds	26	53	22	290
Manure nutrient management	29	47	24	286
On-farm biosecurity protocols	18	46	36	284
Buying sound animals	17	28	55	275
Grazing and economics	17	25	58	289
Management of organic production	8	8	87	285

the recommended milking practices (prestrip, pre- and postdip, and use of a single towel per cow to dry teats). Herds that did not use these 4 procedures had almost 2 times higher odds of having *Staph. aureus* in their BTM than herds that did. Some of the benefits of these practices are associated with improving milk letdown, assistance in identification of abnormal milk, and reduction of the microbial count on teats before use of predip (Zucali et al. 2011). Teat dipping and use of a single towel per cow can be important, as *Staph. aureus* teat colonization can be significantly associated with *Staph. aureus* IMI (da Costa et al., 2014).

Some practices, such as milking known infected cows last, have proven to reduce the spread of contagious pathogens and to significantly decrease the prevalence of *Staph. aureus* mastitis and bulk tank SCC (Wilson et al., 1995; Zecconi et al., 2003). Segregation of infected cows can be logistically demanding in some farms, as creation of such an additional group would require extra space, time, and effort, in addition to the initial cost of diagnosing those infected cows. This could be one reason why only 38% of the producers in our study reported to milk infected animals last.

Procedures involved in mastitis prevention may appear simple, but the consistency of protocol implementation over time is crucial for good herd performance, increased parlor efficiency, and reduction of mastitis incidence (Taylor, 2012). In the current study, producers appeared to be aware of the importance of proper milking practices to prevent and control mastitis, as the percentage of responders performing these practices individually was high. Still, these milking procedures together were implemented much less frequently. Implementation of recommended management practices depends on the ability of the producer to motivate farm employees (Rodrigues et al., 2005). Jansen and Lam (2012) reported that producers who do not recognize having a mastitis problem in their herd, are unaware of the magnitude and the economic impact of the problem, or do not perceive the effectiveness of management practices to control this disease are probably less motivated to change their mastitis management. Practices used on farms likely remain constant over time, contrary to attitudes, motivation, and knowledge that have the tendency to progress (Dufour et al., 2012). Limited studies have focused on investigating the relationship between the communication strategies by veterinarians, personnel motivation, and milk quality, but frequent training of employees has been reported to result in better milking performance and lower incidence of clinical mastitis cases (Rodrigues and Ruegg, 2005; Rodrigues et al., 2005; Jansen and Lam, 2012). When comparing herds where only hired employees were responsible for milking with herds where the owner was also involved

in milking, detection of *Staph. aureus* was 12% higher in the former herds and the odds of finding *Staph. aureus* were almost double. This suggests that regular training and motivation of employees may be beneficial to improving or maintaining good udder health in the herd.

In the univariate screening, increased detection of *Staph. aureus* was found in herds that provided access to pasture to all cows (OR = 1.6) and that did not use sand as their only bedding (OR = 1.5); however, these variables did not remain in the final model. A study from the southern United States has highlighted the role of flies as a source of *Staph. aureus* when cattle have access to pasture, as flies could be involved in the transmission of *Staph. aureus* between infected and uninfected heifers (Anderson et al., 2012). Another report showed that presence of biting flies (*Haematobia irritans*) that cause teat lesions is associated with a high level of *Staph. aureus* IMI, and suggested that fly control should be included in a mastitis management plan (Ryman et al., 2013). Heifers from herds using fly control had lower prevalence of IMI when compared with herds where no fly control was used (Nickerson et al., 1995). No questions were asked about flies or fly control in the current study. The benefits of sand as inorganic bedding material for udder health and milk quality have been reported before (Hogan et al., 1989; Dufour et al. 2011; van Gastelen et al., 2011); reasons this association was not significant in the current study could be due to the sample size and high prevalence of *Staph. aureus* in the current study as well as its contagious nature in general.

As US dairies grow in size, with a consequent acquisition of replacement animals from outside herds, implementing biosecurity measures can be beneficial to herds in reducing the entry and spread of contagious pathogens (Dinsmore, 2002). More than a third of the responding producers in the current study showed no or little interest in this topic, independent of their status as a closed or open herd. The results obtained in this study imply that interest, or lack thereof, in biosecurity overall does not differ based on whether herds are open or closed. Possible explanation for this can only be speculated: maybe herd managers that know the importance of biosecurity chose to be closed or producers with closed herds simply do not consider biosecurity an important issue for them. Similarly, some open herds recognize the importance of biosecurity measures to help them keep diseases out. Conversely, they may not have interest in the topic, which was reflected in *Staph. aureus* prevalence among these herds. In the current study, open herds that did not quarantine purchased animals were more frequently found to be positive for *Staph. aureus* in BTM than herds where quarantine was

applied. It is likely that herds quarantining purchased animals are also testing them for infectious diseases. Intramammary infections caused by *Staph. aureus* are usually subclinical; thus, *Staph. aureus* can remain unnoticed by producers if no testing is done. Furthermore, testing newly purchased animals before introducing them into the farm would be crucial for preventing and reducing the spread of *Staph. aureus* and keeping the occurrence of new infections to a low level. Although biosecurity practices have been a recommendation for mastitis control (Dinsmore, 2002), it is recognized that their implementation on farms may be challenging.

The questionnaire was an essential instrument for collecting data in the current study and every attempt was made to reduce inherent potential biases. Although the overall response rate of 49% in a mailed survey can be considered acceptable or even good, a response bias is a possibility; however, its potential direction is unknown. For instance, producers responding to the questionnaire may have been experiencing problems with udder health, and *Staph. aureus* specifically, and were responding for that reason. Conversely, they may have also been generally more progressive producers, running well-managed herds, who were simply seeking for new information. To reduce information bias and ensure high-quality, reliable data, questionnaire results were treated with extreme caution; for example, responses were cross-checked for consistency between different questions (e.g., organic herds should not be treating cows during dry-off; these results not presented in the current paper). Additionally, herd-average SCC reported by the responding producers was compared with corresponding SCC values provided by their milk-testing laboratory. No problems were detected through these checks. Due to the number of responses and because participating producers remained anonymous to the authors, face-to-face interviews or visits to farms, as done in other studies (Barkema et al., 1998; Barnouin et al. 2004; Ritter et al., 2015), were not possible in the current study. As discussed by Dufour et al. (2010), terms can be interpreted differently by different individuals and clear definitions or a glossary defining important terms used in the questionnaire should be included to minimize misinterpretations. Producers or herd managers involved in the pilot testing of the questionnaire, but not in the actual survey, did not express concerns about the questions; for a validation of the questionnaire, their responses were compared with the situation in their herds and were found to be reflective of the reality on the farm. Also, attention was given when entering results on a spreadsheet, with multiple questionnaires being randomly selected to be rechecked for data entry errors. Due to logistical challenges, some BTM samples were missing, but not in any systematic

manner; thus, this was not considered to have biased the results with respect to the association between management practices and presence of *Staph. aureus* in BTM. This is supported by the observation that results using the full data set regardless of the number of samples cultured from a herd were very similar to those using a subset of data from herds with 3 BTM samples.

Bulk tank milk samples for the current study were collected from herds located across the state, and considering the characteristics, such as herd size and milk production, these herds were representative of Ohio dairy herds in general (Ohio herds having on average 97 cows and yearly milk production of 9,216 kg or 20,318 pounds; <http://ohiodairyfarmers.com>).

CONCLUSIONS

The prevalence of *Staph. aureus* was found to be 69 to 72% in Ohio BTM. The odds of having *Staph. aureus* in BTM in herds with SCC $\leq 150,000$ cells/mL was significantly lower when compared with herds with higher BTM SCC. Adoption of the commonly recommended milking procedures individually (prestrip, pre- and postmilking teat dip, and use of single towels) was relatively high in the herds responding to this survey, and farms where they all were implemented simultaneously had lower odds of having *Staph. aureus* detected in their BTM. Herds where owners were involved in milking were also associated with lower detection of *Staph. aureus* in contrast to herds where only hired employees were milking, indicating that frequent training of employees and review of the milking procedures and their implementation may be needed to maintain good udder health.

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