Adaptation of ELISA test specific for tick-borne encephalitis human IgG antibodies to detect antibody response in goat sera

Włodzimierz Gut,[1] Jarosław Kaba,[2] Mariusz Nowicki,[2] Emoke Ferenczi,[3] Paweł Stefanoff[4]

1) Department of Virology, National Institute of Hygiene, Warsaw, Poland.
2) Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland
3) Department of Virology, Johan Bela Center for Epidemiology, Budapest, Hungary
4) Department of Epidemiology, National Institute of Hygiene, Warsaw, Poland.

Introduction

- During previous decade several outbreaks of tick-borne encephalitis (TBE) have been associated with consumption of raw milk in Poland, Baltic states, Slovak and Czech Republics.
- To assess the risk of transmission of TBE virus via milk or milk-borne products serologic surveys and field studies are planned in Poland.
- To achieve these results, a valid and simple laboratory method is necessary allowing testing of materials from different household animals using widely available ELISA tests for humans.

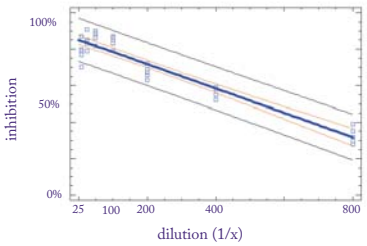
Material and Methods

- The validation based on blocking of human TBE-specific IgG antibodies binding by virus-specific antibodies present in goat sera.
- The reference material used was a goat serum sample confirmed for TBE with HI titre 1:128 obtained from dr Milan Labuda from the Institute of Zoology, Slovak Academy of Sciences.
- As detection system pooled human sera positive for TBE IgG (180 VIEU/ml, +/- 14.6) were used.
- A dilution of TBE-positive goat sample was chosen to block a minimum of 80% reactivity of human IgG antibodies. Reactivity of goat sera with conjugate against human IgG was tested.
- Finally, sensitivity and reproducibility of elaborated method was assessed using a panel of goat 341 sera and control samples. Additionally, possible interfering factors were investigated.
- Positive, equivocal and a sample of negative samples, as well as control serum were sent for retestation by HI and IF methods at the Arboviral Laboratory at the Johan Bela Centre for Epidemiology (JBCE).

Results

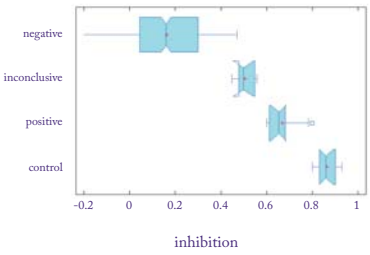
- Goat TBE-positive serum in dilution 1/50 was entirely blocking human IgG antibodies binding (human IgG-negative sample: OD=0.096, cut-off=0.196, +/- 0.037, human IgG-positive sample without blocking: OD=0.614, +/- 0.089, and after blocking: OD=0.080, +/- 0.006).
- The goat TBE-positive serum was blocking 85% of human IgG antibodies activity in dilution 1/100, 67% in dilution 1/200, and 50% in dilution 1/400. No reactivity of goat sera with conjugate against human IgG was observed.

Figure 1. Analysis of inhibition of human IgG by antibodies from positive control goat serum.



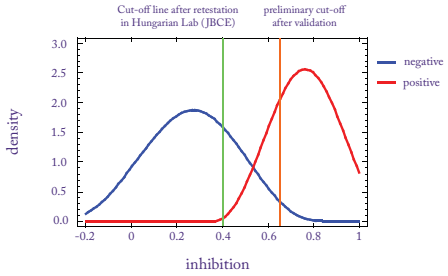
- Based on blocking function curve the following critical parameters of the method were established. The positive result was obtained when inhibition was above 40%, the equivocal result (equivalent to HI<=4) was obtained when inhibition was 25-40%, and negative result was obtained when inhibition was below 25% (Figure 2).

Figure 2. Characteristic of inhibition by different group of goat sera (negative, inconclusive and positive).



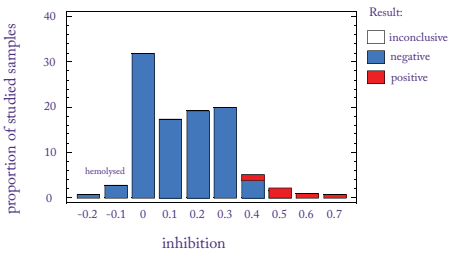
- Retesting of all positive, equivocal, and randomly selected negative sera show 97% precision of the test compared to classical method (HI, IF). As a result of retestation the criteria of sample classification were adjusted (Figure 3).

Figure 3. Density trace for result of inhibition obtained on a panel of 341 goat sera, adjustment of cut-off level based on retesting in independent laboratory (JBCE).



- The uncertainty of detection system was established at 12.5%, the reproducibility of positive results 100%, of equivocal results 72% (Figure 4). Strong haemolysis was potential source of false negative results.

Figure 4. Distribution of inhibition test values by serologic test result for 341 goat sera, Poland, 2006



Conclusions

- The presented results indicate the usefulness of the validated test in detecting antibodies in goat sera.
- This method can be easily adapted to test sera of other animal species, as well as other body fluids (i.e. milk from household animals).
- Retestation in independent laboratory is crucial for assessing reliability of obtained results.
- The validated test should be used by public health laboratories to test samples from animals during epidemiologic investigation of TBE milk-borne outbreaks