Herd-level seroprevalence and risk-mapping of bovine hypodermosis in Belgian cattle herds

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Abstract

Our objective was to determine the seroprevalence of \textit{Hypoderma} spp. and to develop a spatial model describing the risk surface of warble-fly infection in Belgian cattle herds (adjusting for herd size, herd type, local temperature, rainfall, relative air humidity and land-cover).

This survey was carried out in 390 selected herds of all types (dairy, mixed and beef) from December 1997 to March 1998, which were included in a national infectious bovine rhinotracheitis and paratuberculosis (Johne’s-disease) survey. All animals >24 months old were blood sampled and an ELISA was used on pooled serum samples (10 animals per pool).

The herd seroprevalence was 48.7% (95% confidence interval: 43.6–53.8); positive herds were mainly in the south of the country and along the North Sea coast.
The logistic multiple-regression model of herd-level seropositivity indicated that mixed-type and beef-cattle herds have more than four-fold and two-fold increases in the odds of being *Hypoderma*-positive, respectively, compared with dairy herds.

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**Keywords:** Prevalence; Risk-mapping; Spatial analysis; Hypodermosis; Belgium

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### 1. Introduction

Warble flies (*Hypoderma* spp.) are common parasites of cattle in the Northern Hemisphere. Infestations of cattle with the larvae of this fly cause serious damage to hides and occasional deaths (due to anaphylactic shock or toxaemia or damage to the central nervous system or oesophagus). The adult flies are nuisances, causing reduction in milk yield and/or subnormal weight gains (Tarry, 1986). Moreover, larvae produce enzymes causing immunodepression and subsequent diseases (Boulard et al., 1997).

*Hypoderma*'s economic importance is reflected in the eradication schemes of several countries, especially Belgium’s neighbouring countries (The Netherlands (Sol, 1997), France (Boulard, 1999), Germany (Liebisch et al., 1995), Great Britain (Webster et al., 1997; Phipps and Webster, 1999; Phipps and Swallow, 2001). Belgium discontinued its control campaign in the early 1980s, but has been under increasing pressure to reinstate it.

Very few data are available about hypodermosis in Belgium. The only survey showed a high seroprevalence in two provinces: Liège and Luxemburg (43 and 86%, respectively) (Lonneux et al., 1994).

The ecology of the vector or the parasite and the environmental determinants of their distribution are of prime importance in the transmission, surveillance and control of vector-borne and parasitic diseases (Kitron, 1998). However, the relationship between the development of warble flies and climatic risk factors has been studied only in vitro (Pfadt et al., 1975; Minář and Breev, 1982; Karter et al., 1992; Benakhla et al., 1993, 1999). The factors found to be of interest were a high temperature during the pupal period, moisture, age and dairy type of breed.

Our aim was to determine the herd-level seroprevalence of *Hypoderma* spp. and to develop a spatial model predicting prevalence risk in Belgian cattle herds from local climatologic, geographic and demographic risk factors. This is the first study of this type about warble fly ever run at a national level.

### 2. Background

The fly’s life cycle is approximately 1 year, of which 9 months are spent in cattle as internal parasites. Adult flies appear in the spring and summer (they are active only in warm weather). In Belgium, adults are active from May to August (peaks: June and July) (Grégoire, 1952). Adults do not feed; they are active for only a few days. Mating occurs off the host at aggregation points where females are intercepted in flight (Hall and Wall, 1995). Mating influences flight behaviour; unmated female flies stay at the birth site and fly away only once mated. The flight range varies between 3 and 5 km and probably rarely exceeds 5 km (Lonneux et al., 1991; Sol and Sampimon, 1997).
An ELISA to detect antibodies against *Hypoderma bovis* and *Hypoderma lineatum* infections was developed to screen cattle; the ELISA uses individual sera, pooled sera of up to 10 animals or milk (Sinclair and Wassall, 1983; Boulard, 1985; Colwell and Baron, 1990; Boulard and Villejoubert, 1991). The test most likely will detect seropositive animals between late October and February/March in northern Europe. The animals do not necessarily have to show clinical signs of disease (e.g. the larvae might not have completed their life cycle because they were not viable, the host had developed a protective immune response or the host had been treated; Boulard, 1985). Antibodies are not detectable until 3–5 months following the appearance of the flies—and then, only for <1 year (Boulard, 1975; Sinclair et al., 1984; Boulard and Villejoubert, 1991). Therefore, positive serology is a specific indicator of current and recent warble-fly activity and also can be interpreted on a herd basis (Webster, 1996).

Using sera from clinically negative cattle from warble-free areas (therefore unexposed and considered to be true negative) and sera from clinically infested cattle (true positives), the sensitivity of the serologic test on individual sample was 94% and the specificity 98% (Webster, 1998). The specificity data were probably fairly accurate—but could have been over-estimated; in contrast, sensitivity in a screening situation probably will be less accurate because it only applied to animals with clinical signs of warble-fly disease (Chauvin et al., 1988).

### 3. Materials and methods

#### 3.1. Survey design

The data were collected as part of a national infectious bovine rhinotracheitis and paratuberculosis (Johne’s-disease) survey from December 1997 to March 1998 (Boelaert et al., 2000a,b). The survey used a stratified one-stage cluster-sample design, with province as the stratification variable and proportional allocation of the number of herds amongst provinces according to total number of herds (Thrusfield, 1995). The total number of herds to be sampled was set at 384 herds according to Cannon and Roe (1982), to detect a prevalence of 50% with 5% precision at 95% confidence level. This number was doubled to represent 1% of the total number of Belgian cattle herds to get more precise estimates of prevalence when stratifying by herd type.

The sampling frame consisted of all cattle-keeping holdings in Belgium, which were included in the SANITEL-Cattle database. This database is maintained by the Dierengezondheidszorg Vlaanderen (DGZ) and the Association Régionale de la Santé et de l’Identification Animale (ARSIA), and all cattle holdings are required to report stock numbers and any movements. The database also has the geographic coordinates of the main building of the farm.

All animals >24 months old were blood sampled in the selected herds. The samples were aggregated into pools of ≤10 animals and 60% of the herds sampled had at least two serum pools tested (maximum = 22). Pooling samples has no important effect on sensitivity and the test is still of value in the detection of animal-level infestation prevalence as low as 2% because the ELISA can detect one positive animal amongst a pool of 10 (Chauvin et al., 1988).
The blood samples were taken by the local veterinary practitioners and sent to the Veterinary and Agrochemical Research Centre (VAR, Federal Public Service of Health, Food Chain Safety and Environment) for diagnosis. The pooled serum samples were tested for antibodies against *Hypoderma* spp. with a commercially available ELISA kit (Calfcheck-hypodermose®, Vétoquinol, France; pooled sensitivity 91.5% according to the manufacturer). All samples were tested according to the manufacturer’s instructions.

During the farm visit, the veterinary practitioners conducting the sampling also recorded herd type (dairy, mixed or beef herd) and size (number of cattle on the premises). Beef type was defined as a cow–calf herd, a young-stock herd, a fattening-cow herd, a fattening-steer herd, a fattening-calf herd or a hobby holding. Mixed type represents herds with both dairy and beef cattle on the premises. The age of the animals was available from the SANITEL-Cattle database.

Information about climatic risk factors was provided by the Royal Meteorological Institute of Belgium (3 Avenue Circulaire, 1180 Brussels, Belgium) for the years 1996–1997. Only data from April 1 to November 30 were used because in Belgium, the flies are active only during this period of the year. We also used climatic data from the actual fly season (1997) as well as from the previous year because 1997 flies were the result of the previous fly generation in 1996. Data consequently were smoothened over these 2 years and conditions met in the different regions could be generalized. Data from the ground stations included minimum and maximum daily temperatures (112 stations), daily rainfall (200 stations) and daily relative air humidity (12 stations). The mean daily minimum, maximum, average and range of temperature and the mean daily rainfall and mean daily relative air humidity from April 1 to November 30 were calculated for each station.

The National Geographic Institute of Belgium (13 Abbaye de la Cambre, 1000 Brussels, Belgium) provided land-cover information from the Belgian CORINE (Co-ordination of Information on the Environment) database. These data are recorded at a resolution of 1:100,000 for 44 different land-cover types, of which 32 are present in Belgium. Homogeneous cover of one single class is digitized down to a smallest area of 25 ha. The land-cover type covering at least 75% of the cell was assigned when mixed land-cover types were present in a 25-ha cell.

### 3.2. Descriptive statistics

The seroprevalence was estimated based on the survey results from the seropositive herds. A herd was defined as *Hypoderma*-positive if at least one positive pooled serum sample was detected. The test result was interpreted on a herd basis.

The point locations of all herds were displayed by serological status on a map of the province boundaries of Belgium, using the geographical information system software (GIS) ArcView for Windows Version 3.2 (ESRI, Redlands, CA, USA). The herd-location data were converted into continuous raster surfaces using the kernel-density interpolation function of ArcView, expressing the case occurrence as intensity per square km. The kernel density was estimated using a bandwidth (τ) of 15 km and a cell size of 1500 m.
3.3. Data analysis

The data were analysed in two steps: exploratory analysis based on spatial cluster analysis and then development of predictive regression models.

Disease clustering was investigated using the spatial scan statistic (SaTScan 2.1, National Cancer Institute, MD, USA) based on 999 Monte Carlo iterations (Raftery and Lewis, 1992, 1995) and a maximum spatial cluster size set to 50% of the total population at risk.

The kriging technique was used to generate raster surfaces from the various climatic variables with the ArcView add-in software Kriging Interpolator 3.2 (http://www.nieuwland.nl). The kriging estimates were converted into vector contour data (contour interval 0.05 °C, 0.05 mm or 0.05%). The spatially relevant environmental and land-cover data were linked to each herd record using GIS overlay functions.

Based on this database, logistic multiple-regression was used to model the seroprevalence of warble-fly exposure in cattle with Stata 7.0 (Stata Corp., College Station, TX, USA). For this analysis, continuous-scale variables (herd size, minimum temperature, maximum temperature, average temperature, average temperature range, rainfall, relative air humidity) were categorised into quintiles. The 32 land-cover types present in Belgium were grouped into six classes (urban, agricultural, pastures, forests, waste land and wet land). Multicollinearity amongst putative risk factor variables was assessed using Spearman Rank correlation coefficients, Pearson correlation coefficients, unpaired t-tests, ANOVAs, Kruskall–Wallis tests and Wilcoxon rank-sum tests. One variable in a pair was dropped if $R$ was greater than $0.8$ or if $P < 0.05$. Multicollinearity also was checked using the variance-inflation factor and the mean-variance inflation factor; values $>4$ were considered worrisome and these variables were withdrawn (Neter et al., 1996; Katz, 1999). Confounding, interaction and departure from a linear trend were assessed using the likelihood-ratio test (LRT) (Neter et al., 1996). We used backward stepwise variable selection, based on $P < 0.05$. Land-cover and herd type were kept in the analysis, whatever their level of significance. Spatial dependence in the data was taken into account through use of the robust variance estimator for clustered data (White, 1980; Gourieroux et al., 1984; Liang and Zeger, 1986, 1993; Williams, 2000). Administrative boundaries (provinces) were used to identify these subgroups or clusters.

Hosmer–Lemeshow test was performed to assess the model’s goodness-of-fit (Hosmer and Lemeshow, 1989).

The significant environmental factors, i.e. climatic and land-cover variables, were modelled separately using logistic multiple-regression. The final-model regression equation was used to generate a GIS raster layer expressing disease probability based on local environmental conditions. This was done using the map–calculator function of ArcView.

4. Results

4.1. Descriptive statistics

A total number of 556 herds were sampled. Of those, 166 herds were withdrawn because insufficient serum was available for testing, the herds were being used as part of a national
IBR and Johne’s-disease survey or because the test gave erroneous results (i.e. doubtful results, insufficient available sera, haemolysed blood, data wrongly encoded). Also, some herds had to be excluded because no geographic-location information was recorded in the SANITEL-Cattle database. As a result, a total of 390 herds remained in the analysis, of which 362 had geographic information, 361 had data about size and 349 about herd type.

The herd seroprevalence of hypodermosis was 48.7% (95% confidence interval: 43.6–53.8). For the two federal regions of Belgium, Flanders has a 29% herd seroprevalence (23–35) and Wallonia 85% (79–91). Moreover, in Flanders, herds are mainly negative along the Dutch border (Antwerp province)—but seroprevalence is higher in the West-Flanders province along the North Sea coast. The kernel density map of positive herds (τ = 15 km, cell size 1500 m) shows this (Fig. 1).

4.2. Cluster analysis

The most likely cluster based on the spatial scan statistic represented negative herds (relative risk (RR) 0.29, \( P = 0.001 \)) in the provinces of Antwerp and Limburg. There are three significant secondary clusters which are (ordered according to their likelihood ratio):

- East of Belgium (province of Liège, Herve, German border) with RR 1.88 (\( P = 0.001 \)) compared to the surrounding area, and 45 cases (23.96 expected) out of a population of 49.

Fig. 1. Serological hypodermosis herd infection status and kernel estimate of positive density of infected holdings: A, Antwerp; B, Brussels; L, Liège; N, Namur.
- Province of Hainaut (west of Wallonia), RR = 1.84 ($P = 0.001$), 35 cases (19.1 expected) out of 39.
- Province of East-Flanders (along the North Sea), RR = 0.20 ($P = 0.006$), three cases (15.2 expected) out of 31.

### 4.3. Risk-factor analysis

The herd-level seroprevalences for all significant risk factors are displayed in Table 1. A logistic-regression model was developed, ignoring the dependence in the data structure and modelling the quintiles of the herd size and climatic variables as continuous data. This model included the variables mixed herds (versus dairy herds), minimum temperatures, maximum temperatures, rainfall and pastures land-cover (urbanized areas being the baseline). There was no association between *Hypoderma* herd serostatus and average temperatures, range of temperatures and relative air humidity.

Table 1

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Category level</th>
<th>Number of herds</th>
<th>Herd-level seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd type</td>
<td>Dairy</td>
<td>89</td>
<td>44.9</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>77</td>
<td>72.7</td>
</tr>
<tr>
<td></td>
<td>Beef</td>
<td>183</td>
<td>39.9</td>
</tr>
<tr>
<td>Herd size (animals)</td>
<td>First quintile (1–8)</td>
<td>73</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td>Second quintile (9–30)</td>
<td>72</td>
<td>40.3</td>
</tr>
<tr>
<td></td>
<td>Third quintile (31–59)</td>
<td>72</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>Fourth quintile (60–98)</td>
<td>72</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>Fifth quintile (99–271)</td>
<td>73</td>
<td>61.6</td>
</tr>
<tr>
<td>Daily minimum temperature ($^\circ$C)</td>
<td>First quintile (5.95–7.75)</td>
<td>73</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>Second quintile (7.76–8.25)</td>
<td>72</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Third quintile (8.26–8.35)</td>
<td>72</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>Fourth quintile (8.36–8.45)</td>
<td>72</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>Fifth quintile (8.46–8.65)</td>
<td>73</td>
<td>42.5</td>
</tr>
<tr>
<td>Daily maximum temperature ($^\circ$C)</td>
<td>First quintile (14.55–17.25)</td>
<td>73</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>Second quintile (17.26–17.6)</td>
<td>72</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Third quintile (17.61–17.75)</td>
<td>72</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>Fourth quintile (17.76–17.85)</td>
<td>72</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>Fifth quintile (17.86–18.25)</td>
<td>73</td>
<td>30.1</td>
</tr>
<tr>
<td>Mean daily rainfall (mm)</td>
<td>First quintile (2.00–2.15)</td>
<td>73</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>Second quintile (2.16–2.20)</td>
<td>72</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>Third quintile (2.21–2.25)</td>
<td>72</td>
<td>50.0</td>
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<tr>
<td></td>
<td>Fourth quintile (2.26–2.30)</td>
<td>72</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Fifth quintile (2.31–2.95)</td>
<td>73</td>
<td>90.4</td>
</tr>
<tr>
<td>Land-cover</td>
<td>Urbanized area</td>
<td>207</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td>Agricultural land</td>
<td>117</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>Pastures</td>
<td>31</td>
<td>45.2</td>
</tr>
<tr>
<td></td>
<td>Forests</td>
<td>4</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Wet land</td>
<td>1</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Taking account of dependence between observations in the model changed the output to some extent (log likelihood = −171.61). The beef herd became significant whereas maximum temperature was not anymore (Table 2).

The log odds of herd seropositivity increased linearly with the quintiles of herd size, minimum temperature, maximum temperature and rainfall, according to their likelihood-ratio tests ($\chi^2$ and $P$ for the LRT ranging from 4.22 to 6.49 and 0.09 to 0.24, respectively).

The Hosmer–Lemeshow goodness-of-fit statistic was computed and returned a $\chi^2$ of 5.61 and a corresponding $P$ value of 0.69.

The pasture variable was the only significant land-cover risk factor. All land-cover types other than pasture were used as the reference category and a new logistic-regression model including only climatic variables and this binary land-cover variable was produced (log likelihood = −197.45). The coefficients expressed at a log odds scale were used to produce a disease risk map based on the most important environmental risk factors (Fig. 2).

5. Discussion

Hypodermosis has high seroprevalence in Belgium and is widely— but not homogeneously—distributed across the country. The high prevalence in the south resulted in a blank space in the province of Namur when performing the cluster analysis because all herds sampled in that province were positive and no comparison was possible between cases inside and outside the circles generated by the scan-statistic test.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI (OR)</th>
<th>S.E.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd type$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy herd</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mixed herd</td>
<td>4.32</td>
<td>2.55, 7.31</td>
<td>1.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beef herd</td>
<td>1.97</td>
<td>1.03, 3.77</td>
<td>0.65</td>
<td>0.040</td>
</tr>
<tr>
<td>Herd size$^c$</td>
<td>1.42</td>
<td>1.15, 1.74</td>
<td>0.15</td>
<td>0.001</td>
</tr>
<tr>
<td>Climatic factors$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily minimum temperatures</td>
<td>0.68</td>
<td>0.55, 0.84</td>
<td>0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily maximum temperatures</td>
<td>0.60</td>
<td>0.33, 1.12</td>
<td>0.19</td>
<td>0.111</td>
</tr>
<tr>
<td>Mean daily rainfall</td>
<td>1.32</td>
<td>1.01, 1.73</td>
<td>0.18</td>
<td>0.041</td>
</tr>
<tr>
<td>Land-cover factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urbanized area</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Agricultural land</td>
<td>0.74</td>
<td>0.36, 1.51</td>
<td>0.27</td>
<td>0.402</td>
</tr>
<tr>
<td>Pastures</td>
<td>0.35</td>
<td>0.17, 0.75</td>
<td>0.14</td>
<td>0.007</td>
</tr>
</tbody>
</table>

$^a$ Robust estimator.

$^b$ 349 herds.

$^c$ The quintiles of these variables were modelled as continuous data; LR chi-square = 130.14 (d.f.: 8).
Mixed and beef herds had increased odds of being *Hypoderma*-positive compared with dairy herds—even though dairy breeds are at a particular risk (Benakhla et al., 1993, 1999) because of drug restrictions. For dairy cattle, only eprinomectin can be used (which makes dairy cattle less likely to be treated against this condition).

It appears that larger herds are at increased risk of being *Hypoderma*-positive than smaller ones. Management of parasites control in a large herd could be more difficult and also more expensive.

Heavy rainfall increased the odds—contrary to the literature (Tarry, 1980; Minář and Breev, 1982; Lonneux et al., 1991; Karter et al., 1992). With increasing minimum temperature, the odds declined. However, the upper limiting temperature might not be reached in Belgium because maximum temperature was not significant.

The observed effect of the different land-cover types was unexpected at first sight. Compared to the baseline urban area, agricultural land was not significantly different and pastures were at decreased risk. The baseline consisted of continuous or discontinuous urban areas, industrial zones and sport and leisure equipments. Cattle are more likely to be present on agricultural land (especially on pastures) than in urban zones. This would suggest an odds ratio >1 for agricultural areas. However, Belgium is a densely populated country and a large part of the countryside is really just a discontinuous urban area (according to the CORINE database, which codes land-cover types according to a European standard). In fact, according to this classification, most of the farms were considered to be in an urban area! A more accurate analysis using land-cover classification data (other than from the CORINE database) would require a higher spatial resolution—but this is not currently available for Belgium.

Fig. 2. Predicted hypodermosis odds map based on environmental data: A, Antwerp; B, Brussels; L, Liège; N, Namur.
The risk map generated on the basis of the model equation could be an effective tool for strategic implementation of a control program. The south of the country is largely positive and this region represents a largely suitable environment for the fly. In contrast, Flanders is mainly negative and the risk according to environmental data is lower. But, some zones in Flanders do represent a favourable habitat for the fly (e.g. along the Dutch border and along a north–south axis joining two highly favourable areas).

6. Conclusion

Although herd-level seroprevalence of hypodermosis in Belgium is 48.7%, the habitat of warble flies in Belgium is not homogeneous. Larger herds and higher daily rainfall are also important contributing factors and lower minimum temperature appears to be protective.

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