Results of Bluetongue sentinel surveillance program and cross-sectional serological survey in cattle in Belgium in 2010-2011

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INTRODUCTION
Bluetongue virus serotype 8 (BTV-8) emerged in Central Western Europe in 2006 and caused a large scale epidemic in 2007 involving several EU countries including Belgium. Vaccination against BTV-8 with inactivated vaccines was initiated in Belgium in spring 2008 as in several other EU member states. Since 2009, no clinical cases of Bluetongue (BT) have been reported in Belgium and BTV-8 circulation seemed to have completely disappeared by spring 2010 (Garigliany et al., 2011). Based on this, a BT sentinel surveillance program was initiated in dairy cattle towards the end of 2010 with the aim to demonstrate the absence of BTV circulation and to consequently re-gain Belgium’s BTV free status in accordance with EC Regulation No 1266/2007. In addition, a cross-sectional serological survey in cattle, the BT winter screening, was carried out between January and March 2011 to assess the level of herd immunity against BTV-8 achieved by 3 consecutive years of compulsory BTV-8 vaccination. This report describes the results of this serological survey and the results of the first two sampling rounds of the BT sentinel surveillance program carried out in October-November 2010 and January-February 2011, respectively.

MATERIALS AND METHODS
For the BT sentinel surveillance program, a total of 300 dairy herds, 30 herds per province, were selected randomly and a target number of 15 non-vaccinated animals between 4 and 12 months of age were tested in each sentinel herd on each sampling occasion by non-serotype specific BTV RT-qPCR (pan-BTV RT-qPCR, in-house test NRL for BTV) performed on EDTA blood. For the BT winter screening, these 300 BT sentinel herds were selected in addition to another 800 randomly selected dairy, beef or mixed herds; stratified by province. In each herd, a target number of 10 animals between 6 and 12 months of age and 10 animals between 12 and 24 months of age were tested for the presence of serum antibodies against BTV by means of a commercially available competitive ELISA (ID Screen® Blue Tongue Competition, ID VET). All doubtful serological results were classified as positive in the data analysis. Estimations of the within-herd seroprevalence were obtained using a Generalized Estimating Equation (GEE) via the GENMOD procedure in SAS® taking into account the possible correlation among animals within the same herd. Apparent within-herd seroprevalence estimates were converted into true seroprevalence estimates by means of the Rogan and Gladen estimator (1978) assuming a sensitivity and specificity for the c-ELISA of 92.0% and 95.7%, respectively (Vandenbussche, personal communication, August 31, 2011). For the estimation of the seroprevalence at herd level, a herd was considered seropositive if at least one of the sampled animals had a positive or doubtful c-ELISA test result.

RESULTS
In October-November 2010 and January-February 2011, respectively, a total of 3684 animals (from 264 sentinel herds) and 2150 animals (from 202 sentinel herds) were tested by pan-BTV RT-qPCR. In October-November 2010, 5 animals tested positive and 12 animals tested doubtful. In January-February 2011, 17 animals tested positive; 5 animals tested doubtful. All positive/doubtful samples were also tested by means of serotype specific RT-qPCR assays; BTV-8 was the only serotype that was detected. The positive/doubtful animals from both rounds belonged to a total of 17 different herds spread over Belgium. Verification of the vaccination status revealed that a number of these animals had been vaccinated against BTV-8. All positive/doubtful animals were re-sampled 2-4 weeks after the original sampling and tested negative by pan-BTV RT-qPCR.

A total of 16616 animals from 1030 herds (292 BT sentinel herds and 738 additional herds) were tested for BTV antibodies by c-ELISA. A total of 10823 animals (65.14%) tested positive and 557 animals (3.35%) tested doubtful. The overall true within-herd seroprevalence in Belgium was estimated at 73.38% (95% CI: 71.27-75.42). The within-herd seroprevalence stratified by production type was 73.16% (95% CI: 70.47-75.75) for dairy herds (n=612) and 73.71% (95% CI: 70.27-76.98) for non-dairy herds (n=418). Higher within-herd seroprevalence estimates were found in the Northern provinces than in the Southern provinces. The within-herd seroprevalence in the sampled herds ranged between 0 and 100%. Approximately half (54.47%) the herds sampled had a true within-herd seroprevalence equal to or above 80%, the recommended herd level immunity for protection against BT. The true within-herd seroprevalence was higher in the 12-24 month old animals (79.47%, 95% CI: 77.44-81.42) than the 6-12 month old animals (66.26%, 63.38-69.07). The overall BTV seroprevalence at herd level in Belgium was estimated at 97.38% (95% CI: 96.20-98.20). The herd seroprevalence was very high in each province and ranged between 94.95% (95% CI: 88.49-97.55) in Liege and 99.44% (95% CI: 96.10-99.92) in West Flanders.

CONCLUSIONS
The results of the BT sentinel surveillance program demonstrate the absence of BTV circulation in Belgium in 2010. The next sampling in the surveillance program is scheduled to take place after the main period of vector activity in October 2011 in order to exclude virus circulation in 2011 and to consequently re-gain Belgium’s BTV free status. The BTV seroprevalence at herd level after 3 years of compulsory BTV-8 vaccination was very high (97.38%). The overall true within-herd BTV seroprevalence in Belgium early 2011 was estimated at 73.38%.

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