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A CASE-CONTROL STUDY ON RISK FACTORS FOR BOVINE BRUCELLOSIS IN THE VENETO REGION (ITALY)

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Une étude cas-témoin a été conduite dans la région de Veneto (Italie) dans le but d'identifier les facteurs limitants de l'éradication de la brucellose bovine, l'élevage y ayant une importante économique majeure. Dans cette région, des cas ont été enregistrés non seulement chez les bovins, mais aussi chez les ovins, les caprins, les buffles et les humains. Parmi 63 troupeaux de bovins officiellement infectés en 1993, 1994 et 1995, 30 ont été sélectionnés comme cas sur la base de la disponibilité de données épidémiologiques valides. 74 troupeaux officiellement indemnes de brucellose au moins depuis 1992 ont été sélectionnés comme élevages témoins par 2 étapes à partir de l'ensemble de la population des troupeaux officiellement indemnes dans la région. Les facteurs étudiés pour leur association probable avec un risque accru d'avoir au moins un animal infecté étaient: la taille du troupeau, le type d'élevage, le type de bâtiment, les pratiques de pâturage, l'introduction d'animaux, les contacts avec d'autre troupeaux de bovins, d'ovins ou de caprins, ou d'animaux sauvages. Les données ont été analysées par régression logistique multiple. Les facteurs de risque statistiquement associés avec la brucellose bovine (p<0.052) étaient la taille du troupeau (OR=1,79; intervalle de confiance à 95%, 1,00-3,23) et les contacts (directs ou indirects) avec des ovins ou des caprins (OR=3,83: 1,28 - 11,46). Ce dernier résultat est en conformité avec l'isolement presque systématique de Brucella melitensis à partir des cas de brucellose chez les bovins dans la région de Veneto pendant la période 1993-1995.

INTRODUCTION

Since 1968 bovine brucellosis has been submitted in Italy to a national control programme in breeding herds, which in 1994 became specifically aimed at eradication. The programme operates on a regional basis. In those areas where eradication of the infection is within reach, the test and slaughter policy should be supported by a strict veterinary surveillance based on the knowledge of the risk factors for brucellosis. This case-control study is aimed at such factors identification in the Veneto Region, where animal husbandry is a prominent activity and brucellosis eradication is by now achievable. In this Region in fact vaccination of cattle was suspended more than 10 years ago and infected farms prevalence was 0.24%, 0.09% and 0.07% in 1993, 1994 and 1995, respectively.

MATERIALS AND METHODS

Sixty-three farms in which during the period 1993-1995 one animal at least gave a positive result on the two official tests (Rose Bengale plate test, confirmed by complement fixation test) were considered. Thirty of these (47.6%) were included in the study since reliable information concerning exposure to risk factors under study was available. Animals that had given a positive result on official tests were submitted to bacteriological examination for *Brucella spp.*, which led to isolation in 12 of the farms considered as cases. Animals from these herds that had resulted positive to serological tests and negative to bacteriological examination for *Brucella* spp. were also bacteriologically screened for the presence of *Yersinia enterocolitica*, and all of them gave a negative result. Seventy-four farms selected by means of a two stages sampling among the officially brucellosis free ones (OBF) were considered as controls. In the first stage 5 out of 22 Local Health Unit (LHU) were selected by stratified sampling by LHU size (number of breeding herds registered). In the second stage, in each of the LHU previously selected, 16 herds were randomly drawn from the list of the OBF herds. Due to the lack of reliable information, 6 out of the 80 selected control herds could not participate in the study. Information concerning exposure to risk factors have been collected by investigation on site in the control herds. As cases are concerned, data were drawn from those routinely generated by the eradication programme. The possible risk factors were chosen on the basis of their epidemiological relevance (Nicoletti, 1980) and of the local livestock characteristics. The factors considered in the study were: farm enterprise (dairy or mixed dairy and beef); housing (free or tied); grazing (no grazing or summer mountain pasture); possible contacts with local roe deer and/or chamois populations (yes or not); introduction of one animal at least since the last official diagnostic test (yes or not); indirect contacts with other cattle herds throughout exchange of manpower and/or objects (yes or not); herd size (the log-transformation of the number of cattle on herd was used in order to normalise the distribution); direct or indirect contacts with sheep and/or goats flocks (yes or not); presence of sheep and/or goats on farm (yes or not). Unconditional multiple logistic regression (SPSS Inc., Chicago, USA) was performed in order to estimate the effect of the exposure to these factors. A Spearman correlation coefficients matrix was examined for collinearity among the independent variables. No variable was highly correlated (highest correlation coefficient = 0.62). All the factors were therefore included into the logistic model. To build it both a simultaneous entry of all the variables at once and a reduction procedure (backward stepwise, with removal based on the likelihood-ratio

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statistic and P<0.05) were performed. The goodness of the fit of the models was assessed by the likelihood ratio statistic.

RESULTS

In Table I multiple logistic regression outcomes are shown. Data reported are referred to the model where all the variables were entered simultaneously, as the outcomes of the backward model presented only slight differences. The likelihood ratio χ^2 for the goodness of the fit is 21.8 (P = 0.01). Herd size and contacts with sheep and/or goats flocks appear to be associated with an increased risk for herds of having at least one infected animal.

Table I

Distribution of the 9 risk factors for bovine brucellosis considered among the 30 cases and the 74 control herds and odds ratio (OR) values with a 95% confidence interval (CI) and P values resulting from the multiple logistic regression analysis.

Variable		Cases	Controls	OR (95% CI)	P
Farm enterprise	dairy	24	69		
	mixed	6	5	2.52 (0.62-10.28)	0.19
Herd size (Log)	median	23	13		
	(min-max)	(3-172)	(2-250)	1.79 (0.99-3.23)	0.05
Sheep and/or goats on farm	no	23	68		
	yes	7	6	1.92 (0.43-8.53)	0.39
Type of stall	Tied	23	56		
	free	7	18	0.33 (0.08-1.42)	0.14
Summer mountain pasture	no	23	54		
	yes	7	20	1.49 (0.32-6.98)	0.62
Contacts with wild ruminants	no	22	48		
	yes	8	26	0.84 (0.23-3.01)	0.78
Introduction of cattle	no	20	56		
	yes	10	18	1.85 (0.65-5.29)	0.25
Contacts with sheep and/or goats flocks	no	17	64		
	yes	13	10	3.83 (1.28-11.46)	0.02
Indirect contacts with other cattle herds	no	25	53		
	yes	5	21	0.45 (0.09-2.12)	0.32

DISCUSSION

The risk factor which resulted as the most important, namely contacts with sheep and/or goats flocks, is confirmed by laboratory examinations results, as *Brucella melitensis* biovar 2 has been isolated in 10 out of the 12 bacteriologically positive farms. In the two other cases we isolated *B.abortus* biovar 1. The infection has not been eradicated yet from Veneto sheep and goats flocks and prevalences in the years considered were 1.63%, 0.81% and 1.43%. Moreover, the possibility of direct and indirect contacts between sheep and/or goats flocks and cattle farms is enhanced by practices that are typical of sheep husbandry, such as roaming pasture and transhumance. Also in other parts of Italy as well as in other countries brucellosis in sheep and goats has been demonstrated to represent a risk for cattle infection (Verger *et al.*, 1989; Farina *et al.*, 1992; Garin-Bastuji *et al.*, 1993; Garin-Bastuji *et al.*, 1994).

The success of bovine brucellosis eradication programme can be considered to be linked to infection eradication in these species. Regulations concerning flocks movements should in any case take account of these epidemiological factors.

The second highlighted risk factor is only weakly associated to the presence of infection. The risk can be considered just to depend on the natural increase of infection probability consequent on the increase in the number of exposed animals. The higher the number of animals is, the higher is the probability of having false positive results at the herd level. We anyway believe that cases included in the study have a very low probability of having been misclassified. In fact the execution in series of Rose Bengale plate test and complement fixation test makes the diagnostic procedure highly specific (Dohoo *et al.*, 1986; Godfroid, 1992). On the other hand, in 12 farms *Brucella* isolation has been achieved, and the other 18 ones gave a negative results when

bacteriologically screened for *Y.enterocolitica*, the major cause of false positivity in the serological diagnosis of brucellosis (Kittelberger *et al.*, 1995).

Cattle introduction, one of the most frequently involved risk factors (Kellar *et al.*, 1976; Nicoletti, 1980; Crawford *et al.*, 1990), does not seem to represent a risk factor in bovine brucellosis diffusion. This observation could be explained by the low prevalence of the disease and by the preclusion of introducing cattle in the farm unless they come from an OBF farm and they have been submitted again to serological tests during the month before. This result strengthens the hypothesis that moving infected sheep and/or goats flocks represent the major source of infection for bovine herds.

On the contrary, the presence of sheep and/or goats on farm does not seem to play an important role for bovine brucellosis eradication, since the introduction of these species in cattle herds is submitted to the same regulations as cattle introduction. In any case, for this factor and for farm enterprise, which resulted not to be associated to bovine brucellosis, it should be noted that the low exposition frequency, mostly in controls, makes analysis power very low. This study therefore does not allow to exclude the possibility that these factors play a role in brucellosis infection spreading in this area.

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