



**HAL**  
open science

## Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium

E. Méroc, C. Faes, C. Herr, B. Verheyden, T. Vanbinst, F. Vandebussche, J. Hooyberghs, M. Aerts, K. de Clercq, K. Mintiens, et al.

### ► To cite this version:

E. Méroc, C. Faes, C. Herr, B. Verheyden, T. Vanbinst, et al.. Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium. *Veterinary Microbiology*, 2008, 131 (1-2), pp.133. 10.1016/j.vetmic.2008.03.012 . hal-00532409

**HAL Id: hal-00532409**

**<https://hal.science/hal-00532409>**

Submitted on 4 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium

Authors: E. Méroc, C. Faes, C. Herr, B. Verheyden, T. Vanbinst, F. Vandebussche, J. Hooyberghs, M. Aerts, K. De Clercq, K. Mintiens, C. Staubach



PII: S0378-1135(08)00108-9  
DOI: doi:10.1016/j.vetmic.2008.03.012  
Reference: VETMIC 3990

To appear in: *VETMIC*

Received date: 18-9-2007  
Revised date: 14-3-2008  
Accepted date: 18-3-2008

Please cite this article as: Méroc, E., Faes, C., Herr, C., Verheyden, B., Vanbinst, T., Vandebussche, F., Hooyberghs, J., Aerts, M., De Clercq, K., Mintiens, K., Staubach, C., Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.03.012

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1     **Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium**

2

3     E. Méroc<sup>1</sup>, C. Faes<sup>2</sup>, C. Herr<sup>1</sup>, B. Verheyden<sup>1</sup>, T. Vanbinst<sup>3</sup>, F. Vandebussche<sup>3</sup>, J.

4     Hooyberghs<sup>4</sup>, M. Aerts<sup>2</sup>, K. De Clercq<sup>3</sup>, K. Mintiens<sup>1</sup>, C. Staubach<sup>5</sup>

5

6     <sup>1</sup>. Veterinary and Agrochemical Research Centre, Co-ordination Centre for  
7     Veterinary Diagnostics, Groeselenberg 99, B-1180 Brussels, Belgium.

8     <sup>2</sup>. Hasselt University, Center for Statistics, Agoralaan Block D, B-3590  
9     Diepenbeek, Belgium

10    <sup>3</sup>. Veterinary and Agrochemical Research Centre, Department of Virology,  
11    Groeselenberg 99, B-1180 Brussels, Belgium.

12    <sup>4</sup>. Federal Agency for the Safety of the Food Chain, Directorate General of Control  
13    Policy. S. Bolivarlaan 30, B-1000 Brussels, Belgium.

14    <sup>5</sup>. Friedrich-Loeffler-Institut, Institute of Epidemiology, Seestr. 55, D-16868  
15    Wusterhausen, Germany.

16

17    Correspondence address:

18    E. Méroc

19    Veterinary and Agrochemical Research Centre

20    Co-ordination Centre for Veterinary Diagnostics

21    Groeselenberg 99, B-1180 Brussels, Belgium

22    Tel:        +32 2 379 04 61

23    Fax:        +32 2 379 06 70

24 E-mail: [esmer@var.fgov.be](mailto:esmer@var.fgov.be)

Accepted Manuscript

25     **Abstract**

26

27     Bluetongue (BT) was notified for the first time in several Northern European  
28 countries in August 2006. The first reported outbreaks of BT were confirmed in herds  
29 located near the place where Belgium, The Netherlands and Germany share borders.  
30 The disease was rapidly and widely disseminated throughout Belgium in both sheep  
31 and cattle herds. During the epidemic, case-reporting by the Veterinary Authorities  
32 relied almost exclusively on the identification of herds with confirmed clinical  
33 infected ruminants. A cross-sectional serological survey targeting all Belgian  
34 ruminants was then undertaken during the vector-free season. The first objective of  
35 this study was to provide unbiased estimates of BT-seroprevalence for different  
36 regions of Belgium. Since under-reporting was suspected during the epidemic, a  
37 second goal was to compare the final dispersion of the virus based on the  
38 seroprevalence estimates to the dispersion of the confirmed clinical cases which were  
39 notified in Belgium, in order to estimate the accuracy of the case-detection based on  
40 clinical suspicion. True within-herd seroprevalence was estimated based on a logistic-  
41 normal regression model with prior specification on the diagnostic test's sensitivity  
42 and specificity. The model was fitted in a Bayesian framework. Herd seroprevalence  
43 was estimated using a logistic regression model. To study the linear correlation  
44 between the BT winter screening data and the case-herds data, the linear predicted  
45 values for the herd prevalence were compared and the Pearson correlation coefficient  
46 was estimated. The overall herd and true within-herd seroprevalences were estimated  
47 at 83.3 (79.2-87.0) and 23.8 (20.1-28.1) per cent, respectively. BT seropositivity was  
48 shown to be widely but unevenly distributed throughout Belgium, with a gradient  
49 decreasing towards the south and the west of the country. The analysis has shown

50 there was a strong correlation between the outbreak data and the data from the survey  
51 ( $r=0.73$ ,  $p<0.0001$ ). The case detection system based on clinical suspicion  
52 underestimated the real impact of the epidemic, but indicated an accurate spatial  
53 distribution of the virus at the end of the epidemic.

54

#### 55 **Keywords**

56 Bluetongue; Survey; Seroprevalence; Correlation; Belgium; Epidemics

57

#### 58 **1. Introduction**

59 Bluetongue (BT), a vector-borne viral disease, is transmitted in ruminant populations  
60 almost exclusively by several species of biting midges of the genus *Culicoides*  
61 (Diptera: Ceratopogonidae) (Cêtre-Sossah et al., 2004; Pili et al., 2006). BT virus  
62 (BTV) is a species of the genus *Orbivirus* within the *Reoviridae* family. To date, 24  
63 distinct BTV-serotypes have been identified (Gorman, 1990; Takamatsu et al., 2003).  
64 BT can cause spectacular outbreaks and has an adverse impact on worldwide trade due  
65 to restrictions on the source of animals (Green et al., 2005; FAO, 2006). It thus appears  
66 on the list of diseases notifiable to the World Organisation for Animal Health (OIE).  
67 Influenced by several factors such as geographical location, the incidence of clinical  
68 disease is highly variable. BT disease is uncommon in many areas where BTV is  
69 endemic (MacLachlan, 2004). The virus is traditionally known to be distributed around  
70 the world in countries lying in the tropics and subtropics, although it may extend further  
71 north like in parts of Western North America and Xinjiang, China (Dulac et al., 1989;  
72 Gibbs et al., 1994; Qin et al., 1996). The virus has been documented as far as 45°N in  
73 Southern Europe (Caporale et al., 2004).

74 In August 2006, very unexpectedly, BT was for the first time notified in The  
75 Netherlands, Belgium and Germany. (OIE Animal Health Department, 2006;  
76 Toussaint et al., 2006). Later on during the epidemic, related cases were also reported  
77 in France and Luxembourg. The virus incriminated was identified as BTV-serotype 8  
78 (CRL, 2006; Toussaint et al., 2007a), which prior to this epidemic had only occurred  
79 in Africa, Central America, Malaysia, and India/Pakistan (Herniman et al., 1980;  
80 Hassan, 1992; Mo et al., 1994; Daniels et al., 2004; Gerdes, 2004). Although the  
81 possible routes of introduction were investigated, the exact origin remains unknown  
82 (Mintiens et al., 2007). Based on the data from the early stages of the epidemic, the  
83 rate of the local spread was estimated to be around 15 km/week, partially reflecting  
84 the rapid extension of BTV in Northern Europe (Gerbier et al., 2007).

85 In Belgium, the first 11 ever reported BT outbreaks were confirmed in the near  
86 East-border part of the country on the 19th of August 2006, in both sheep and cattle  
87 herds (Toussaint et al., 2007b). Despite the measures implemented banning animal  
88 movement, the disease was rapidly and widely disseminated throughout the Belgian  
89 territory. By December 2006, a total of 695 herds or flocks were declared “case  
90 herds” of which 297 were cattle herds. During the epidemic, case-reporting by the  
91 Belgian Veterinary Authorities relied almost exclusively on the identification of herds  
92 with confirmed clinical infected ruminants. Laboratory diagnoses were mostly used  
93 for confirmation of BTV infections in ruminants reported with BT-like clinical signs.  
94 Therefore under-reporting was suspected.

95 During the winter of 2006/2007, it was assumed that climatic conditions were  
96 unfavourable for further propagation of BTV. The last cases of the epidemic in  
97 Belgium were reported by the Veterinary Authorities on 15 December 2006. A  
98 serological and virological cross-sectional survey (BT winter screening) targeting all

99 Belgian ruminants was undertaken in January-February 2007 in order to establish the  
100 true final dispersion of the virus across the country. The first objective of the study  
101 was to provide unbiased estimates of BT-seroprevalence for different regions of  
102 Belgium. A second objective was to compare the final dispersion of the virus based  
103 on the seroprevalence estimates to the dispersion of the confirmed clinical cases  
104 which were notified in Belgium, in order to estimate the accuracy of the case-  
105 detection based on clinical surveillance. This paper presents the descriptive  
106 epidemiology of the BT winter screening 2007.

107

## 108 **2. Material and Methods**

### 109 *2.1 Sampling design for the BT winter screening*

110 The study population of the winter screening consisted of dairy cattle of more than  
111 two years old which were housed in dairy farms with on-farm delivery of dairy  
112 products. Cattle were sampled because of expected higher prevalence in this species  
113 compared to sheep (Ward et al., 1994). Only dairy cattle were considered for  
114 sampling since serologically negative animals that were to be identified by the BT  
115 winter screening would participate afterwards in a longitudinal BT sentinel animals  
116 monitoring programme (logistically dairy animals are sampled more easily). The  
117 sampling frame was provided by the list of 1245 dairy herds with on-farm delivery of  
118 dairy product previously identified for the official Belgian Leucois-Brucellosis winter  
119 screening. In this programme, all animals of more than two years were sampled.

120 Since no prior information on the herd prevalence was available, the number of  
121 herds to be sampled was based on an expected prevalence of 50 per cent (maximal  
122 variance), a desired absolute precision of 5 per cent and 95 per cent confidence level.  
123 Since at the time of sample size's selection, no information was available on the

124 diagnostic test's sensitivity and specificity, these were assumed to be perfect. A  
125 sample of 384 herds was set to be selected (Cannon and Roe, 1982). A one-stage  
126 cluster sampling design was performed with stratification of the herds by province  
127 and proportional allocation according to province surface.

128

## 129 *2.2 Diagnostic methods*

130 Samples were collected by the official farm veterinarians and conditioned to serum  
131 samples at the regional laboratories of 'Dierengezondheidszorg Vlaanderen' and the  
132 'Association Régionale de Santé et d'Identification Animales'. The serum samples  
133 were assayed using a commercially available competitive ELISA (c-ELISA) kit (ID  
134 Screen® Blue Tongue Competition for detection of anti-VP7 antibodies; ID.VET,  
135 Montpellier, France) which was carried out according to the OIE Manual of Standards  
136 (OIE, 2004) and to the procedure described by the manufacturer. Results were  
137 expressed as percentage negativity (PN) compared to the negative kit control and cut-  
138 off settings considered were those provided by the manufacturer. Samples which  
139 presented a PN less or equal to 35 per cent, between 35 and 45 per cent, and greater  
140 than 45 per cent were considered as positive, doubtful and negative, respectively.  
141 Doubtful results were classified positive in the data analysis. Using RT-qPCR as  
142 reference test during the epidemic, the diagnostic sensitivity and specificity of the c-  
143 ELISA was estimated at 87.4 per cent (95%CI: 83.5-90.4) and 99.0 per cent (95%CI:  
144 97.2-99.6), respectively (Vandenbussche et al., 2007).

145

## 146 *2.3 Case herds*

147 Case herds were mostly herds (cattle or ovine) for which the veterinary practitioner,  
148 who has been consulted by the animal owner, identified suspicious clinical cases and

149 where at least one of those animals was subsequently confirmed positive using a  
 150 laboratory test (c-ELISA and/or real-time PCR) and then notified to the veterinary  
 151 authorities (EFSA, 2007). A maximum of three animals were sampled per herd. In  
 152 addition, herds without clinical signs but with seropositive animals which were then  
 153 confirmed positive with real-time PCR (Toussaint et al., 2007a) were also included.  
 154 For example, animals could be detected when tested serologically for certification  
 155 prior to trade between zones with different BTV-8 status within the country or prior to  
 156 export. EDTA blood and serum samples were tested at the Belgian National  
 157 Reference Laboratory (VAR).

158

#### 159 *2.4 Statistical methods*

160 The estimation of within-herd seroprevalence was based on a logistic-normal  
 161 regression model. For the BT winter screening data, let  $Z_i$  be the number of positive  
 162 tested animals out of  $N_i$  tested animals from herd  $i$ . It was assumed that the number  
 163 of positive animals followed a binomial distribution:

$$164 \quad Z_i \sim \text{Bin}(N_i, p_i^a), \quad (1)$$

165 with  $p_i^a$  the apparent prevalence. The true prevalence  $p_i^t$ , reflecting the true  
 166 serological status of the animals, was derived from the following equation (Rogan and  
 167 Gladen, 1978) taking the sensitivity and specificity of the c-ELISA test into account:

$$168 \quad p_i^a = Se \times p_i^t + (1 - Sp) \times (1 - p_i^t), \quad (2)$$

169 or,

$$170 \quad p_i^t = \frac{p_i^a + Sp - 1}{Se + Sp - 1},$$

171 where  $Se$  is the test sensitivity and  $Sp$  is the test specificity.

172 To account for possible correlation among the animals from the same herd, the  
 173 seroprevalence in herd  $i$  was modelled as

$$174 \quad \text{logit}(p_i^t) = \beta + u_i, \quad (3)$$

175 with  $u_i \sim \text{Normal}(0, \sigma^2)$  being the normally distributed random intercepts for each  
 176 herd. This model is a special form of a generalized linear mixed model as described  
 177 by Molenberghs and Verbeke (2005). The Intraclass Correlation Coefficient (ICC)  
 178 given by

$$179 \quad ICC = \frac{\sigma^2}{\sigma^2 + \pi^2/3},$$

180 was estimated to establish the correlation between the infection status of two animals  
 181 within a herd. Since the sensitivity and specificity were no fixed or known values, a  
 182 prior distribution for the sensitivity and specificity was assumed. Thus, model  
 183 specification was further extended by assuming a beta-distribution for the  $Se$  and  $Sp$   
 184 parameters:

$$185 \quad Se \sim \text{beta}(a_1, b_1), \quad (4)$$

$$186 \quad Sp \sim \text{beta}(a_2, b_2), \quad (5)$$

187 where  $a_1, b_1, a_2, b_2$  were chosen based on literature material (Vandenbussche et al.,  
 188 2007). In summary, the within-herd seroprevalence estimation was based on a  
 189 logistic-normal regression model, accounting for the test sensitivity and specificity of  
 190 the test. The model is given by equations (1) to (5). Because of its hierarchical  
 191 structure, it was fitted in a Bayesian framework, using WinBUGS software  
 192 (<http://www.winbugs-development.org.uk>): Non-informative priors were used for all  
 193 model parameters. Posterior seroprevalence distributions and 95 per cent central  
 194 credibility intervals were generated. A density plot of the predicted within-herd

195 seroprevalence estimates, was produced based on the logistic-normal regression  
196 model (1)-(5).

197 The herd seroprevalence (probability that a herd was infected) was estimated using  
198 a logistic regression model:

$$199 \quad Y_i \sim \text{Bernoulli}(p^h), \quad (6)$$

$$200 \quad \text{Logit}(p^h) = \beta,$$

201 where  $p^h$  is the apparent herd prevalence. This model was extended by allowing  
202 different  $\beta$  's for the different provinces, in order to estimate the province-specific  
203 herd seroprevalences. For the purpose of this study, a herd was considered as positive  
204 if at least one of the sampled animals had a positive ELISA test result, otherwise it  
205 was considered negative.

206 In both models described above, the design-effect was taken into account by  
207 weighting each observation by the inverse of the sampling probability. Provinces  
208 which were under-represented in the sample were attributed a higher weight, whereas  
209 the over-represented received smaller weight. Herd density data was extracted from  
210 the Belgian animal identification and registration system (SANITEL) to provide  
211 estimates of the population at risk. A map showing herd density for cattle at  
212 municipality level was produced using ArcView GIS 3.2. (ESRI).

213 A map showing the distribution of within-herd seroprevalence in the country was  
214 produced. The true within-herd prevalence estimates of the farms which were sampled  
215 were interpolated by Inverse Distance Weighting (IDW) (Shepard, 1968; ESRI,  
216 1996).

217 In order to estimate the accuracy of the case-detection based on clinical  
218 surveillance, the linear correlation between the BT winter screening data and the case  
219 data was estimated. For both data sets, herd prevalence per municipality was

220 estimated based on logistic regression models, as in equation (6). In order to account  
221 for spatial differences, a flexible smoothing method was used to estimate the spatial  
222 trend. It is assumed that

$$223 \quad \text{Logit}(p^h) = f(x, y),$$

224 where  $f(x, y)$  is some unspecified smooth function of the  $x$ - and  $y$ -coordinates. The  
225 method used penalized splines with radial basis function, fitted as a generalized linear  
226 mixed model (Eilers et al., 1996; Ruppert, Wand and Carroll, 2003). This method was  
227 implemented in the SAS procedure GLIMMIX (9.1.3. SAS, Inc.).

228 To study the linear correlation between the two datasets, the linear predicted  
229 values  $f(x, y)$  for herd prevalence, resulting from the logistic regression models  
230 applied to the two datasets, were compared and the Pearson correlation coefficient  
231 was estimated. For the outbreak data to be comparable with the winter screening data,  
232 solely cattle results were used for this part of the analysis. Maps showing the  
233 distribution of herd prevalence estimates at municipality level were produced.

234 Daily meteorological data on the mean temperature were collected at 247 weather  
235 stations in The Netherlands, Belgium, Germany and France. The raw temperature  
236 values were interpolated between the locations of the weather stations using IDW. A  
237 model of the German meteorological service for large scale maps was used to adjust  
238 the interpolated temperature values for the correlation between temperature and  
239 altitude (Müller-Westermeier, 1995; Ahrens, 2006). In a first step, the raw  
240 temperature data were reduced to sea level and interpolated on a grid cell size of 250  
241 m using the IDW algorithm. Finally, the interpolated values were adjusted for the  
242 height in the actual topography using altitude data with a resolution of 90 m (NASA  
243 SRTM data). Maps of the spatially interpolated mean daily temperature were  
244 produced for six dates at monthly intervals.

245

246 **3. Results**247 *3.1 Bluetongue winter screening*

248 A total of 25,846 cattle from 344 herds were sampled between the first and the 31<sup>st</sup> of  
249 January 2007. An average of 75 animals (standard deviation, 51), ranging from 1 to  
250 370, were sampled per herd. Among those samples, 5008 gave positive results. The  
251 overall herd seroprevalence was estimated at 83.3 per cent (95%CI 79.2-87.0).  
252 Province-specific herd seroprevalence estimates are shown in Figure 1. The highest  
253 estimates were found in provinces located in the north-east of Belgium, near the place  
254 where the epidemic started. The true overall within-herd prevalence was 23.8 per cent  
255 (95%CI 20.1-28.1). The spatial distribution of the within-herd prevalence is presented  
256 in Figure 2. The highest provincial within-herd prevalence estimates were found in  
257 Limburg and Liege provinces. There was a second focus around the city of Ghent in  
258 East Flanders province. On the other hand, the lowest within-herd seroprevalence was  
259 found in Hainaut province. Figure 3 shows the density plot of within-herd  
260 seroprevalence estimates. A large variability in within-herd seroprevalence was  
261 observed. However, in most herds, within-herd seroprevalence was between 0 and 20  
262 per cent. The within herd ICC was estimated at 0.41 (95%CI 0.36-0.47). This shows  
263 that correlation between the infectious statuses of two animals within a herd was high.  
264 Figure 4 presents herd density at municipality level. High herd densities in 2006 were  
265 mainly observed in the western part of the country. Figure 5 shows maps of the  
266 spatially interpolated temperature for the 30 June, 30 July, 30 August, 30 September,  
267 30 October and 30 November 2006. Around the possible time of introduction (June-  
268 July), the temperature was high in Belgium. The global temperature trend during the  
269 BT epidemic consisted of two warm time periods (June-July and End-September)

270 separated by a cooler period. Local differences in mean temperature were also  
271 suggested by the maps.

272

### 273 *3.2 Case herds*

274 Between the 18<sup>th</sup> of August and the 31<sup>st</sup> of December 2006, a total of 1445 cattle  
275 and 893 sheep samples were analysed. The overall BT herd prevalence was estimated  
276 at 0.7 per cent (95%CI 0.7-0.8) for cattle herds and 1.3 per cent (95%CI 1.2-1.4) for  
277 sheep flocks. Herd prevalence estimates at the provincial level for cattle and sheep are  
278 shown in Table 1. Estimates were found to be more or less twice higher for sheep than  
279 for cattle. However, the distributions of herd prevalence estimates at the provincial  
280 level were found to be similar in the two species.

281

### 282 *3.3 Comparison of winter screening and cattle case herd results*

283 Based on the linear predicted values for herd prevalence of the winter screening  
284 and the cattle case herd data, the Pearson correlation coefficient was 0.73 and  
285 significant ( $p$ -value $<0.0001$ ). Figure 6 consists of two maps showing the distribution  
286 of BT herd prevalence estimates at municipality level based on cattle case-herd and  
287 winter screening data. The patterns indicated by the two maps seemed to be similar to  
288 each other.

289

## 290 **3. Discussion**

291 Starting from the original focus in the area where Belgium, The Netherlands and  
292 Germany share borders, the epidemic gradually disseminated throughout the Northern  
293 European countries. The epidemic predominantly spread horizontally along an East-  
294 West axis (EFSA, 2007). In Belgium, until October 2006, case herds were mainly

295 limited to an area situated in the Eastern part of the country. Early September 2006,  
296 the area of chief concern appeared to be the infectious status of the “still free”  
297 provinces; therefore, a serological screening was conducted and demonstrated  
298 freedom of BTV infection for all the provinces in which no case herd was notified at  
299 that time (Vandenbussche et al., 2007). The first case in East Flanders was notified on  
300 September 18 and the infection then further continued its spread to the west. At the  
301 end of the epidemic, BTV-seropositivity in dairy cattle herds was shown to be widely  
302 but unevenly distributed throughout Belgium. Seroprevalence was found to be the  
303 highest near the area of first infection with a gradient decreasing towards the south  
304 and the west of the country. In The Netherlands, the same distribution was observed,  
305 with, in this case, a gradient decreasing towards the Northern part of the country  
306 (Elbers et al., 2007). Based on case herd data, Gerbier et al. (2007) identified two  
307 spatial clusters of cases in Belgium which centered around the cities of Maastricht  
308 (the Netherlands) and Ghent. The authors stated that a gap between the two clusters  
309 remained by the end of the epidemic. The results of the present study on the other  
310 hand demonstrated a herd seroprevalence gradually decreasing towards the west with  
311 no higher level around the Ghent area. The within-herd seroprevalence map (Figure 2)  
312 revealed areas around Maastricht and Ghent where the within-herd seroprevalence  
313 was high. The highest within-herd seroprevalences were found on farms situated in  
314 Liege, Limburg, Flemish and Walloon Brabant provinces most certainly due to the  
315 fact that those regions were affected at the beginning of the epidemic. However,  
316 further study of specific risk factors such as local temperature, farm management  
317 system, and abundance of vector, is needed to better understand the spatial variation  
318 in the occurrence of BT and to allow a more efficient control of the infection in the  
319 future. The second focus around the city of Ghent could be explained for instance by

320 the high cattle farm density in this area (Figure 4) which could be a risk factor for  
321 within-herd propagation of BT. Visual examination of the temperature maps (Figure  
322 5) suggested that hilly areas were always cooler compared to areas of lower elevation.  
323 Those lower temperatures may have had an influence on the life cycle of *Culicoides*  
324 and the replication of BTV in the vectors. The high ICC reflected the important  
325 correlation between two animals within a herd with respect to the presence/absence of  
326 BTV. A study conducted in Kazakhstan demonstrated also significant clustering at  
327 farm level (Lundervold et al., 2003). The authors pinpointed the fact that this effect  
328 could be related to local variations in the vector's distribution.

329 Clinical signs of BT appear as soon as five days post-infection. Therefore, in the  
330 early stages of an epidemic, infected animals are more quickly detected by clinical  
331 examination than by serology. In Italy, during the 2000-2001 BT-outbreak, sero-  
332 surveillance only debuted in the decreasing phase of the epidemic curve (Giovannini  
333 et al., 2004). In a reporting system such as the one implemented during the course of  
334 the outbreak in Belgium, a succession of events has to occur before a case is detected.  
335 Theoretically, the reporting of suspect cases allows for a view of the situation for the  
336 entire susceptible population which is under owner and veterinary observation. This  
337 first relies on the assumption that the infection will produce clinical signs; hence,  
338 subclinical cases will go unnoticed (Doherr et al., 2001). BTV has in the past been  
339 isolated in several countries without clinical disease being recognised (Gibbs et al.,  
340 1994; Mulhern, 1985). Based on the sparse data from whole-herd-sampling during the  
341 Northern European epidemic, it has been shown that a high proportion of cattle within  
342 a herd could be PCR or seropositive, while not showing any BT-clinical signs.  
343 Moreover, owners and veterinarians in Belgium had never previously experienced this  
344 exotic disease; therefore clinical signs were unfamiliar to them (Elbers et al., 2007).

345 Also, owners may have been reluctant to report cases for fear of consequent loss of  
346 trade. The winter screening revealed indeed a higher prevalence than demonstrated by  
347 the reporting of clinical cases. Results demonstrated a high level of exposure to BTV  
348 in the dairy herds. The results confirm the fact that BTV spreads very quickly in an  
349 immunologically naïve ruminant population. The first Italian epidemic of BT in 2000  
350 in Sardinia has demonstrated a rate of spread of 30 km per week and 80% of the  
351 island had been infected. Both in Sardinia and Sicilia, serological surveillance  
352 detected virus circulation to be wider than shown by clinical surveillance (Calistri et  
353 al., 2004; Giovannini et al., 2004). Serological screening demonstrated a BT animal-  
354 prevalence levels ranging from 3.23 to 61.11% in Albania after recent first infection  
355 (Di Ventura , 2004). In the present study, the obtained Pearson correlation coefficient  
356 showed that the spatial distributions of the virus indicated by the two datasets (Figure  
357 6) were very similar. However, there is was large scale-difference in estimated  
358 prevalences. The case detection system based on clinical suspicion underestimated the  
359 real impact of the epidemic, but indicated an accurate spatial distribution of the virus  
360 at the end of the epidemic.

361 In theory, each individual within the target population, namely the Belgian ruminant  
362 population, should have had an equal chance of being selected for sampling. For  
363 accessibility matters, only dairy herds with on-farm delivery of dairy product were  
364 considered in the sampling frame. Moreover, solely animals older than 24 months  
365 were sampled. Sub sampling presents an opportunity for selection bias which must be  
366 accounted for when willing to extrapolate the results to the target population. From  
367 the outbreak data, BT herd prevalence level in sheep was higher than in cattle. Ovine  
368 BTV infection cases might have been easier to detect since this species is commonly  
369 known to be more prone to develop the clinical form of the disease (Gibbs et al.,

1994). However, the particularity of this BTV-8 epidemic was that the virus was able to induce severe clinical signs in cattle (Thiry et al., 2006). Moreover, the results of the confirmation analyses showed that clinical signs observed in cattle were more specific than those observed in sheep (Toussaint et al., 2007b). In general, seroprevalence is known to be higher in cattle than in small ruminant populations (Ward et al., 1994; Di Ventura et al., 2004). On the other hand, a study conducted on the Indian sub-continent, demonstrated a higher seroprevalence in sheep than in cattle, with 45.71% and 33.4%, respectively (Sreenivasulu et al., 2004). Those findings demonstrate differences which can occur when sampling a particular species instead of another. In the same way, many studies have concluded that older cattle were more likely to be positive to BTV antibodies than younger cattle, related to a greater opportunity for repeated exposure to the virus (Uhaa et al., 1990; Ward et al., 1994; Lundervold et al., 2003). Factors such as breed specific genetics or management methods differ a lot between a beef and a dairy cattle herd; hence, the level of prevalence may not follow identical patterns. Moreover, the “on-farm delivery of dairy products” characteristic of selected herds may be associated for instance to a more artisanal agriculture which may indirectly have consequences on disease control. An analysis performed by Green et al. in the United States (2005), has not proven herd type to be a significant risk factor. However, this conclusion may depend on local conditions and consequently differ for Belgian cattle.

The only indication a positive serological result gives is that the tested animal was at one point infected with the virus. Due to resistance in host population, future outbreaks would probably occur more silently in herds which were already infected during the 2006 epidemic. In this case, the genuine dissemination of the virus would

394 certainly be much more extensive than the distribution of suspected cases (Purse et  
395 al., 2006).

396

#### 397 **4. Conclusion**

398 These findings currently provide the best information available on the  
399 unprecedented occurrence of BT in Belgium and emphasized the rapid and non-  
400 confined spread of the virus in a susceptible ruminant population. Local variations in  
401 estimated prevalence should be further investigated to help identify particular risk  
402 factors and be able to better control future outbreaks. The results of the winter  
403 screening were also used to set up a sentinel program in the country. This study  
404 showed that the case detection system based on clinical suspicion underestimated the  
405 real impact of the epidemic, but provided an accurate indication of the spatial  
406 distribution of the virus at the end of the epidemic.

407

#### 408 **Acknowledgements**

409 This study is part of a contract research programme funded by the Belgian Federal  
410 Public Service of Health, Food Chain Safety, and Environment, the Belgian Fund for  
411 Health and Quality of Animals and Derived Products, and The Belgian Federal  
412 Agency for the Safety of the Food Chain. Partners in this programme are Avia-Gis  
413 (Zoersel, Belgium), the Gembloux Agricultural University (Gembloux, Belgium), the  
414 Institute for Tropical Medicine (Antwerp, Belgium), Liège University (Liège,  
415 Belgium), the Veterinary and Agrochemical Research Centre (Brussels, Belgium),  
416 and the Walloon Agricultural Research Centre (Gembloux, Belgium).

417 The authors gratefully acknowledge all the persons which have contributed to the  
418 collection of data for their cooperation in this study and Kathrin Teske for her  
419 technical assistance to produce the temperature maps.

420

## 421 **References**

- 422 Ahrens, C.D. 2006. Meteorology today. An introduction to weather, climate and the  
423 environment. Thomson Learning, Stamford, CT.
- 424 Calistri, P., Giovannini, A., Conte, A., Nannini, D., Santucci, U., Patta, C., Rolesu, S.,  
425 Caporale, V., 2004, Bluetongue in Italy: Part I. Veterinaria Italiana. 40, 243-  
426 251.
- 427 Cannon, R.M., Roe, R.T., 1982, Livestock Disease Surveys: A field manual for  
428 veterinarians. Australian Bureau of Animal Health, Canberra.
- 429 Caporale, V., MacLachlan, N.J., Pearson, J.E., Schudel, A., 2004, Introduction Third  
430 International Symposium on Bluetongue. Veterinaria Italiana 40, 29-30.
- 431 Cêtre-Sossah, C., Baldet, T., Delécolle, J.-C., Mathieu, B., Perrin, A., Grillet, C.,  
432 Albina, E., 2004, Molecular detection of *Culicoides* spp. and *Culicoides*  
433 *imicola*, the principal vector of bluetongue (BT) and African horse sickness  
434 (AHS) in Africa and Europe. Veterinary Research 35, 325-337.
- 435 CRL, European Commission Reference Laboratory, 2006, Bluetongue virus in the  
436 Netherlands identified as serotype 8 by Institute for Animal Health, Promed,  
437 August 28. Accessed at [www.promedmail.org](http://www.promedmail.org), archive no.: 20060828.2448
- 438 Daniels, P.W., Sendow, I., Pritchard, L.I., Sukarsih, Eaton, B.T., 2004, Regional  
439 overview of bluetongue viruses in South-East Asia: viruses, vectors and  
440 surveillance. Veterinaria Italiana 40, 94-100.

- 441 Di Ventura, M., Tittarelli, M., Semproni, G., Bonfini, B., Savini, G., Conte, A., Lika,  
442 A., 2004, Serological surveillance of bluetongue virus in cattle, sheep and  
443 goats in Albania. *Veterinaria Italiana* 40, 101-104.
- 444 Doherr, M.G., Audigé, L., 2001, Monitoring and surveillance for rare health-related  
445 events: a review from the veterinary perspective. *Philosophical Transactions*  
446 *of the Royal Society B* 356, 1097-1106.
- 447 Dulac, G.C., Dubuc, C., Myers, D.J., Afshar, A., Taylor, E.A., 1989. Incursion of  
448 Bluetongue virus type 11 and Epizootic Haemorrhagic disease of deer type 2  
449 for 2 consecutive years in the Okanagan valley. *Canadian Veterinary Journal*  
450 30, 351.
- 451 Eilers, P.H.C. and Marx, B.D., 1996. Flexible smoothing with B-splines and penalties  
452 (with discussion). *Statistical Science*, 89, 89-121.
- 453 EFSA, 2007. Report on Epidemiological analysis of the 2006 bluetongue virus  
454 serotype 8 epidemic in north-western Europe: provisional findings through 31  
455 January 2007. European Food Safety Authority. Accessed at:  
456 [http://www.efsa.europa.eu/en/in\\_focus/bluetongue/bluetongue\\_report\\_s8.html](http://www.efsa.europa.eu/en/in_focus/bluetongue/bluetongue_report_s8.html)
- 457 Elbers, A.R.W., Backx, A., Méroc, E., Gerbier, G., Staubach, C., Hendrickx, G., van  
458 der Speck, A., Mintiens, K., 2007, Nature and severity of disease in sheep and  
459 cattle herds during the Bluetongue virus serotype 8 epidemic in 2006 in north-  
460 western Europe. *Preventive Veterinary Medicine* (accepted).
- 461 ESRI, 1996, ArcView Spatial Analyst, advanced spatial analysis using raster and  
462 vector data. Environmental Systems Research Institute Inc, Redlands, CA, 81-  
463 111
- 464 FAO, Animal Production and Health Division, 2006, Animal Health, disease cards,  
465 Bluetongue. Accessed at:

- 466 <http://www.fao.org/ag/againfo/subjects/fr/health/diseases->  
467 [cards/bluetongue.html](http://www.fao.org/ag/againfo/subjects/fr/health/diseases-cards/bluetongue.html)
- 468 Gerbier, G., Baldet, T., Tran, A., Hendrickx, G., Guis, H., Mintiens K., Ellbers, A.,  
469 Staubach, C., 2007, Modelling local dispersal of bluetongue serotype 8 using  
470 Random walk. Preventive Veterinary Medicine (accepted).
- 471 Gerdes, G.H., 2004, A south African overview of the virus, vectors, surveillance and  
472 unique features of bluetongue. *Veterinary Italiana* 40, 39-42.
- 473 Gibbs, E.P.J., Greiner, E.C., 1994, The epidemiology of Bluetongue. *Comparative*  
474 *Immunology, Microbiology and Infectious Diseases* 17, 207-220.
- 475 Giovannini, A., Calistri, P., Conte, A., Savini, L., Nannini, D., Patta, C., Santucci, U.,  
476 Caporale, V., 2004, Bluetongue virus surveillance in a newly infected area.  
477 *Veterinary Italiana* 40 (3), 188-197.
- 478 Gorman B.M., 1990. The Bluetongue viruses. *Current Topics in Microbiology and*  
479 *Immunology* 162, 1-19.
- 480 Green, A.L., Dargatz, D.A., Schmidtman, E.T., Herrero, M.V., Seitzinger, A.H.,  
481 Ostlund, E.N., Wagner, B.A., Moser, K.M., Wineland, N.E., Walton, T.E.,  
482 2005, Risk factors associated with herd-level exposure of cattle in Nebraska,  
483 North Dakota, and South Dakota to bluetongue virus. *American Journal of*  
484 *Veterinary Research* 66, 853-860.
- 485 Hassan, A., 1992. Epidemiology of bluetongue virus infection in Malaysia. In:  
486 Walton, T.E., Osburn, B.I. (Eds.), *Bluetongue, African horse sickness &*  
487 *Related Orbiviruses*. CRC Press, Boca Raton, pp. 155-161
- 488 Herniman, K.A., Gumm, I.D., Owen, L., Taylor, W.P., Sellers, R.F., 1980,  
489 Distribution of Bluetongue viruses and antibodies in some countries of the  
490 eastern hemisphere. *Bulletin Office of International Epizootics* 92, 581-586.

- 491 Lundervold, M., Milner-Gulland, E.J., O'Callaghan, C.J., Hamblin, C., 2003, First  
492 evidence of bluetongue virus in Kazakhstan. *Veterinary Microbiology* 92,  
493 281-287.
- 494 MacLachlan, N.J., 2004, Bluetongue: pathogenesis and duration of viraemia.  
495 *Veterinary Italiana* 40 (4), 462-467.
- 496 Mintiens, K., Méroc, E., Mellor, P.S., Staubach, C., Gerbier, G., Elbers, A.,  
497 Hendrickx, G., De Clercq, K., Deluyker, H., 2007, Possible routes of  
498 introduction of bluetongue serotype 8 virus into the epicentre of the 2006  
499 epidemic in north-western Europe. *Preventive Veterinary Medicine*  
500 (accepted).
- 501 Mo, C.L., Thompson, L.H., Homan, E.J., Oviedo, M.T., Greiner, E.C., Gonzales, J.,  
502 Saenz, M.R., 1994, Bluetongue virus isolations from vectors and ruminants in  
503 Central America and the Caribbean. *American Journal of Veterinary Research*  
504 55, 211-215.
- 505 Molenberghs, G. and Verbeke, G., 2005. *Models for Discrete Longitudinal Data*.  
506 pringer Series in Statistics. New York: Springer.
- 507 Mulhern, F.J., 1985, Economic impact of bluetongue and related orbiviruses: Western  
508 Hemisphere. *Progress in Clinical and Biological Research*. 178, 21-25.
- 509 Müller-Westermeier, G., 1995. *Numerische Verfahren zur Erstellung*  
510 *meteorologischer Karten*. Selbstverlag des Deutschen Wetterdienstes,  
511 Offenbach, 55
- 512 OIE, 2004, *Manuel of Standards for Diagnostic Tests and Vaccines*. Office  
513 Internationale des Epizooties 2004 Chapitre 2.1.9. Bluetongue.

- 514 OIE Animal Health Department, 2006. Bluetongue - Netherlands, Belgium, Germany-  
515 OIE. ProMed. August 21, 2006. Accessed at <http://www.promedmail.org>,  
516 archive no.: 20060821.2353.
- 517 Pili, E., Ciucce, S., Culurgioni, J., Figus, V., Pinna, G., Marchi, A., 2006, Distribution  
518 and Abundance of Bluetongue Vectors in Sardinia: Comparison of Field Data  
519 with Prediction Maps. *Journal of Veterinary Medicine B.* 53, 312-316.
- 520 Purse, B.V., Baylis, M., McCormick, B.J.J., Rogers, D.J., 2006, Hindsight and  
521 foresight on the spread of bluetongue virus in Europe. Prepared for the  
522 Foresight Project Detection and Identification of Infectious Diseases.  
523 Department of Trade and Industry, UK.
- 524 Qin, Q., Tai, Z., Wang, L., Luo, Z., Hu, J., Lin, H., 1996. Bluetongue epidemiological  
525 survey and virus isolation in Xinjiang, China. In: St. George, T.D., Kegao, P.  
526 (Eds.), *Bluetongue disease in Southeast Asia and Pacific, 1996, Proceedings of*  
527 *the First South-East Asia and Pacific Regional Bluetongue Symposium.*,  
528 *ACIAR Proceedings (Australia) 66*, 67-71.
- 529 Rogan, W.J., Gladen, B., 1978, Estimating prevalence from the results of a screening  
530 test. *American Journal of Epidemiology* 107, 71-76.
- 531 Ruppert, D., Wand, M. P. and Carroll, R.J., 2003. *Semiparametric Regression.*  
532 Cambridge University Press.
- 533 Shepard, D., 1968. A two-dimensional interpolation function for irregularly-spaced  
534 data. In *Proc. 23<sup>rd</sup> National Conference ACM*, 517-524.
- 535 Sreenivasulu, D., Subba Rao, M.V., Reddy, Y.N., Gard, G.P., 2004, Overview of  
536 bluetongue disease, viruses, vectors, surveillance and unique features: the  
537 Indian sub-continent and adjacent regions. *Veterinary Italiana* 40, 73-77.

- 538 Takamatsu, H., Mellor, P.S., Mertens, P.P.C., Kirkham, P.A., Burroughs, J.N.,  
539 Parkhouse, R.M.E., 2003. A possible overwintering mechanism for bluetongue  
540 virus in the absence of the insect vector. *Journal of General Virology*. 84, 227-  
541 235.
- 542 Thiry, E., Saegerman, C., Guyot, H., Kirten, P., Losson, B., Rollin, F., Bodmer, M.,  
543 Czaplicki, G., Toussaint, J.F., De Clercq, K., Dochy, J.M., Dufey, J.,  
544 Gilleman, J.L., Messeman, K., 2006. Bluetongue in Northern Europe.  
545 *Veterinary Record* 159, 327.
- 546 Toussaint, J.F., Vandebussche, F., Mast, J., Demeestere, L., Goris, N., Van Dessel,  
547 W., Vanopdenbosch, E., Kerkhofs, P., Zientara, S., Sailleau, C., Czaplicki, G.,  
548 Depoorter, G., Dochy, J.M., De Clercq, K., 2006. Bluetongue in Northern  
549 Europe. *Veterinary Record* 159, 327.
- 550 Toussaint, J.F., Sailleau, C., Bréard, E., Zientara, S., De Clercq, K., 2007a.  
551 Bluetongue virus detection by two real-time RT-qPCRs targeting two different  
552 genomic segments. *Journal Virology Methods* 140, 115-123.
- 553 Toussaint, J.F., Sailleau, C., Mast, J., Houdart, P., Czaplicki, G., Demeestere, L.,  
554 Vandebussche, F., van Dessel, W., Goris, N., Bréard, E., Bounaadja, L.,  
555 Thiry, E., Zientara, S., De Clercq, K., 2007b, Bluetongue in Belgium, 2006.  
556 *Emerging Infectious Diseases Journal* 13, Number 4. Accessed  
557 at:<http://www.cdc.gov/eid/content/13/14/614.htm>.
- 558 Uhaa, I.J., Riemann, H.P., Thurmond, M.C., Franti, C.E., 1990, A  
559 seroepidemiological study on bluetongue virus in dairy cattle in the central  
560 valley of California. *Veterinary Research Communications* 14, 99-112.
- 561 Vandebussche, F., Vanbinst, T., Verheyden, B., Van Dessel, W., Demeestere, L., De  
562 Poorter, G., Houdart, P., Bertels, G., Praet, N., Berkvens, D., Mintiens, K.,

563 Goris, N., De Clerq, K., 2007, Evaluation of antibody-ELISA and real time  
 564 RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during  
 565 the epidemic in Belgium in 2006. Veterinary Microbiology (in press).

566 Ward, M.P., Carpenter, T.E., Osburn, B.I., 1994, Host factors affecting  
 567 seroprevalence of bluetongue virus infections of cattle. American Journal of  
 568 Veterinary Research 55, 916-920.

569

570

571 **Table 1. Bluetongue herd prevalence (%) and associated 95%CI in Belgian cattle and**  
 572 **sheep population based on the 2006 case herd data**

573

574

575

576

577

578

579

580

581

582

583

584

585

586

587

Province	Herd-prevalence (%) (95%CI)	
	cattle	sheep
Antwerp	0.7 (0.5-1.0)	1.5 (1.1-2.0)
East Flanders	1.5 (1.3-1.8)	2.2 (1.9-2.5)
Flemish Brabant	0.8 (0.5-1.2)	1.2 (0.9-1.6)
Hainaut	0.1 (0.0-0.3)	0.1 (0.0-0.3)
Liege	1.6 (1.3-2.1)	1.5 (1.1-2.2)
Limburg	1 (0.7-1.4)	3.2 (2.5-4.0)
Luxembourg	0.2 (0.1-0.4)	0.1 (0.0-0.5)
Namur	0.3 (0.2-0.7)	0.5 (0.3-0.9)
Walloon Brabant	1 (0.5-2.1)	0.3 (0.1-1.2)
West Flanders	0.1 (0.1-0.2)	0.4 (0.3-0.7)

588 **Appendix: figures**

589

590 **Figure 1. Bluetongue herd seroprevalence (%) and associated CI95% at the provincial**  
591 **level in Belgian dairy cattle based on the winter screening data, January 2007**

592 **Figure 2. Distribution of within-herd seroprevalence (%) in Belgian dairy cattle based on**  
593 **the winter screening data, January 2007**

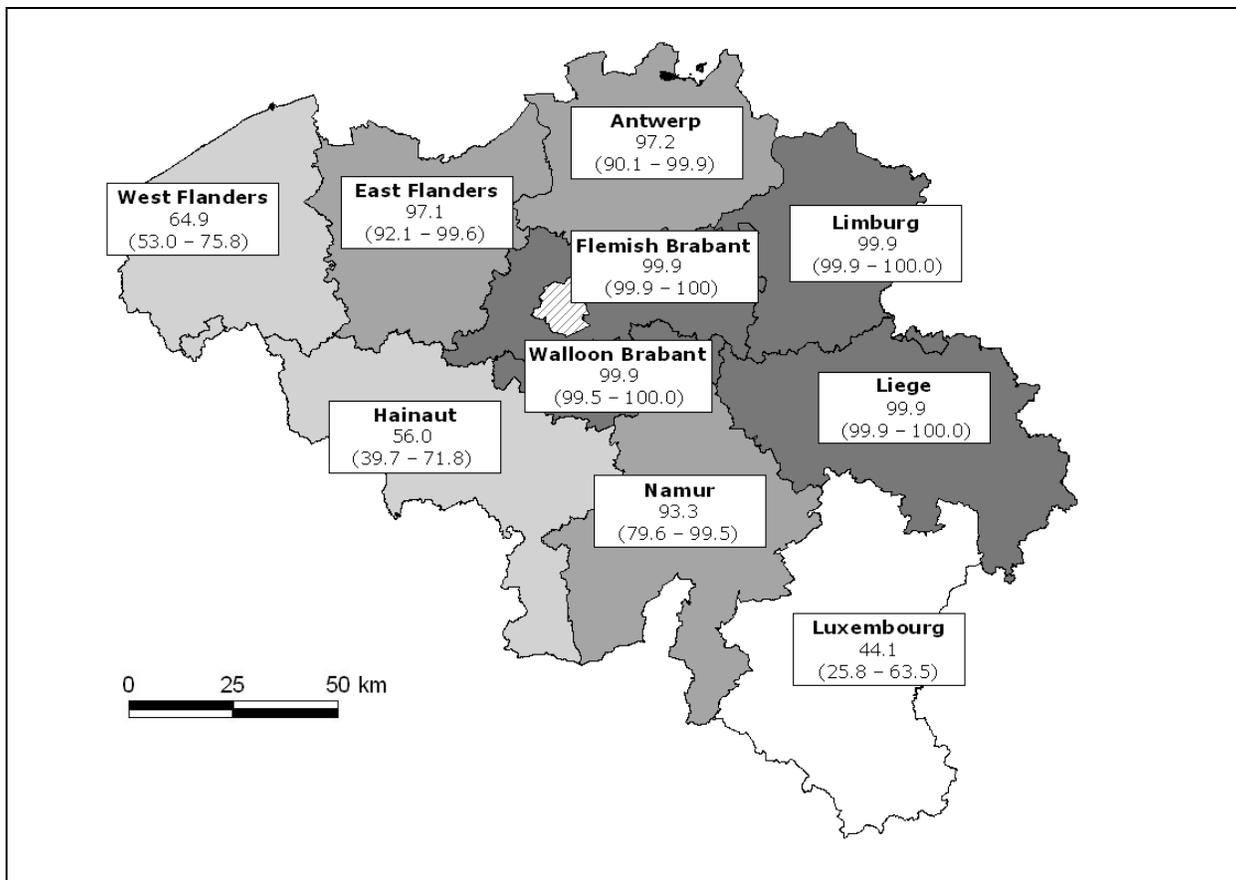
594 **Figure 3. Density plot of the farm-specific within-herd seroprevalence estimates**  
595 **based on the winter screening data**

596 **Figure 4. Cattle herd density at the municipality level in Belgium, 2006.**

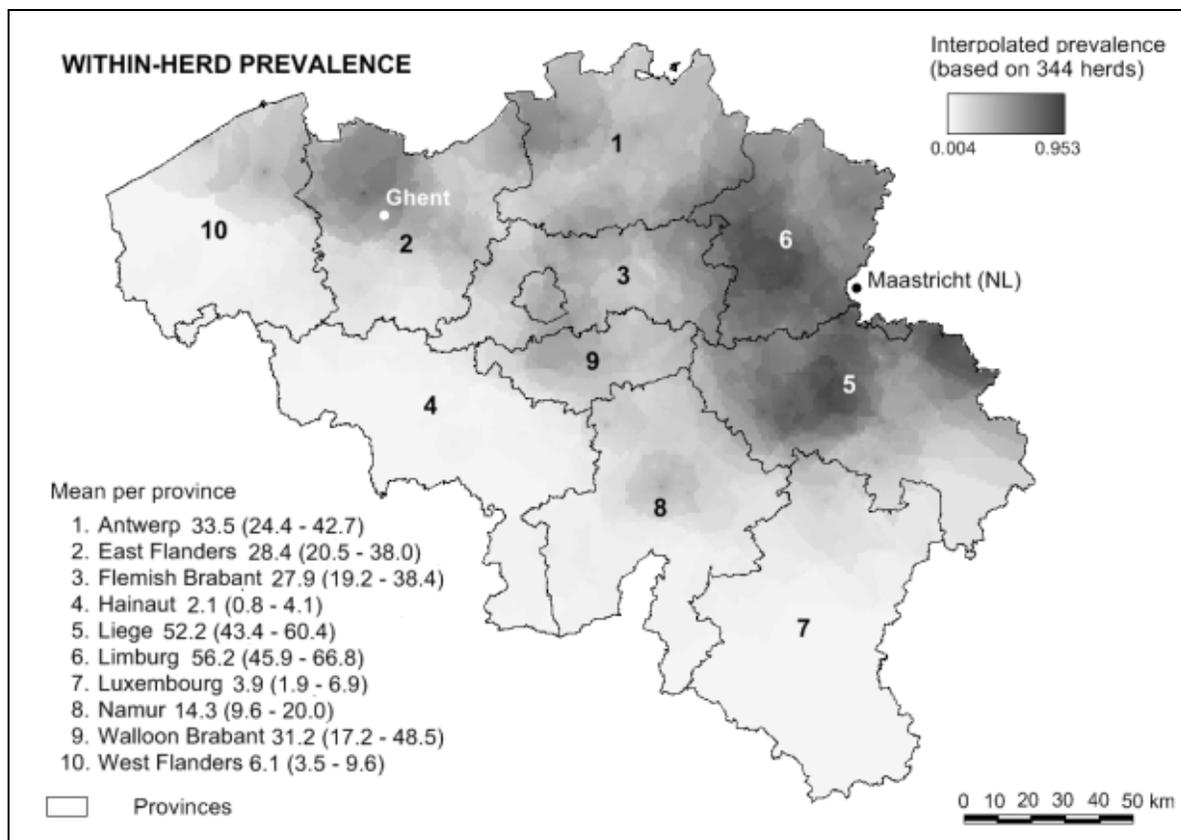
597 **Figure 5. Maps of the spatially interpolated temperature in Belgium on the (a.)**  
598 **30 June (b.) 30 July (c.) 30 August (d.) 30 September (e.) 30 October and (f.) 30**  
599 **November 2006**

600 **Figure 6. Bluetongue herd prevalence at the municipality level in Belgian dairy**  
601 **cattle based on (a.) the 2006 case herd data and on (b.) the winter screening data.**

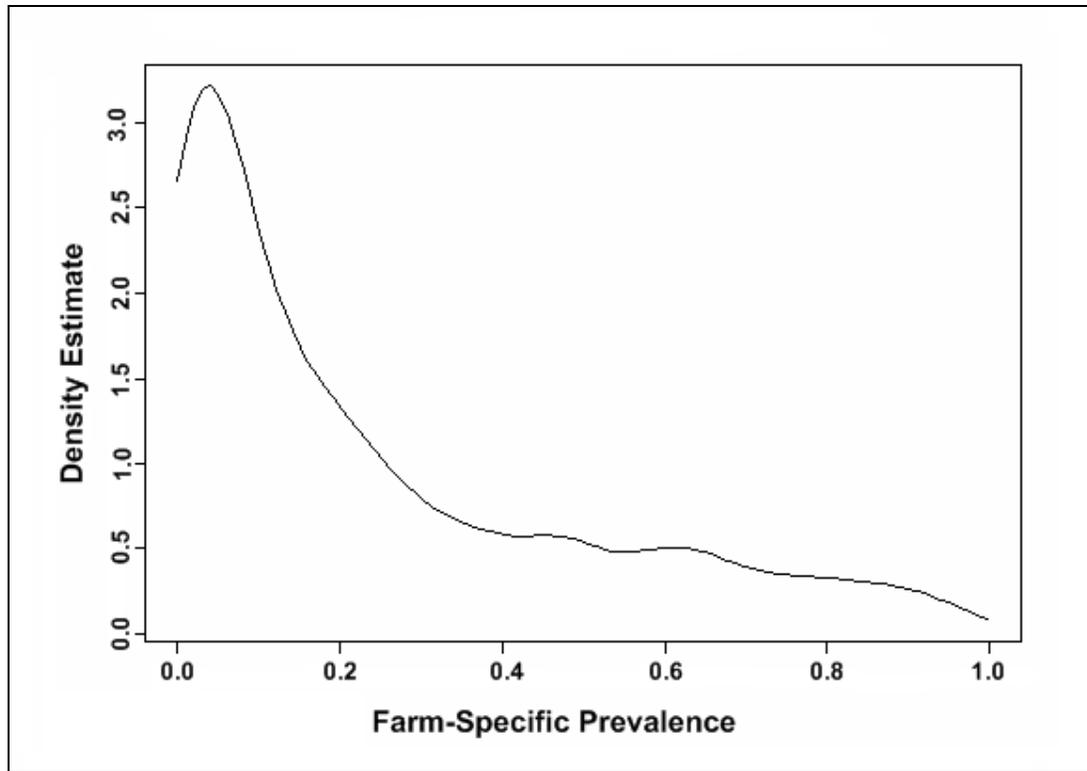
602



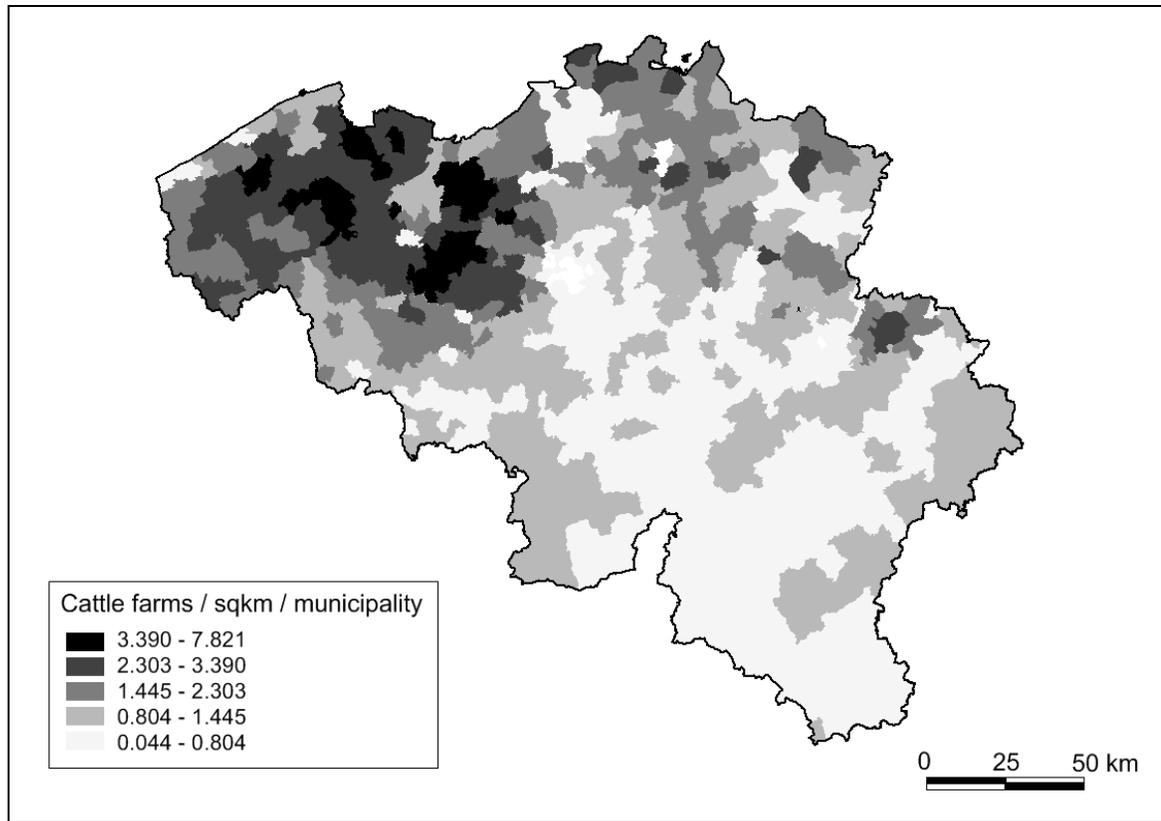
Accepted



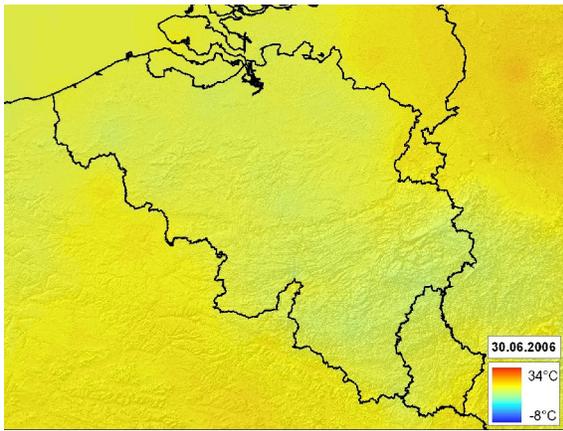
Accepted



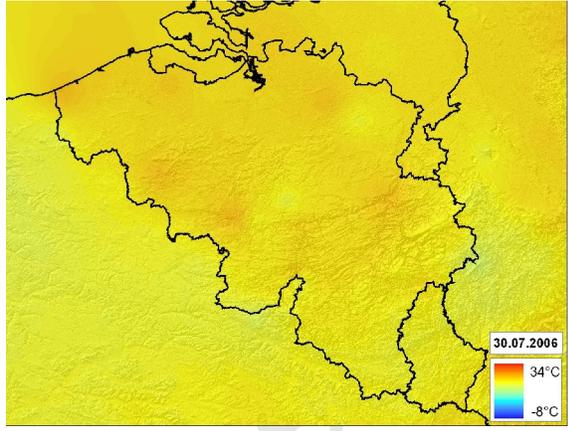
Accepted Manuscript



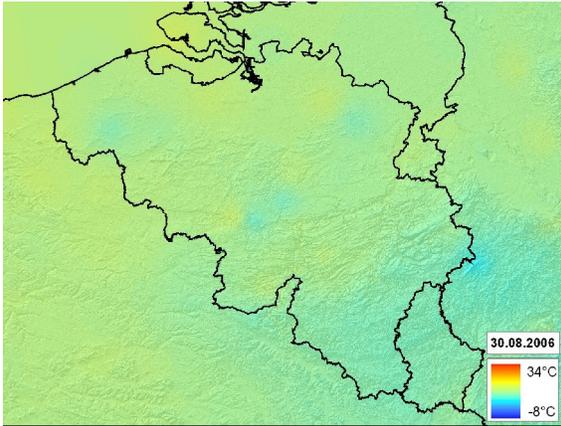
Accepted



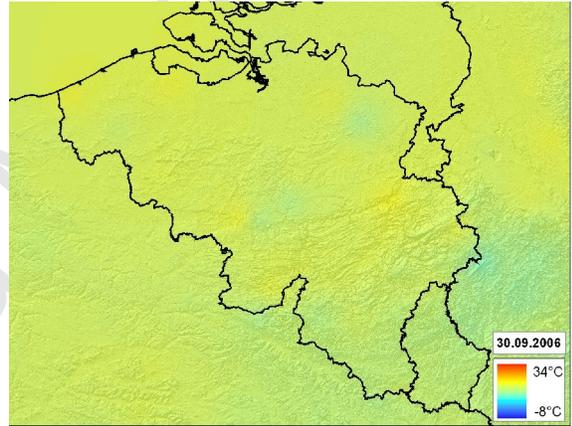
a



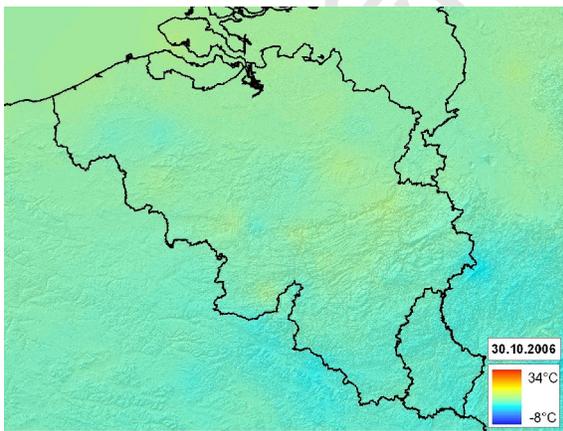
b



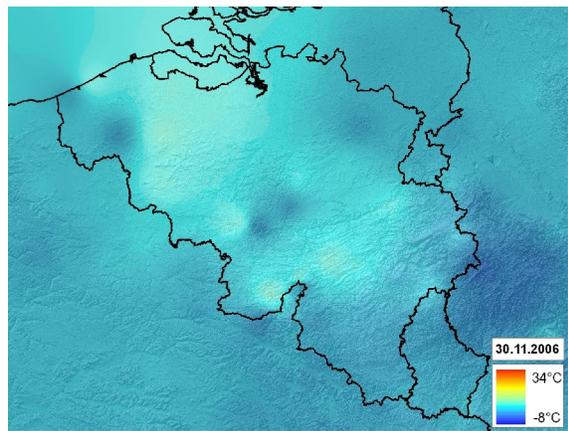
c



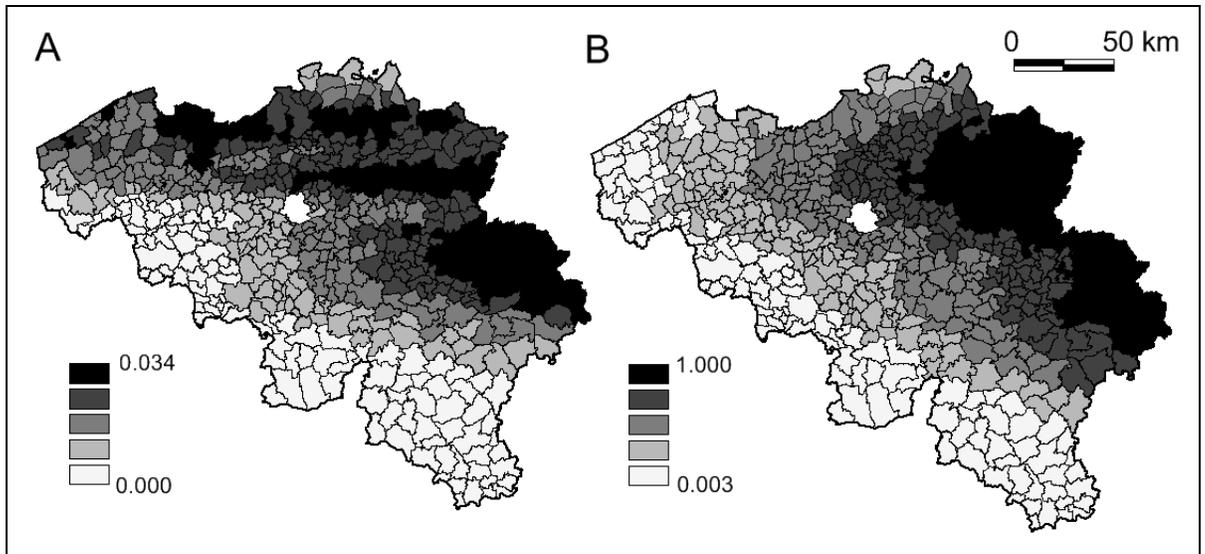
d



e



f



Accepted Manuscript