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INCIDENCE AND RISK FACTORS FOR REPEATED CASES OF CLINICAL *ESCHERICHIA COLI* MASTITIS IN DAIRY CATTLE

Döpfer D.[1], Barkema H.W.[3] , Lam T.J.G.M.[1,2], Schukken Y. H.[2], Gaastra W.[1]

L'incidence et les indicateurs de risque pour les cas répétés cliniques dans le cadre d'une infection intramammaire chronique d'Escherichia coli ont été étudiés dans une grande cohorte d'opérations laitières aux Pays Bas. Une polymerase chain reaction (PCR) a été utilisée pour séparer les cas répétés cliniques pendant une infection intramammaire chronique avec l'étiologie d'E. coli et les DNA fingerprints identiques des cas répétés d'infection intramammaire avec l'étiologie d'E. coli et les DNA fingerprints différents. L'incidence totale des cas des infections intramammaires avec l'étiologie d'E. coli a été déterminée à 24.5 % dans la cohorte.
L'incidence des cas répétés cliniques pendant une infection intramammaire chronique avec l'étiologie d'E. coli dans le même quartier mammaire a été 4.8 % de tous les cas avec E. coli. L'incidence des cas répétés cliniques en quartiers mammaires différents du même animal a été estimé à 3 % de tous les cas avec E. coli. Les cas répétés ont été relativement moins fréquents dans les parités 1 et 2 (OR: 0.39) en relation avec les autres parités. Depuis 60 journées jusqu'à 120 journées depuis le vêlage (OR: 2.7), pendant la période sans extraction du lait (OR: 11.6) et d'avril jusqu'à septembre (OR: 2.0) les cas répétés ont été relativement plus fréquents en relation avec les autres stages de la lactation et saison de l'année. Les cas répétés sont corrélés relativement plus fréquemment avec Corynebacterium bovis (OR: 1.9) et relativement moins fréquemment avec des staphylocoques coagulase négative (OR: 0.04).
Les cas répétés cliniques dans le cadre d'une infection intramammaire chronique dans le même quartier mammaire sont possibles ainsi que la transmission du micro-organisme E. coli entre les quartiers mammaires d'un animal. Il y a besoin des investigations de plus pour décrire le mécanisme pathogénique pour cette infection intramammaire.

INTRODUCTION

The incidence of clinical mastitis due to coliform bacteria is very high in some herds despite of the improvement of udder health. This can be a problem especially in dairy herds with low bulk milk somatic cell count (BMSCC). The incidence of chronic intramammary *Escherichia coli* infections (IMI) in low BMSCC herds has been estimated to be 9.1 % and 7.5 % of all quarter cases (Lam et al. 1996, Hogan et al. 1989). The aim of this study was to quantify the incidence and the epidemiological characteristics of repeated cases of *E. coli* IMI in a large cohort of Dutch dairy farms (Barkema et al. 1997).

MATERIAL AND METHODS

During a study period of 18 months 300 farms were grouped into three classes according to their BMSCC (<150.000 cells/ml, 150.000-250.000 cells/ml and >250.000 cells/ml). A milk sample was taken from each quarter by the farmer when signs of clinical mastitis were observed (Barkema et al. 1997). Samples from the same quarter were excluded from analysis if taken within a period of 4 days from the previous case. The *E. coli* strains were isolated from the milk samples and identified according to the standard of the National Mastitis Council (Harmon et al. 1990). A polymerase chain reaction (PCR) with ERIC (Enterobacterial Repetitive Intergenic Consensus Sequences) primers was used to differentiate repeated mastitis cases due to *E. coli* strains with the same DNA fingerprint from repeated cases due to different *E. coli* strains as described by Lipman et al. (1994).
If the same *E. coli* genotype was found in repeated cases of clinical mastitis it was considered a part of a chronic IMI.
Disease incidences and risk factors for the occurrence of repeated cases of clinical mastitis belonging to one chronic *E. coli* IMI are reported. Risk indicators that predicted the presence of a repeated *E. coli* case from a chronic infection in contrast to a single case were evaluated by using a logistic regression analysis. The odds ratios (OR) are calculated by exponentiating the coefficients from the logistic regression model ($OR=e^{\beta}$, $\alpha<5$ %). Statistical analysis was performed using SAS (Statistical Analysing System 6.08, Gary USA).

[1] Institute of Infectious Diseases and Immunology and
[2] Department of Herd Health and Reproduction, Faculty of Veterinary Medicine, Utrecht University, PO Box 80151, 3508 TD Utrecht, The Netherlands
[3] Animal Health Service, PO Box 361, 9200 AJ Drachten, The Netherlands

RESULTS

During the study period 9186 milk samples of 7845 quarters and 6227 cows were collected from clinical mastitis episodes. *Escherichia coli* was found in 2247 samples of 2108 quarters and 1946 animals. There were 230 repeated cases of the same quarter and 241 repeated cases of different quarters in the same cow. There were 271 samples from repeated cases of 198 quarters and 123 animals available for the PCR.

The distribution of clinical mastitis episodes (quarter cases) according to the PCR result is presented in table 1. In 62 of the 133 repeated *E. coli* cases (46.6%) chronic intramammary infections were found. Assuming that the 133 cases were a random sample of the 230 repeated cases within the same quarter, 107 quarter cases were part of a chronic *E. coli* infection, which corresponds to 4.8 % of all *E. coli* quarter cases in the study (n=2247).

In 39 of 138 *E. coli* cases (28.3 %) from different quarters within the same cow the same genotype of *E. coli* was found (table I). Again assuming that the 138 cases were a random sample of the 241 quarter cases found in different quarters from the same cow, 68 quarter cases correspond to 3 % of all *E. coli* quarter cases in the study.

Table I
Results of the PCR

	cases within the same quarter	contributing quarters/ animals	cases in different quarters*	contributing quarters/ animals
same genotype	62	27 / 27	39	39 / 21
different genotype	71	37 / 37	99	99 / 53
total	133		138	

*same animal

Risk indicators for the occurrence of repeated cases of clinical mastitis as part of a chronic *E. coli* IMI in contrast to single *E. coli* cases were derived by logistic regression. For this analysis 1308 clinical mastitis cases were available, of which 62 repeated cases were part of a chronic *E. coli* infection and 1246 mastitis episodes were single *E. coli* cases. The results of the analysis are presented in table II. Repeated cases of clinical mastitis did occur relatively less in parities 1 and 2 (OR: 0.39) compared to all other parities. During the peak of lactation (60 to 120 days post partum, OR:2.7), the dry period (OR:11.6) and between April and September (OR: 2.0) repeated cases occurred relatively more often compared to other stages of lactation and periods of the year. Those repeated cases correlated with relatively more *Corynebacterium bovis* in the milk sample (OR: 1.9, p=0.059) and relatively less coagulase negative staphylococci (OR:0.04).

Table II
Results of the logistic regression

(n=1308)

risk indicator	coefficient	OR	95 % confidence interval	
C.bovis (yes/no)	0.62	1.86	2.68 - 1.34	*
Coagulase neg. Staph. (yes/no)	-3.19	0.04	0.02 - 0.11	
Parity 1 or 2 (yes/no)	-0.93	0.39	0.29 - 0.54	
April - Sept. (yes/no)	0.70	2.02	1.53 - 2.68	
Peak of lact. (yes/no)	0.97	2.65	1.96 - 3.58	
Dry period (yes/no)	2.45	11.61	8.13 -16.56	
Intercept	-3.46	*: p=0.059, otherwhise p<0.05		

DISCUSSION

As described in this study repeated cases of clinical mastitis as part of a chronic *E. coli* IMI are no exception. The incidence found in this study (4.8 %) is lower compared to 9.1 % found by Lam et al. (1996) and 7.5 % found by Hogan et al. (1989) in low BMSCC farms. This difference in incidences may be due to the fact that the current study included farms of all BMSCC classes. The pathogenesis of these chronic *E. coli* IMI is not clear. A possible explanation for the occurrence of chronic *E. coli* IMI is that the host is unable to eliminate the microorganisms exhaustively from the mammary gland. The *E. coli* have to survive in the udder although the mechanism of persistence is unknown. *E. coli* strains from mastitis are considered to be non-invasive for udder epithelial cells (Valente et al. 1988), but we cannot exclude the possibility of intracellular survival in the mammary gland.

The occurrence of repeated cases of *E. coli* mastitis in different quarters within one animal due to strains with the same genotype can be explained as follows: (a) reinfection of the animals with the same strain from the environment has taken place, (b) the quarters were infected at the same point of time but showed clinical signs of mastitis at different points of time or (c) there has been transmission of *E. coli* between quarters. Reinfection with the same strain from the environment is unlikely because of the large variety of *E. coli* strains in the environment of the animals. Explanation (b) cannot be ruled out completely, yet (c) seems more plausible.

Based on the results of this study the transmission of *E. coli* between quarters is possible during *E. coli* mastitis.

Animals of the parities 1 and 2 have relatively less repeated cases of clinical mastitis as part of a chronic *E. coli* IMI compared to other parities (table 2). This may be due to a better defense mechanism in young animals.

Repeated cases occur relatively more between April and September, which is the warmer season of the year in the northern hemisphere. Mastitis due to *E. coli* is known to occur more often during the warm season (Hogan et al. 1989) and this appeared to be especially the case for the repeated cases of clinical mastitis in this study.

During the peak of the lactation the repeated cases may become clinical relatively more often, because during this stage of lactation there is a great demand for energy, which may result in immunosuppression. During the dry period the cessation of milking may result in the accumulation of udder secretion in which the lack of milk wash-out leads to multiplication of *E. coli* and clinical cases of mastitis.

The minor pathogens *C. bovis* and coagulase negative staphylococci are associated with high and low incidence of chronic infections respectively. Their role in the pathogenesis of bovine mastitis is not clear though. Their presence is thought to be protective for the occurrence of *E. coli* mastitis.

Since chronic *E. coli* IMI could represent a reservoir of infection for other quarters and the pathogens involved could bear special virulence factors resulting in chronic infections it is advisable to identify chronically infected animals using a PCR in order to eliminated them from the farms and to study the pathogenesis of those infections more closely.

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