Spatial risk factors related to outbreaks of contagious bovine pleuropneumonia in northern Italy (1990-1993)

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Summary
In October 1990, an outbreak of contagious bovine pleuropneumonia (CBPP) was reported in Italy after an absence of approximately one century. Since October 1990, ninety-four outbreaks have occurred in Italy, of which forty-seven were concentrated in three areas of northern Italy (Lombardy region). The disease was eradicated in September 1993.
The data used for the analysis were obtained from the epidemiological investigations undertaken during the outbreaks of 1990-1993. The unit of interest for the analysis is the farm. Spatial segregation of infected and uninfected farms within the study area was determined through the Pielou index of segregation. Data from herds within the same set of outbreaks were analysed through logistic regression to identify factors which could be used to discriminate between infected and uninfected herds.
The study indicated a clear spatial segregation between infected and uninfected herds. The results of the analysis do not indicate the mode of disease spread. However, the study demonstrated that both aerosol and indirect transmission of the infection could have occurred, as previously documented in Africa. The possibility that indirect transmission played a prominent role in the spread of CBPP in the region of Lombardy is a completely new suggestion. Further studies are required to understand the epidemiology of CBPP in regions with intensive farming and a relatively cold climate. In particular, three aspects require consideration; firstly, animal movement among neighbouring herds could produce a pattern of disease similar to the one produced by indirect transmission (this possibility was excluded in the epidemiological outbreak investigations conducted by field veterinarians). Secondly, the methods of spatial analysis used in this study have not been previously used in the field of veterinary epidemiology. Further validation of the efficacy of these methods is thus required. Thirdly, the epidemiology of CBPP under conditions of high animal density and a relatively cool climate, as encountered in Lombardy, requires further investigation.

Keywords
Introduction

In October 1990, an outbreak of contagious bovine pleuropneumonia (CBPP) was reported in a dairy herd of 594 animals in the region of Lombardy in Italy. Until this time, Italy had been free of CBPP since 1899. The outbreak was confirmed following isolation and identification of Mycoplasma mycoides subsp. mycoides SC (small colony). The original investigation failed to identify the source of the infection, although the most likely hypothesis is that CBPP was imported through animals originating in infected countries.

Between October 1990 and September 1993, ninety-four outbreaks were reported. Of these, 50% occurred in three locations in northern Italy (Lombardy region), covering a total area of approximately 59 km$^2$ (Fig. 1). The remaining forty-seven outbreaks were scattered in nine regions (Fig. 2). The last outbreaks were eradicated in September 1993.

Fig. 1
High-risk areas in the region of Lombardy
Two high-risk areas were found in Bergamo Province and one high-risk area in Brescia Province

The mode of transmission of CBPP is widely accepted to be direct contact (3, 8, 14), and the disease can be 'conveyed only by droplet infection, that is, by inhalation of finely dispersed bronchial secretions expelled during coughing' (14). Alternative methods of transmission, such as contact with infected urine (5, 13) and indirect transmission through contaminated hay (15) are described in the literature. These alternative modes of transmission are generally considered to be less important than direct contact (8). The resistance of M. mycoides to environmental factors is low, and is influenced by the climate; viability varies from two and three days in tropical regions to one to two weeks in temperate regions (10).

In the outbreaks outside the high-risk area in Italy, epidemiological evidence suggested that the disease spread through direct contact (1, 11). In twenty out of forty-seven outbreaks outside the high-risk area (42.55%), a successful traceback of the infection was possible (Table I). Sixteen of these outbreaks (80%) were due to purchase of infected animals. In two cases, the exchange of agricultural devices was associated with outbreaks, in the absence of trade in infected animals.

In the high-risk area, the traceback of outbreaks was generally unsuccessful (Table I) and the purchase of infected animals was documented in only one case. In most outbreaks in this area, epidemiological investigations revealed that trade in animals among farms did not occur.

This paper describes the outbreaks in Italy and analyses whether modes of transmission other than close direct contact could have occurred within the study area.

Materials and methods

Description of the control programme in Italy

In Italy, before the most recent introduction of CBPP, the law stated the following:

a) health authorities must be notified of confirmed and suspected cases of CBPP and animals must be confined to the farm premises until anatomo-pathological findings or bacteriology are able to confirm or exclude the presence of infection.
Table I
Results of the outbreak investigations
Number of outbreaks with identified source of the infection divided by geographical area with different risk levels (endemic: sets 1, 2 and 3; low risk: rest of Italy)

<table>
<thead>
<tr>
<th>Geographical area</th>
<th>Trade (a)</th>
<th>Number of outbreaks by source of infection</th>
<th>Import (c)</th>
<th>Unknown</th>
<th>Proportion of traceback</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>21</td>
<td>16.00%</td>
</tr>
<tr>
<td>Set 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0.00%</td>
</tr>
<tr>
<td>Set 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0.00%</td>
</tr>
<tr>
<td>Total high-risk areas</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>43</td>
<td>8.51%</td>
</tr>
<tr>
<td>Rest of Italy</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>27</td>
<td>42.95%</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>2</td>
<td>5</td>
<td>70</td>
<td>25.53%</td>
</tr>
</tbody>
</table>

a) purchase of animals from an infected farm was ascertained
b) exchange of agricultural devices with an infected farm was documented
c) direct import without mixing with indigenous animals, although infection was not reported in the exporting countries

b) all animals in infected herds must be slaughtered and destroyed and the premises disinfected.

In June 1991, the Ministry of Health ordered the implementation of a surveillance programme, encompassing the following main activities:
a) random sampling of cattle to assess the distribution of endemic areas in Italy
b) movement restrictions and serological testing of animals to be moved
c) selective slaughter of serological reactors to attempt Mycoplasma isolation
d) ante-mortem and post-mortem inspection in slaughterhouses (2).

Sources of data
Data used for this study derive from epidemiological investigations performed during the outbreaks of 1990-1993. Every herd or farm with at least one confirmed case of CBPP was considered as an outbreak. Isolation of M. mycoides subsp. mycoides SC or animals with pathological lesions typical of CBPP, even in the absence of successful isolation, were considered as confirmed cases. Each time an outbreak occurred, official veterinary service field veterinarians inspected the farm and collected the epidemiological data. The official local Veterinary Services used various forms to collect the epidemiological data, which included at least the following information:
- outbreak number (unique identification on a national basis)
- name of owner
- complete address of the farm
- date of suspected onset of outbreak
- date of laboratory confirmation
- date of eradication of the outbreak
- type of herd (dairy/fattening/mixed/trade)

- number of animals in the herd
- method of detection (pathological changes at slaughterhouse, serology, clinical, follow-up of other outbreaks)
- possible origin of the infection
- movements of animals to and from the farm
- map of the outbreaks.

Analysis of data
Three high-risk areas for CBPP were identified in northern Italy (1, 11). Data from one of the three areas were used for the analysis to test the hypotheses proposed. In the study area, all cattle are permanently housed, therefore the unit of interest for the analysis is the farm.

The analysis of data collected aimed to test if the CBPP outbreaks in the high-risk area could be explained by the hypothesis that close direct contact was not the principal mode of transmission of the infection among herds. The data collected during the epidemiological investigations were not suitable for investigation of spread of infection through airborne particles or indirect contact, mainly due to the lack of specific information related to alternative modes of disease spread.

To test the hypothesis that close direct contact was not the principal mode of transmission of the infection among herds, the spatial arrangements of infected and non-infected herds were analysed using the following:
a) the measurement of spatial segregation of infected and non-infected farms
b) the comparison of different models of disease spread through logistic regression.

Since the geographical co-ordinates of infected farms were available for all three high-risk areas, but co-ordinates of all the non-infected farms were available only for one of the three, the latter was used as the study area.
Spatial segregation of infected and non-infected farms
Spatial segregation of infected and non-infected farms within one of the endemic areas was determined using the Pielou index of segregation (6, 7, 12). In a population consisting of two different types of herds (A = infected; B = non-infected), each individual herd was examined and the status of the herd and that of the nearest neighbour were recorded. This information is summarised in a 2 x 2 contingency table (Table II).

In Table II, the first subscript, associated with the number of observations in a given category, refers to the status of the base herd, and the second subscript to the status of the nearest neighbour. A $\chi^2$ test, with one degree of freedom, is used to assess whether a statistical association exists between the infection status of a herd and the status of the nearest neighbour, or whether a random mixing occurs. If $n_{AA}$ differs significantly from the expectation of random mixing, $E(n_{AA}) = n_A n_B N$, status A herds have other A herds as the nearest neighbour more often than would be expected under random conditions. Thus, the two statuses of infection are partly segregated. In addition, Pielou's coefficient of segregation ($S$) (6, 7) was calculated as follows:

$$S = 1 - \frac{N * (n_{AB} + n_{BA})}{n_A n_B + n_B n_A}.$$ 

In an unsegregated population, the expected value of $S$ is 0, while in a fully-segregated population, in which no A's have Bs as nearest neighbours, $n_{AB} = n_{BA} = 0$, and $S = 1$. Pielou also notes that negative segregation is, at least theoretically, possible (6, 7). In the case of negative segregation, $S$ has an expected value of $-1$. Thus, $S$ ranges from $-1$ for a maximum negative segregation, to a value of 0 for non-segregation, to a value of 1 for maximum positive segregation.

Comparison of different models of disease transmission through logistic regression
Logistic regressions (4) were used to evaluate the effect of a number of outbreaks simultaneously occurring around each herd in the study area.

The dependent variable was the herd infection status (infected versus non-infected). Three different functions of the distance between the study herd and all the outbreaks in the study area were considered as independent variables. The three models were based on the following considerations:

a) in the case of airborne spread of the infection and in the absence of prevailing winds (which was the case in the study area), the concentration of infectious particles is expected to decrease according to the square distance from each outbreak

b) in the case of indirect spread of infection due to co-operation among farmers in the study area (exchange of agricultural devices, hay, etc.), a more linear decrease of the risk of infection with distance is expected

c) the summation of effects of a number of outbreaks simultaneously occurring around each herd in the study area has to be considered

d) since the disease remained in the study area for three years, and during that period, new outbreaks were continuously detected and eradicated, a time co-ordinate must be included in the models.

One bivariate and two univariate models were considered and compared with each other. The dependent variables considered for logistic regression were as follows:

a) average distance between each study herd and the outbreaks, and the number of outbreaks contributing to the calculation of that average distance

b) sum of the inverses of the distances between each study herd and the outbreaks

c) sum of the squared inverses of the distances between each study herd and the outbreaks.

For each infected study herd, the midpoint between detection and eradication was considered as the time co-ordinate of each outbreak. An outbreak neighbouring a study farm was considered only if the interval between the two midpoints was less than six months, that is the maximum incubation period for CBPP recorded in the literature (9). For each non-infected study herd, the period studied was centred on a date in such a way as to include the maximum value for the independent variables of the models b) and c), and the minimum value for the average distance in the model a).

Statistical analyses (excluding Pielou's index) were performed using a statistical package for social sciences (SPSS for UNIX, version 5.0).

**Results**

**Description of outbreaks**

**Outbreaks in the region of Lombardy**

Between the first reported outbreak in October 1990 and eradication of the last outbreak in September 1993, sixty-two confirmed outbreaks were recorded in Lombardy. Forty-seven of these can be grouped into three sets, according to location (Fig. 1).
Set 1
Twenty five outbreaks occurred within an area of 5.0 km × 4.0 km in Bergamo Province, corresponding to 1.25 outbreaks/km². In this group two outbreaks occurred in 1990, thirteen in 1991, three in 1992 and seven in 1993. The disease remained active until September 1993 and six cases of reinfection were recorded following restocking of farms on which stamping-out and disinfection had been performed. In this area, in early 1993, the cattle population consisted of 2,872 animals, with a density of 143.6 animals/km². The cattle present in these outbreaks accounted for 37.4% of the total number of animals slaughtered for CBPP in Italy. The area in which set 1 of outbreaks occurred is the study area of this paper.

Set 2
Eight outbreaks occurred within an area of 6.0 km × 3.5 km in Bergamo Province, corresponding to 0.38 outbreaks/km². In this group, two outbreaks occurred in 1990, five in 1991 and one in 1992. The animals present in these outbreaks accounted for 10.1% of the total number of animals slaughtered for CBPP in Italy.

The distance between the two clusters described (set 1 and set 2) was less than 10 km. Both areas are under the jurisdiction of the same local veterinary service (local health unit).

Set 3
Fourteen outbreaks occurred in Brescia Province within an area of 6.0 km × 3.0 km, corresponding to 0.78 outbreaks/km². In this group, five outbreaks occurred in 1991 and nine in 1992. The last outbreak of this set was eradicated on 15 April 1992. The animals present in these outbreaks accounted for 11.2% of the total number of animals slaughtered for CBPP in Italy.

Traceback of outbreaks
The number of outbreaks in the endemic areas and in the rest of Italy is reported in Table I. Outbreaks are listed according to source of infection, either ascertained or suspected.

Set 1
No hypothesis can be proposed as to the source of infection in twenty-one of the twenty-five outbreaks (84%). In one case, animals had been purchased from a farm where an outbreak was confirmed. In three cases, imported cattle were suspected, although the presence of the disease was not reported in the countries of origin of these cattle. In these three cases of direct imports, imported animals were not in direct contact with local animals. One of the animals was from France and two were from Poland.

Sets 2 and 3
No hypotheses can be proposed regarding the origin of the infection in these sets.

Results of the outbreak analysis in the study area
The determination of Pielou’s index of segregation for herds in set 1 gave a statistically significant value ($S = 0.7422$, $P < 0.0005$). Therefore, infected and non-infected herds can be considered spatially segregated.

The results of logistic regression are reported in Tables III, IV and V.

Two models had a significant $\chi^2$, namely model a), based on the average distance ($\chi^2 = 85.054$, $P = 0.0000$), and model b), based on inverse squared distances ($\chi^2 = 15.797$, $P = 0.0001$).

Table III
Results of logistic regression analysis for infected and non-infected farms in set 1
Model a: $Y = B_0 + B_1 \times (\text{mean distance}) + B_2 \times (\text{number of outbreaks})$
Model $\chi^2 = 85.054$; degree of freedom = 2; significance = 0.0000

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient (B)</th>
<th>Standard error</th>
<th>Significance</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean distance</td>
<td>-0.0038</td>
<td>0.0011</td>
<td>0.0009</td>
<td>0.9962</td>
</tr>
<tr>
<td>Number of outbreaks</td>
<td>-1.6207</td>
<td>0.5922</td>
<td>0.0062</td>
<td>0.1978</td>
</tr>
<tr>
<td>Constant</td>
<td>233.501</td>
<td>7.5276</td>
<td>0.0019</td>
<td>-</td>
</tr>
</tbody>
</table>

Classification table

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>Correct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>Infected</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Overall:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table IV
Results of logistic regression analysis for infected and non-infected farms in set 1
Model b: $Y = B_0 + B_1 \times (\text{sum of 1/distance}^2)$
Model $\chi^2 = 15.797$; degree of freedom = 1; significance = 0.0001

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient (B)</th>
<th>Standard error</th>
<th>Significance</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of 1/distance²</td>
<td>127901.1</td>
<td>60309.596</td>
<td>0.0339</td>
<td>-</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.5871</td>
<td>0.3695</td>
<td>0.0000</td>
<td>-</td>
</tr>
</tbody>
</table>

Classification table

<table>
<thead>
<tr>
<th>Observed</th>
<th>Predicted</th>
<th>Correct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Infected</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Overall:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table V
Results of logistic regression analysis for infected and non-infected farms in set 1
Model c): \( Y = B_0 + B_1 \times (\text{sum of } 1/\text{distance}) \)
Model \( \chi^2 = 2.657; \text{degree of freedom} = 1; \text{significance} = 0.1031 \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient (B)</th>
<th>Standard error</th>
<th>Significance</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of 1/distance</td>
<td>114.0672</td>
<td>71.0763</td>
<td>0.1085</td>
<td>3.46 x 10^8</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.5243</td>
<td>0.4796</td>
<td>0.0015</td>
<td>-</td>
</tr>
</tbody>
</table>

Classification table

<table>
<thead>
<tr>
<th></th>
<th>Predicted</th>
<th>Correct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td>60</td>
<td>100.00</td>
</tr>
<tr>
<td>Infected</td>
<td>22</td>
<td>12.00</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>74.12</td>
</tr>
</tbody>
</table>

Of the three models studied, the one with the best fit was model a), based on the average distance between the study herd and the outbreaks (Table III), which showed a sensitivity of 92% and a specificity of 97% in discriminating the health status of the study farms. Of the other two models studied, model b), based on inverse squared distances (Table IV) had a sensitivity of 28% and a specificity of 98% in discriminating the health status of the study farms, while model c), based on inverse distance (Table V) had a sensitivity of 12% and a specificity of 100%.

Discussion and conclusions

The value of Pielou’s index of segregation for herds in set 1 suggests that in this high-risk area, infected and non-infected herds are spatially segregated.

This clustering of outbreaks may suggest the existence of a mode of transmission other than close direct contact. Such an assumption might explain the poor success rate in the traceback of infection based on the sole hypothesis of close direct contact as the mode of transmission within the area. If one assumes that CBPP entered Italy two years before detection (i.e. in late 1988), five additional outbreaks could be traced to animal trading. Three outbreaks in set 1, one in set 2, and one in set 3 could have been caused by trading of animals which occurred in 1989 and 1990 with farms later found to be infected with CBPP. This improvement in the ability to trace outbreaks allows the source of the infection in three high-risk areas to be traced to a single lot of imported infected animals. However, this hypothesis is not sufficient to explain the links between all the outbreaks.

The high density of the bovine population within the study area (143.6 animals/km²) and the possible intensive interactions between specialised cattle breeding farms, are likely to contribute to direct, as well as indirect transmission of the infection (exchange of devices, vehicles, fodder, etc.).

To test this hypothesis, logistic regressions were performed. Of the models considered, the first two (i.e. average distance and sum of the inverse distance) were consistent with an indirect method of transmission of infection. The third model (sum of the inverse squared distances) was consistent with aerosol transmission. The results of the analysis were not able to indicate clearly the mode of transmission, due to the significant value found for the \( \chi^2 \) statistics for the goodness of fit and for the Wald statistics for the coefficients for the three models. However, the first model was more efficient in discriminating health status. An indirect mode of transmission occurring in the study area cannot, therefore, be excluded.

The negative coefficient for both the average distance between each study farm and the surrounding outbreaks can be explained as follows: the closer the outbreaks to the study farm, the higher the risk of infection of the farm. The negative value of the coefficient for the number of surrounding outbreaks could be due to increased caution by farmers in terms of co-operation when a number of outbreaks are detected in the neighbourhood.

The possibility of indirect transmission of CBPP has already been documented in Africa by Windsor and Masiga (15). Previous reports have not indicated that indirect transmission could play an important role in the spread of infection, as appears to be the case in Lombardy. Additionally, the epidemiology of CBPP has never been studied under the climatic conditions and high animal density typical of northern Italy.

The following factors must be considered in evaluating the results reported:

a) epidemiological outbreak investigations conducted by field veterinarians excluded the possibility of animal movements between herds. Should animal movement be considered in the spread of the disease, a model similar to the one fitting indirect transmission is the logical consequence

b) methods of spatial analysis used in this study have not previously been used in the field of veterinary epidemiology. Further validation of the efficacy of these methods is required

c) the epidemiology of CBPP under the high animal density and climatic conditions encountered in Lombardy requires further investigation.
Facteurs de risque spatiaux favorisant l’apparition de foyers de pérripneumonie contagieuse bovine en Italie du Nord (1990-1993)

A. Giovannini, S. Bellini, M.D. Salman & V. Caporale

Résumé

Mots-clés

Factores espaciales de riesgo ligados a brotes de perineumonia contagiosa bovina en el norte de Italia (1990-1993)

A. Giovannini, S. Bellini, M.D. Salman & V. Caporale

Resumen
Tras aproximadamente un siglo de ausencia de la enfermedad, en octubre de 1990 se declaró un brote de perineumonia contagiosa bovina en Italia. Desde entonces y hasta septiembre de 1993, cuando la enfermedad quedó erradicada, se
comunicaron en el país noventa y cuatro brotes, cuarenta y siete de ellos concentrados en tres áreas septentrionales (región de Lombardía).

Los datos utilizados para el análisis provienen de las investigaciones epidemiológicas emprendidas durante los brotes de 1990-1993. La unidad considerada en el análisis es la explotación. Mediante el índice Pielou de segregación se determinó la segregación espacial de las explotaciones infectadas y no infectadas dentro del área de estudio. Además se analizaron por regresión logística los datos de rebaños afectados por brotes de un mismo periodo, con el fin de determinar factores que permitan distinguir entre rebaños infectados y no infectados.

Aunque el estudio revela una clara segregación espacial entre rebaños infectados y no infectados, los resultados del análisis no permiten deducir el mecanismo por el que se propaga la enfermedad. Aun así, el estudio sugiere la posibilidad de que la transmisión haya ocurrido por inhalación (aerosol) o de manera indirecta, como ya se había observado anteriormente en África. La posibilidad de que la transmisión indirecta desempeñara un papel preponderante en la propagación de la enfermedad en Lombardía es una hipótesis completamente nueva. Para comprender cabalmente la epidemiología de la perineumonía contagiosa bovina en regiones con ganadería intensiva y clima relativamente frío es preciso estudiar más a fondo la cuestión, teniendo en cuenta sobre todo tres consideraciones: primero, el movimiento de animales entre rebaños vecinos podría dar lugar a un patrón epidemiológico similar al resultante de la transmisión indirecta (esta posibilidad quedó descartada durante las investigaciones epidemiológicas realizadas sobre el terreno por veterinarios); segundo, los métodos de análisis espacial utilizados para este estudio son inéditos en el campo de la epidemiología veterinaria, por lo que es preciso comprobar más exhaustivamente su validez; y tercero, es necesario estudiar más a fondo la epidemiología de la perineumonía contagiosa bovina en las condiciones que se dan en Lombardía, a saber, gran densidad de ganado y clima relativamente frío.

Palabras clave

References


