Tick-borne encephalitis (TBE): First seropositive dog detected during screening of the Belgian canine population as sentinels

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Introduction

Western subtype tick-borne encephalitis (TBEV, flavivirus) is the most important tick-borne virus infection in humans in Europe and causes meningo-encephalitis with high morbidity and long term sequelae. TBEV can also cause a febrile illness with lethargy, anorexia and multifocal neurological signs in 50% of canine cases. The best diagnostic test is IgG detection by ELISA, though positive results should always be confirmed by e.g. seroneutralisation (SN), due to common occurrence of flavivirus cross-reactions and non-specific false positives.

Human TBE is emerging in several Northern and Western European countries and cases were diagnosed in dogs living in or visiting endemic areas. Though Belgian citizens (and their dogs) travel to endemic areas, clinicians do not routinely test for TBE in viral meningo-encephalitis cases and surveillance is currently almost non-existent in Belgium.

Until now, no autochthonous human or canine cases were reported in Belgium, but if TBE were to emerge, it could pose an important threat to canine and public health. Targeted serological screening of sentinel animals such as dogs, cattle and wildlife (e.g. deer, rodents) would contribute in a cost-effective way to a continuous epidemiosurveillance program for TBEV in Belgium. In European endemic areas, canine seroprevalence ranges from 0.97-24%.

Materials and Methods

AIM: Adaptation of a commercially available ELISA for the detection of TBEV-specific IgG antibodies in canine serum (Immunozym FSME/TBE IgG All Species-ELISA®, Progen Biotechnik GmbH, Heidelberg, Germany) for the purpose of early detection of TBEV-presence in Belgium.

METHODS: The kit has been used for human TBEV testing (IgG: DSe = 97% and An.Sp = 99%). The kit was adapted using a known positive canine serum (In Vitro Labor für Veterinärmedizinische Diagnostik und Hygiene GmbH, Vienna, Austria) and a mix of five TBEV-negative sera (SPF laboratory beagle, Janssen Pharmaceutica N.V., Beerse, Belgium) alongside human calibrator and control samples of the kit. The manufacturer's instructions were followed. A standard curve was generated using the five human calibrator samples. Sample concentrations were read from this standard curve in Vienna units per ml (VIEU/ml). (conc. <63 VIEU/ml = negative; conc. >126 VIEU/ml = positive; 63< conc. <126 VIEU/ml = borderline).

DESIGN: Serum of 960 Belgian dogs from three diagnostic laboratories from Northern (n=768) and Southern (n=192) Belgium. All samples were taken by local veterinary surgeons between 15/03/2009 and 22/06/2009 and submitted to the laboratories for a variety of diagnostic tests.

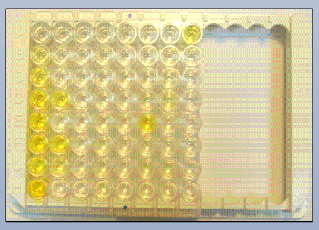

Figure 1. Sample results

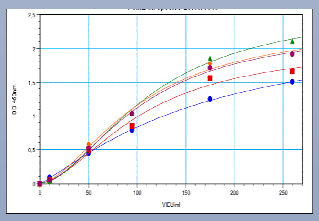

Figure 2. Standard curves

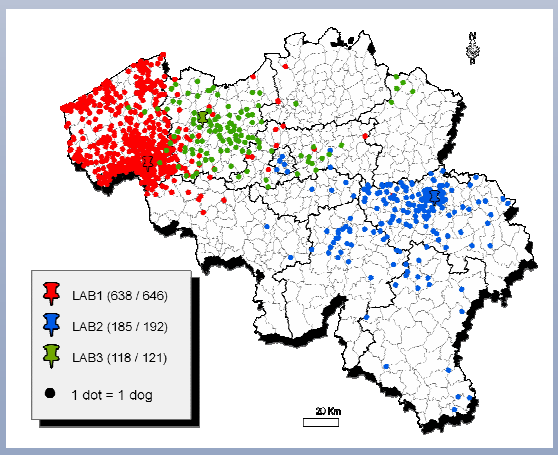

Figure 3. Samples per lab

Results

Two dogs were ELISA 'positive' (0.21%), three samples were 'borderline' (0.31%) and five samples (0.52%) scored VIEU/ml within 15% of the borderline cut-off. These reactive samples were subjected to a i) TBEV-RFFIT confirmation test (rapid fluorescent focus inhibition test; Vene et al., 1998) ii) WNV-SNT (West Nile virus) and iii) a LIV-SNT (Louping Ill virus).

One Belgian dog was found RFFIT-positive at low titre (1:5) and was therefore exposed to and infected with TBEV, either abroad or in Belgium. Further epidemiological investigation of this seropositive « case » is pending. All samples were confirmed as negative for WNV and LIV. Presently, in our laboratory the TBEV-ELISA diagnostic specificity is 99.5%.

Discussion

This pilot study has used sufficient samples to detect 0.35% TBEV prevalence in the Belgian canine population with 95% confidence. Figure 3 shows that some Belgian areas are as yet over- or underrepresented in the sampling, which was opportunistic.

ELISA positive/borderline samples need to be subjected to a TBEV-RFFIT/SN confirmation test to rule out false positives and optionally to an WNV-SNT and an LIV-SNT to differentiate cross-reactions. When true positive samples are found, a retrospective descriptive epidemiological analysis (owner questionnaire and telephone interview) needs to determine whether these canine « cases » are autochthonous or acquired abroad, since the discovery of an autochthonous case is more worrisome. Such an investigation is currently underway.

This ELISA was previously used as a TBEV screening test in foxes (Wurm et al., 2000) and is adaptable for use in other mammalian species involved in TBEV epidemiology. We plan to adapt the test in the future, to enable screening of deer sera. Preliminary tests on Belgian deer sera (n=30) were negative.

References

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