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INFECTIOUS BOVINE RHINOTRACHEITIS (I.B.R.): SEROLOGICAL SURVEY ON DAIRY HERDS IN *LOMBARDIA REGION*

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Un échantillonnage à plusieurs degrés a été réalisé afin d'estimer la prévalence des anticorps contre la rhinotrachéite infectieuse bovine dans la région de Lombardie. Les données relatives aux vaccinations et aux statuts sanitaires des troupeaux ont été collectées par questionnaire. Trois cent cinquante deux troupeaux laitiers ont été visités et 5309 sérums ont été examinés par un test indirect ELISA. Cent quatre vingt douze troupeaux (54,5%; 95% C.I. 49,2-59,8) et 2113 sérums (39,8%; 95% C.I. 38,5-41,1) ont été positifs.
Dans 55 troupeaux (16,7%) (C.I. 12,9 - 20,3), la vaccination contre le syndrome respiratoire a été réalisée dans les 5 années précédentes, et par conséquent ces troupeaux ont été diagnostiqués positifs. Cent vingt six des 275 troupeaux laitiers non vaccinés (46,0%; 95% C.I. 40,0 - 51,7) ont été trouvés positifs. La prévalence animale était de 29,7% (95% C.I. 28,3 - 31,1), alors que la prévalence entre troupeaux variait de 3,0% à 100% (moyenne: 42% - médian: 35%).
Les prévalences des syndromes respiratoires et des problèmes de fertilité ont été respectivement de 15,2% (95% C.I. 11,5 - 19,6) et 26,7% (95% C.I. 22,0 - 31,8). Une analyse de régression logistique multivariée a été réalisée dans le but de sélectionner les variables les plus importantes associées avec le statut sérologique BHV-1 des troupeaux. Le modèle final incluait la taille du troupeau, l'achat d'animaux et la localisation géographique (provinces) des troupeaux. Dans le but d'étudier les variables affectant la prévalence intra-troupeau, un modèle linéaire a été formulé. La vaccination et la taille des troupeaux ont été les seules variables significatives dans le modèle final.

Infectious bovine rhinotracheitis (IBR) is caused by bovine herpesvirus type 1 (BHV-1) and its importance is both of sanitary (respiratory and infertility syndrome) and economic level (trade limitations). The BHV-1 seroprevalence is not homogeneous in Europe: some countries like Switzerland, Denmark and Finland declare to be IBR free; others, like Sweden and Austria have national eradication plans allowing them to ask for additional trade guarantees; while in France and Germany there are regions with a low BHV-1 prevalence; the remaining countries have the intention in carrying out regional or national control plans.
It is necessary to have prevalence data to decide whether how to set up a control or eradication plan in Italy. Only the autonomous province of Bolzano started a voluntary eradication program from 1991 (based on a cognitive survey in 1990) and since then this program has become compulsory. Now there are 0,75% of the herds infected and 2% of the animals infected (Zambotto et al., 1995). In the other regions of Italy IBR control plans include only hygienic-sanitary and vaccinal measures.
The precise BHV-1 seroprevalence in Italy is unknown but from regional surveys it is thought to be at low or medium levels. In herds of Northern Italy, particularly in the Padana plain, the seroprevalence may vary from 25 to 80 per cent (Belletti et al., 1995; Cavirani et al., 1992; Nardelli, 1995). This study deals with assessing the seroprevalence in the region of Lombardia. In this region there are 16.235 dairy herds and about 1 million dairy cows, the milk production was almost of 4 million tonnes in 1994-95, representing 37,5% of the national production (Pieri R., 1996). Until today, there was no available representative data about the BHV-1 seroprevalence in Lombardia region except for the province of Brescia (Massirio et al., 1993).
In order to estimate the BHV-1 serological status in dairy herds and to plan a voluntary IBR control program in the region of Lombardia, a serological survey was carried out between 01.09.1995 and 31.08.1996. Also some herd characteristics were assessed in order to determine their relation with the herd serological status.

MATERIALS AND METHODS

A two-stage sampling design was used (Thrusfield, 1986). In the first stage, a stratified (to herdsize) random sample of 384 dairy farms in Lombardia region was selected, based on an expected prevalence of 50%, a precision of 5% and a confidence level of 95%. In the second stage, from each farm that participated, a random sample was taken in order to detect the presence of at least one reactor to BHV-1 (expected within-herd prevalence ≥ 10%; confidence level of 95%). Samples sizes were determined using an updated version of EPISCOPE (Frankena et al., 1990).
Blood samples were collected for the serological examination using an indirect ELISA test having a relative sensitivity and specificity, respectively, of 97.7% and 95.9%, compared to a 24 hours serum-neutralization test (Bitsch V., 1978).

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A herd was considered to be BHV-1 positive if at least one animal reacted positive.
Data about geographic location (provinces), herd-size, breed, hygienic status of herds, past history in respiratory and infertility syndromes, purchasing of animals and BHV-1 vaccination strategy during the last 5 years, were collected by questionnaire.
Data from 275 herds that claimed not to vaccinate to BHV-1 were analyzed to assess the statistical association of serological status with the 8 variables mentioned above by using multivariate logistic regression and applying the backward deletion method as described by Hosmer and Lemeshow (1989). Goodness of fit of the model was assessed using the Hosmer and Lemeshow statistic. A second analysis was performed on herds with at least one positive animal in order to determine which variables were significantly related to the within herd seroprevalence. All statistical analyses were performed using SPSS 6.1 (Norusis M.J., 1994).

RESULTS

352 of **384** dairy farms selected complied with the serological survey and were sampled. **330** of these **352** farms filled in the questionnaires. A total of **5.309** blood sera from 352 dairy farms were tested.
192 of the **352** dairy farms (54.5%; 95% CI: 49.2-59.8) showed at least one positive animal. Of the **5.309** blood samples, **2.113** (39.8%; 95% CI: 38.5-41.1) were found being BHV-1 positive. **55** farmers out of the **330** (16.7%), pointed out they had vaccinated against BHV-1 during the last 5 years, so all these herds were tested as being positive. **126** out of the **275** non-vaccinated dairy herds (46.0%; 95% CI: 40.0- 51.7) were found positive. The animal level prevalence was 29.7% (95% CI: 28.3-31.1). The within herd seroprevalence ranged from 3.0% to 100% (mean: 42% - median: 35%)
In 330 herds, the prevalences for respiratory syndrome and fertility problems were, respectively; 15.2% (C.I. 95% 11.5-19.6) and 26.7% (C.I. 95% 22.0-31.8). Hygienic conditions of herds were good in 47.0%, sufficient in 46.3%, bad in 6.7%. The prevalent breeds were Holstein Friesian and Brown Swiss.
The final logistic regression model included the variables: "geographic location", "herd size" and "purchasing of animals". The Hosmer and Lemeshow statistic indicated a sufficient fit of the model to the data (p=0.84). Results in terms of Odds Ratios (OR) were as follows.
Herds that used to purchase animals were about 3 times (OR 2.6; 95% CI: 1.3-5.4) more likely to be seropositive than herds where no animals were introduced. The risk of being seropositive increased with herd size, particularly in medium (10-100 heads) and large (> 100 heads) herds; OR's were, respectively, 9.0 (95% CI: 4.3-19.0) and 24.5 (95% CI: 6.6-91.6) versus small herd size (< 10 heads).
The dairy herds in the provinces of Bergamo (OR 8.2, 95% CI:2.0-33.1) and Brescia (OR 6.4, 95% CI:1.6-24.9) showed a significantly increased risk compared to the dairy herds in the province of Sondrio, which showed the lowest regional prevalence.
In the analysis of variance, using positive herds only, the variables "herd size" and "vaccination" showed a statistically significant relation with the within-herd prevalence and they account for 20% of the variation in prevalence. The analysis of residuals showed a Normal distribution.

DISCUSSION

A BHV-1 prevalence of 54.5% on the herd level and 39.8% on animal level agrees with the results obtained in other surveys in Italy (Belletti et al., 1995; Cavirani et al., 1992; Nardelli, 1995). This high seropositivity is partly influenced by the presence of vaccinal antibodies, which could not be distinguished from those due to natural infection, because conventional IBR vaccines were used. Since IBR vaccination is not very common in Lombardia region, the high prevalence of seropositive dairy herds might be merely due to endemic virus diffusion.
The lack of significant associations between serological status of dairy herds and the presence of respiratory and infertility syndromes might be explained by BHV-1 infections that do not cause notable clinical signs (Vilain et al., 1994). The results from the multivariate analysis show the importance in the purchasing of animals as the principal risk factor associated with BHV-1 introduction which is in agreement with Vilain et al. (1994) and Wentink et al. (1993).
The significant association between "herd-size" and seropositive status of the dairy farms might be due to the sampling design. In larger herds, more samples were taken compared to smaller herds and so the probability of finding a false positive was increased, i.e. the herd specificity of the test decreases with increasing sample size (Martin et al., 1992). However, it has also been shown that infections in larger herds have a higher probability to maintain themselves compared to smaller herds. In smaller herds the infection might disappear as chance processes play a more important role (Willeberg et al., 1994).
We can only speculate about the association between BHV-1 status and geographic location. This provincial effect might be due to herd or animal densities. Higher densities might result in higher prevalences, due to more intensive direct and/or indirect contacts between herds. The highest herd densities in Lombardia are in the provinces of Mantova, Bergamo and Brescia, with Bergamo and Brescia showing significantly increased risks. In the province of Mantova, the vaccination rate is higher compared to one in the other two, so a probable benefit regarding "virus pressure" was thought to be present in this province.
The within-herd prevalence was very variable in not-vaccinating herds. This might be due to differences in herd size and/or to difference in housing, which can both have an effect on the direct contact between infected and negative animals. Furthermore, it is possible that cattle that are infected with BHV-1 subtype 1, which shows much more higher titres than BHV-1 subtype 2b are more likely to transmit infection (Edwards et al. 1991).
The fact that only 20% of the variation was explained by the analysis of variance model is due to missing information, for example age, which has been shown to be strongly associated with spread of infection (Van Wuyckhuise et al., 1993; Vilain et al., 1994).

The control strategy of BHV-1 infection needs to consider the high regional seroprevalence as well as the very different serological and epidemiological status in the single dairy herds. Before deciding to eradicate, the seroprevalence should be lowered in a voluntary program which should include vaccination, stamping-out of the seropositive animals and hygienic-sanitary measures.These sanitary actions should be modulated on the different herd situations (e.g. low or middle-high seroprevalences). The use of marker vaccines would be very useful to discriminate between a positive serological status due to natural infection or to vaccination. In this case it could be possible to lower the spread of virus and to proceed with a rational stamping out.

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