Comparison of transmission dynamics between *Streptococcus uberis* and *Streptococcus agalactiae* intramammary infections

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ABSTRACT

The objectives of study were to determine the transmission parameters (β), durations of infection, and basic reproductive numbers (R_0) of both *Streptococcus agalactiae* and *Streptococcus uberis* as pathogens causing mastitis outbreaks in dairy herds. A 10-mo longitudinal study was performed using 2 smallholder dairy herds with mastitis outbreaks caused by *Strep. agalactiae* and *Strep. uberis*, respectively. Both herds had poor mastitis control management and did not change their milking management during the entire study period. Quarter milk samples were collected at monthly intervals from all lactating animals in each herd for bacteriological identification. The durations of infection for *Strep. uberis* intramammary infection (IMI) and *Strep. agalactiae* IMI were examined using Kaplan-Meier survival curves, and the Kaplan-Meier survival functions for *Strep. uberis* IMI and *Strep. agalactiae* IMI were compared using log rank survival-test. The spread of *Strep. uberis* and *Strep. agalactiae* through the population was determined by transmission parameter, β , the probability per unit of time that one infectious quarter will infect another quarter, assuming that all other quarters are susceptible. For the *Strep. uberis* outbreak herd (31 cows), 56 new infections and 28 quarters with spontaneous cure were observed. For the *Strep. agalactiae* outbreak herd (19 cows), 26 new infections and 9 quarters with spontaneous cure were observed. The duration of infection for *Strep. agalactiae* (mean = 270.84 d) was significantly longer than the duration of infection for *Strep. uberis* (mean = 187.88 d). The transmission parameters (β) estimated (including 95% confidence interval) for *Strep. uberis* IMI and *Strep. agalactiae* IMI were 0.0155 (0.0035–0.0693) and 0.0068 (0.0008–0.0606), respectively. The R_0 (including

95% confidence interval) during the study were 2.91 (0.63–13.47) and 1.86 (0.21–16.61) for *Strep. uberis* IMI and *Strep. agalactiae* IMI, respectively. In conclusion, the transmission parameter and R_0 values were not different between both pathogens; however, the duration of infection for *Strep. agalactiae* was longer than *Strep. uberis*. These suggest that *Strep. uberis* may have a different transmission dynamic compared with *Strep. agalactiae*.

Key words: *Streptococcus uberis*, *Streptococcus agalactiae*, mastitis, outbreak, duration of infection

INTRODUCTION

Bovine mastitis is considered to be one of the most economically important diseases in the dairy industry (Fetrow et al., 2000; Pyörälä, 2002; Seegers et al., 2003; Petrovski et al., 2006), due to reduced milk production and lower milk quality, increased cost of labor, drugs, and veterinary services (Wang et al., 1999). The *Streptococcus* spp., including *Streptococcus agalactiae* and *Streptococcus uberis*, are major mastitis pathogens in the dairy industry worldwide (Zadoks and Fitzpatrick, 2009; Shome et al., 2012).

Streptococcus agalactiae is a highly contagious obligate pathogen causing a long duration of subclinical mastitis, especially when the disease has not been identified (National Mastitis Council, 1998). Transmission of this pathogen occurs directly from an infected udder to an uninfected udder during the milking process, via milkers' hands, liners, and common udder cloths (Keefe, 1997). *Streptococcus uberis* is considered to be an environmental pathogen, as its primary reservoir and the source of new IMI is the environment (Radostits et al., 1994). However, cow-to-cow transmission of *Strep. uberis* has also been reported (Phuektes et al., 2001; Zadoks et al., 2003; McDougall et al., 2004; Pullinger et al., 2007; Rato et al., 2008). Zadoks et al. (2001) suggested that the outbreak of *Strep. uberis* mastitis in their study was caused by contagious transmission.

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For infectious pathogens, mathematical models are useful for analysis of the dynamics of the diseases (Anderson and May, 1992). Mathematical models are used to describe the transmission of the disease, and can be used for the estimation of R_0 , the basic reproductive number (Zadoks et al., 2002). R_0 , the average number of secondary infections resulting from the introduction of one infectious individual into a fully susceptible population (Anderson and May, 1992; Diekmann and Heesterbeek, 2000), depends on the duration of infection and the transmission rate (β). The velocity of an outbreak is the rate of change from susceptible to infected individuals in a population. The R_0 has to be >1 for sustained transmission to occur and for the pathogen to spread through a population (Anderson and May, 1992).

Mastitis control interventions are intended to reduce the duration of infection and prevent new infections. However, information about the duration of infection, transmission rate, and basic reproductive number of mastitis for *Strep. agalactiae* and *Strep. uberis* in natural outbreaks is limited, especially in relation to poor herd management. Therefore, the objective of the present study was to evaluate the values of transmission parameter (β), duration of infection, and basic reproductive number (R_0) of *Strep. agalactiae* and *Strep. uberis* in herds with poor mastitis control programs.

MATERIALS AND METHODS

Herds and Animals

A 10-mo longitudinal investigation was performed using data collected from 2 smallholder dairy herds in Phatung dairy cooperative, Chiang Mai, Thailand, from January to October 2013. On November 2012, both herds were required by their cooperative to improve of their milk quality because they had a bulk milk somatic cell counts (BMSCC) of more than 1,000,000 cells/mL for a month. The herds were investigated by the Faculty of Veterinary Medicine, Chiang Mai University, through the udder health section of the clinical rotation course for fifth-year veterinary students. Data on cow characteristic, herd management, milking management, and milking machines were collected. All quarters were tested for subclinical mastitis using the California mastitis test, and milk samples from quarters with California mastitis test score ≥ 1 were aseptically collected for bacterial identification. Results showed that both herds had mastitis outbreaks, one from *Strep. uberis* and the other from *Strep. agalactiae*. After the pathogen identification and data evaluation, the mastitis problems caused by *Strep. uberis* and *Strep. agalactiae* outbreaks were reported to the veterinary team. Adjusting herd

management based on mastitis control strategies had been recommended to the owners to improve their milk quality. On January 2013, a local veterinarian reported that BMSCC remained higher than 600,000 cells/mL in both herds, and found that milking routines had not been improved.

In both *Strep. uberis* and *Strep. agalactiae* outbreak herds, cows were crossbred Holstein-Friesians and were housed in tie-stall barns with concrete floors. Lactating cows were milked twice daily with bucket type milking machines. Poor husbandry and milking practice without regular maintenance of the milking machines were observed in both herds. Use of individual paper towels, immediately postmilking teat disinfection, segregating infected cows and milking them last, dry cow therapy, and routine culling of cows with chronic or recurrent mastitis were not practiced. Antibiotic treatments of clinical cases of mastitis were applied for selected cows by a local veterinarian. The *Strep. uberis* outbreak herd had 31 milking cows with an average milk production of 6.5 kg/d, whereas the herd with *Strep. agalactiae* outbreak had 19 milking cows with an average milk production of 8.7 kg/d. The BMSCC throughout the 10-mo study period had been more than 600,000 cells/mL.

Milk Sample Collection and Bacteriologic Analysis

Quarter milk samples were aseptically collected from all quarters of all lactating cows at approximately monthly intervals from January to October 2013. Data on clinical mastitis, antibiotic treatment and dates of calving, dry-off, or culling were recorded. Bacteriological procedures were conducted according to National Mastitis Council standards (Harmon et al., 1990). Milk samples (0.01 mL) were cultured onto one quarter of a 5% blood agar plate and incubated for up to 48 h at 37°C. Colony morphology and biochemical examination were used for bacterial identification. Preliminary identification of *Strep. uberis* and *Strep. agalactiae* was based on colony morphology and esculin hydrolysis on Edward's medium. The number of colony-forming units was recorded for all samples.

Definition of Intramammary Infection

The definition of IMI was determined according to Lam et al. (1996a) with slight modifications. A quarter was considered to have an IMI when $\geq 1,000$ cfu/mL of the pathogen was cultured from a single sample, or when ≥ 100 cfu/mL was cultured from a sample of a clinical case. Samples containing more than 3 bacterial species were considered contaminated and were not informative of IMI status. A previously infected quarter

was considered recovered from IMI for a species if none of the above definitions were found and the sample was free from the original pathogen (Zadoks et al., 2001). A quarter was eligible for a new episode of infection after it had recovered from IMI (Zadoks et al., 2003). When the IMI at the first sampling period of a new lactation was caused by the same pathogen as before drying off, it was defined as a persistent infection of the same episode of infection due to the absence of dry cow therapy.

Duration of Infection

Duration of infection was calculated based on the midpoint estimation method previously described by Zadoks et al. (2003). Dates for the start and end of IMI were estimated halfway between the 2 adjacent sampling dates. Duration of infection was defined as the interval from the midpoint between the last negative and first positive sample and the midpoint between the last positive and first negative sample. For IMI that was first detected at the first time of study, at calving, or as a clinical mastitis finding, the sample date was assumed to be the date of onset of infection. For IMI that was last detected at drying off or at culling, the sample date was taken as the endpoint of infection.

Infection status was defined into 4 categories:

- Cure: infections with starting and ending point of infection known, as infection ended with spontaneous cure;
- L-censored cure: infections with only the ending point of infection known, as infections occurred before the study commenced (left-censored) and ended with spontaneous cure;
- R-censored: infections with only the starting point of infection known, as infection remained at the end of study (right-censored) or ended due to drying off, culling, or antibiotic treatment;
- L-R censored: infections without starting and ending point of infection known, as infections occurred before the study commenced (left-censored), and remained at the end of the study (right-censored) or ended due to drying off, culling, or antibiotic treatment.

Statistical Analysis

Incidence rates were calculated as the number of new IMI per quarter days at risk (Barkema et al., 1998; Zadoks et al., 2001), and then reported as a normalized value by using the denominator (30 quarter-days at risk). Statistical analysis was conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). The durations of infection for *Strep. uberis* IMI and *Strep. agalactiae*

IMI were estimated using Kaplan-Meier survival curves, accounting for censored observations. Potential differences in the Kaplan-Meier survival function between *Strep. uberis* IMI and *Strep. agalactiae* IMI were tested using log-rank test. Means of duration of infection for each species were estimated from the distribution of the failure times. To compute the mean of the Kaplan-Meier if the longest observation is censored, the longest survival time should be treated as an event (Miller, 1981). Due to the underestimation on mean duration of infection from Kaplan-Meier survival function, a period of 30 d was added to the longest survival time, and treated as an event before the calculation.

The spread of *Strep. uberis* and *Strep. agalactiae* through the population was determined by transmission parameter, β , the probability per unit of time that one infectious quarter will infect another quarter, assuming that all other quarters are susceptible (Lam et al., 1996a). To estimate the parameter β , a susceptible-infected-susceptible model for the transmission of the pathogen between quarters was used. The infected quarters are capable of being re-infected after recovery. Therefore, the population was separated into S (noncases) and I (cases) states. New quarter infections with the pathogen were assumed to occur at the rate $\beta \times S \times I/N$, where β is the transmission parameter, S the number of susceptible quarters, I the number of infected quarters, and N is the total number of quarters (Zadoks et al., 2002). Transmission parameters (β_i) were estimated from a generalized linear model with the number of new IMI events in each sampling interval (IMI, the number of new IMI divided by the sampling interval) as the outcome, a log link, assuming a Poisson error distribution, and $\ln(S \times I/N)$ used as the offset (Lam et al., 1996a; Zadoks et al., 2002; Mweu et al., 2012; Barlow et al., 2013),

$$\varepsilon[\ln(\text{IMI})] = \beta'_i + \ln(S \times I/N),$$

where ε = expected value, $\beta' = \ln(\beta)$, S = number of susceptible quarter days, I = number of infected quarter days, and N = total number of quarter days in each interval. The transmission parameters (including 95% confidence intervals) for *Strep. uberis* and *Strep. agalactiae* were estimated for the individual herds. Data used for the analysis are shown in Table 1. The better fitted model for the I state was determined by comparing Akaike information criterion (AIC) values between models with and without the I state, and a model with lower AIC was determined as the better model.

R_0 varies with β_i (Lam et al., 1996a) as

$$R_0 = \beta_i \times \tau,$$

Table 1. Data from *Streptococcus uberis* outbreak herd and *Streptococcus agalactiae* outbreak herd showing the number of susceptible quarter days, infected quarter days, total number of quarter days, and number of new IMI of *Streptococcus uberis* and *Streptococcus agalactiae* during a 10-mo observation period containing 9 consecutive sampling intervals (sampling time 1 was baseline)

Sampling interval	<i>Streptococcus uberis</i> IMI ¹					<i>Streptococcus agalactiae</i> IMI ¹				
	Interval length (d)	S	I	N	IMI	Interval length (d)	S	I	N	IMI
1	31	90.5	11.5	102	3	36	34	26.5	60.5	9
2	38	90.5	11.5	102	7	23	27.5	28.5	56	0
3	33	82	18	100	12	32	26.5	28.5	55	4
4	30	78.5	19	97.5	4	32	26.5	29.5	56	1
5	27	84.5	14.5	99	6	28	26.5	27.5	54	4
6	34	69.5	14	83.5	9	35	29.5	20	49.5	0
7	28	57	15	72	3	27	33.5	15.5	49	3
8	35	53	16	69	8	33	34	19	53	4
9	37	53	15	68	4	37	39	20	59	1

¹S = number of susceptible quarters (the total number of susceptible quarter days divided by number of days in the interval); I = number of infected quarters (the total number of infected quarter days divided by number of days in the interval); N = number of quarters (the total number of quarter days divided by number of days in the interval); IMI = number of new IMI.

where τ = the mean duration of the infectious period, which equals $1/\alpha$ (α = the cure rate of IMI). The R_0 was calculated for each of individual herds for *Strep. uberis* and *Strep. agalactiae* IMI. The 95% confidence interval lower and upper limits were calculated for R_0 estimates:

$$\text{Exp} [\ln (R_0) \pm 1.96 \times \text{SD} (\ln (R_0))],$$

where $\text{SD} [\ln (R_0)] = \sqrt{\text{Var} [\ln (R_0)]}$, $\text{Var} [\ln (R_0)] = \text{Var} [\ln (\beta_i)] + \text{Var} [\ln (\tau)] + 2\text{Covar} (\text{Covaris equal to zero})$, $\text{Var} [\ln (\beta_i)] = [\text{SD} (\text{estimate of } \beta_i)]^2$, and $\text{Var} [\ln (\tau)] = \{\text{SD} [\ln (\tau)]\}^2$.

RESULTS

A total of 1,397 quarter milk samples were collected from the 2 herds. For the *Strep. uberis* outbreak herd, 851 quarter samples were taken from 31 cows including 4 new cows and 8 culling cows during the study period, and 546 samples were taken from 19 cows including 3 new cows and 1 culling cows during the study period for the *Strep. agalactiae* outbreak herd. Most cows were culled because of reproductive problems. During the study, 14 and 9 cows in *Strep. uberis* and *Strep. agalactiae* outbreak herds, respectively, were dried off with the duration ranging between 1 to 5 mo. Antibiotic treatment was administered on a cow with clinical mastitis in the *Strep. uberis* outbreak herd. The treated animal was not included for the calculation.

For the *Strep. uberis* outbreak herd, the observed numbers of total quarters ranging from 96 to 104 during January to June decreased to 62 to 76 during July to October. The prevalence of *Strep. uberis* IMI at starting was 0.33 (9 out of 27) and 0.125 (13 out of 104) for cow and quarter levels, respectively, and most infected cows

(66.7%) had only one *Strep. uberis* IMI quarter. For the entire study, 12 from 31 cows were free from *Strep. uberis* throughout the study period, but 19 cows had *Strep. uberis* IMI including 5, 5, 4, and 5 cows, with their 4, 3, 2, and 1 IMI quarters, respectively. From 10 monthly sampling times, a mean interval calculated from 9 sampling intervals was 32.6 d (SD 3.8). Figure 1 shows the quarter prevalence of *Strep. uberis* IMI, numbers of new infections, spontaneous cures, and censored quarters due to culling, treatment, and drying off during each sampling time. Prevalence (proportion of infected quarters per 30 quarter-days at risk) for the entire study ranged from 0.09 in third sampling time to 0.22 in eighth sampling time. From a total of 56 new *Strep. uberis* infections, the new IMI occurred mainly in April ($n = 12$), July ($n = 9$), and September ($n = 8$). Spontaneous cure of infection was observed in 28 quarters, whereas end of infection from drying off, culling, or treatment was observed in 27 quarters. The rates of spontaneous cure (percentage of cure quarters per total infected quarters) between sampling times ranged from 0% in 7th and 9th sampling times to 47% in sixth sampling time.

For *Strep. agalactiae* outbreak herd, the observed numbers of total quarters ranged from 54 to 63, except in September ($n = 47$) and August ($n = 51$). The prevalence of *Strep. agalactiae* IMI at starting was 0.63 (10 out of 16) and 0.36 (23 out of 63) for cow and quarter levels, respectively. For the entire study, 5 from 19 cows were free from *Strep. agalactiae* throughout the study period including 3 newly introduced cows. Six cows experienced *Strep. agalactiae* IMI in all quarters. During the 10-mo observation period at a mean interval of 31.4 d (SD 4.6), spontaneous cure of infection was observed in 9 quarters, whereas right-censored IMI due to drying off or culling was observed in 21 quarters. Prevalence

Figure 1. Quarter prevalence (proportion of infected quarters per 30 quarter-days at risk), numbers of new infections, spontaneous cures, and censored quarters due to culling, treatment, and drying off during each sampling time in a *Streptococcus uberis* outbreak herd.

Figure 2. Quarter prevalence (proportion of infected quarters per 30 quarter-days at risk), numbers of new infections, spontaneous cures, and censored quarters due to culling, treatment, and drying off during each sampling time in a *Streptococcus agalactiae* outbreak herd.

during the study ranged from 0.27 in tenth sampling time to 0.66 in the third sampling time (Figure 2). From a total of 26 new *Strep. agalactiae* infections, the new IMI occurred mainly in February ($n = 9$), April ($n = 4$), June ($n = 4$), and September ($n = 4$). The rates of spontaneous cure between sampling times ranged from 0% in 3rd, 4th, 8th, and 9th sampling times to 14.3% in 10th sampling time.

During the 10-mo sampling times (contained 9 sampling intervals), the majority of cases occurred in a limited time interval, covering sampling interval 3 for *Strep. uberis* outbreak herd and sampling interval 1 for *Strep. agalactiae* outbreak herd. These are the outbreak period for each herd.

The average of incidence rates of *Strep. uberis* and *Strep. agalactiae* IMI (number of new IMI per quarter-day at risk) were 0.0026 and 0.0029, respectively. Incidence rates of new IMI per 30 quarter-days at risk by sampling time for both herds are shown in Figure 3. Overall incidence rates of new IMI per 30 quarter-days at risk of *Strep. uberis* and *Strep. agalactiae* outbreak herds were 0.08 and 0.09, respectively. Ranges of *Strep. agalactiae* incidence rates (0–0.22) were broader than that of *Strep. uberis* (0.03–0.13).

Duration of infection was estimated for 69 infections in *Strep. uberis* outbreak herd. Infections that were present at the start or the end of the study, and infections in animals that were dried off, culled or treated (censored) were observed in 48 of 69 infections (70%). The duration of infections ranged from 15.5 to 293 d.

The mean duration of *Strep. uberis* IMI was 187.88 d (SE 23.29). In *Strep. agalactiae* outbreak herd, duration of infection was estimated for 49 infections. Infections that were present at the start or the end of the study, and infections in animals that were dried off, or culled (censored) were observed in 43 of 49 infections (88%). The duration of infections ranged from 18 to 283 d. The mean duration of *Strep. agalactiae* IMI was 270.84 d (SE 17.29).

Distribution of duration of infection in 4 infection categories (cure, L-censored cure, R-censored, and L-R

Figure 3. Incidence rates of new IMI per 30 quarter-days at risk by sampling time for both herds. *Strep.* = *Streptococcus*.

Figure 4. Distribution of duration of infection in 4 infection categories [cure, left (L)-censored cure, right (R)-censored, and left-right (L-R) censored] for *Streptococcus uberis* and *Streptococcus agalactiae* IMI.

censored) for *Strep. uberis* and *Strep. agalactiae* IMI are shown in Figure 4. Most infections caused by *Strep. agalactiae* were in R-censored (20/49, 41%) and L-R censored (20/49, 41%), which ranged from 18.5 to 265 d and 18 to 283 d, respectively. Most *Strep. uberis* infections were in R-censored (35/69, 51%) and cure (21/69, 30%), ranging from 18.5 to 207.5 d and 28.5 to 95.5 d, respectively.

A Kaplan-Meier curve on duration of *Strep. uberis* IMI and *Strep. agalactiae* IMI is shown in Figure 5. Approximately 80% of *Strep. agalactiae* were censored at the end of the study, therefore most *Strep. agalactiae* IMI could not be defined as spontaneous cures. In contrast, approximately 50% of *Strep. uberis* IMI spontaneously cured within 100 d following the start of the IMI. When comparing the 20% of the cured population, the survival time, indicating duration of IMI with spontaneous cure, for *Strep. uberis* (approximately 36 d) was shorter than that for *Strep. agalactiae* (approximately 190 d). In comparing infection duration between *Strep. uberis* and *Strep. agalactiae* using log-rank test, IMI quarters with *Strep. agalactiae* had significantly longer infection duration than IMI quarters infected with *Strep. uberis* ($P < 0.05$).

Figure 5. Kaplan-Meier survival curve of duration of *Streptococcus uberis* IMI (full line) and *Streptococcus agalactiae* IMI (dashed line) observed during a 10-mo period. Lines are significantly different (log rank test, $P < 0.05$); + indicates censoring of observation.

The model including the number of infected quarters (I) was the lower AIC value, indicating the better fit of the model compared with the model without this parameter. The transmission parameter (β) estimates (including 95% confidence interval lower and upper limits) for *Strep. uberis* IMI and *Strep. agalactiae* IMI were 0.0155 (0.0035–0.0693) and 0.0068 (0.0008–0.0606), respectively. The estimates for transmission parameters, mean duration of infection, and basic reproductive number (R_0), calculated from the matching β and the mean duration of infection period, are shown in Table 2. The R_0 (including 95% confidence interval) during the study were 2.91 (0.37–22.87) and 1.86 (0.12–28.59) for *Strep. uberis* IMI and *Strep. agalactiae* IMI, respectively. The R_0 during the study exceeded 1 for both pathogens, whereas R_0 for *Strep. uberis* IMI was higher than R_0 for *Strep. agalactiae* IMI. The 95% confidence intervals for R_0 of *Strep. uberis* and *Strep. agalactiae* overlapped, and they were not significantly different.

DISCUSSION

This study uses longitudinal serial sampling and mathematical modeling to describe the transmission dynamics of *Strep. agalactiae* and *Strep. uberis* in 2 separate herds. Both outbreak herds had poor mastitis

Table 2. Estimates of transmission parameters (β) including 95% confidence interval lower and upper limits, mean duration of infection (SE), and basic reproductive number (R_0) including 95% confidence interval lower and upper limits, for *Streptococcus uberis* and *Streptococcus agalactiae* IMI during the study

Parameter	<i>Streptococcus uberis</i>	<i>Streptococcus agalactiae</i>
β (95% CI)	0.0155 (0.0035–0.0693)	0.0068 (0.0008–0.0606)
Mean duration of IMI (SE)	187.88 (23.29)*	270.84 (17.29)*
R_0 (95% CI)	2.91 (0.63–13.47)	1.86 (0.21–16.61)

*Indicates significantly different at $P < 0.05$ using log-rank test.

control management: for example, using the same cloth to wash or dry all cows, no postmilking teat dipping (PMTD), no dry cow therapy, no segregating infected cows and milking them last, and no culling of cows with chronic mastitis. The result of our previous study in the *Strep. uberis* herd using pulsed-field gel electrophoresis (PFGE) showed that most isolates of *Strep. uberis* (9 of 12) from the first sampling time belonged to the same PFGE type (Leelahapongsathon and Suriyasathaporn, 2015), supporting cow-to-cow transmission of a dominant PFGE type both within and between cows, which is generally reported in the case of contagious mastitis pathogens such as *Strep. agalactiae* and *Staphylococcus aureus* (Lam et al., 1996b; Baseggio et al., 1997; Wang et al., 1999). This might indicate that the transmission characteristics of both pathogens in this study were comparable.

Incidence rates of *Strep. uberis* and *Strep. agalactiae* IMI were in similar ranges, but higher than the rates previously described for both pathogens causing clinical mastitis (Barkema et al., 1998) and for *Strep. uberis* IMI (Zadoks et al., 2001). In this study, the Reed-Frost transmission function was estimated, and improved goodness of fit of the model that included the number of infected quarters suggests cow-to-cow transmission of *Strep. uberis* rather than pathogen exposure from the environment. In addition, inadequate mastitis control programs in both herds were at known risk factors associated with increased IMI and contagious transmission (Dargent-Molina et al., 1988; Mungube et al., 2004).

The transmission parameter (β) for *Strep. uberis* infection was higher than the transmission parameter for *Strep. agalactiae* infection, whereas higher prevalence and longer duration of infection were observed with *Strep. agalactiae*. High prevalence with low number of new infections can occur when infections have long duration (Zadoks et al., 2002), as was observed in the *Strep. agalactiae* herd. The transmission parameter is related to an effective contact between infected and susceptible subjects that would be sufficient to lead to a new infection (Abbey, 1952). The effective contact depends on the method of transmission (Vynnycky and White, 2010). *Streptococcus agalactiae* is considered as a highly contagious obligate pathogen of the bovine mammary gland (Keefe, 1997). Intramammary infections occur primarily during milking, when milk from an infected cow comes into contact with the teat end of an uninfected cow. The infection is spread by contaminated milk on milking equipment, towels, or milker's hands. On the other hand, *Strep. uberis* infections can originate from exposure to environmental sources and spread from cow-to-cow during the milking process

(Zadoks et al., 2003). This characteristic provides for both contagious and noncontagious transmission, the latter occurring at any times including other sources than that during milking. A combination of contagious transmission and environmental exposure may increase the effective contact for *Strep. uberis* infection. The lower transmission parameter of *Strep. agalactiae* was unexpected because the herd did not perform proper milking management such as using separate udder cloths for each cow and PMTD (Neave et al., 1969; Pankey et al., 1983; Boddie and Nickerson, 1997).

The transmission parameter of mastitis pathogens has been estimated in a limited number of reports. White et al. (2006) reported the estimation of β for major gram-positive pathogens with or without PMTD, ranging from 0 to 0.089. Estimates of β for *Strep. uberis* from a single herd ranged from 0.005 to 0.246 (Zadoks et al., 2001).

The duration of infection in the present study might have been underestimated as some cows could have been infected before starting observations began (left-censored) and some remained infected at the end of study or end of infection due to drying off, culling, or antibiotic treatment (right-censored), even if 30 d was added to the longest right-censored events to inflate the estimated survival time. A period of 30 d was assumed from the longest duration of *Strep. uberis* infection from the previous studies (Todhunter et al., 1995; Zadoks et al., 2003; Pullinger et al., 2007). Durations of *Strep. uberis* infection ranged between 50 to 260 d in a UK study (Pullinger et al., 2007), 1 to 309 d in a Dutch study (Zadoks et al., 2003), and 1 to 370 d in a US study (Todhunter et al., 1995). Our longest duration of *Strep. uberis* infection, after adding 30 d, was similar to that reported by Zadoks et al. (2003), and in between those reported by Pullinger et al. (2007) and Todhunter et al. (1995). However, a longer duration of infection was observed in *Strep. agalactiae* infection when compared with *Strep. uberis* infection. As shown in Figure 4, most infections caused by *Strep. agalactiae* were not spontaneously cured, and this supports the study of Keefe (1997) showing that *Strep. agalactiae* infection has a very low spontaneous cure rate and therefore needs to be treated with antibiotics. In this study, the spontaneous cure rate of *Strep. uberis* infection was higher than that of *Strep. agalactiae*. The spontaneous cure rate without treatment of *Strep. uberis* infection was almost 50% (Kirk, 1998), and 45.7% of most streptococcal IMI other than *Strep. agalactiae* were spontaneously cured (Hogan and Smith, 2003). Craven (1987) suggested that the immune mechanism of the udder could be stimulated to enhance spontaneous elimination of infection.

The confidence interval of R_0 included 1 for both *Strep. agalactiae* and *Strep. uberis* infections. When $R_0 < 1$, only minor outbreaks occur; if $R_0 > 1$, major outbreaks are possible, and minor outbreaks can still occur by chance (DeJong and Diekmann, 1992; DeJong et al., 1994). The infections of *Strep. agalactiae* and *Strep. uberis* were observed throughout the 10-mo study period in each herd, and support our observation that if R_0 equal unity the infection is likely to persist in the population (Vynnycky and White, 2010), which suggests the difficulty of controlling the outbreak without any control methods. The main input components of R_0 are the rate of new infections and duration of infections (Zadoks et al., 2002). Thus, R_0 is a summary indicator of the efficacy of mastitis control strategies. Calculation of R_0 has been used to estimate the effectiveness of PMTD during an outbreak of *Staphylococcus aureus* mastitis in a dairy herd (Lam et al., 1996a). The R_0 value for undipped quarters (range from 1.09 to 7.55) was higher than the R_0 value for dipped quarters (range from 0.27 to 1.41). This was in agreement with our observations that without mastitis control strategies the outbreaks of mastitis were persistent.

In the study of the epidemiology of mastitis outbreaks, the mathematical model can provide the number for evaluating the transmission of the infection. However, the use of molecular technique can determine variability in the strain of the pathogen. The combination of mathematical and molecular approaches can support the understanding of the difference of transmission dynamic between strains of each species, especially for *Strep. uberis*, for which the mode of transmission can be characterized as intermediate between contagious and environmental (Zadoks et al., 2001). Thus, the mathematical and molecular techniques both need to be used to identify the epidemiological problem and investigate the nature of transmission of specified pathogens. In addition, parameter estimates might be improved from longer observational studies or by studying more herds or larger herd sizes.

CONCLUSIONS

The persistent infections of both *Strep. agalactiae* and *Strep. uberis* that consequently infect susceptible quarters were characterized as contagious mastitis. The transmission parameter (β) for *Strep. uberis* infection was higher than that for *Strep. agalactiae* infection, indicating higher infection rate of *Strep. uberis*. Longer duration of infection was observed with *Strep. agalactiae* with a lower spontaneous cure rate when compared with *Strep. uberis*. R_0 during the study were not different between either pathogens.

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