Praca oryginalna *Original paper*

Comparison of four ELISAs for the detection of antibodies against bluetongue virus

WIESŁAW NIEDBALSKI

Department of Foot-and-Mouth Disease, National Veterinary Research Institute, Wodna 7, 98-220 Zduńska Wola

Niedbalski W.

Comparison of four ELISAs for the detection of antibodies against bluetongue virus

Summary

The aim of this study was to determine the diagnostic specificity, sensitivity and repeatability of four commercially available ELISA kits for the detection of antibodies against bluetongue (BT) virus. The relative specificity of ELISAs was estimated using a panel of sera originated from healthy cattle never vaccinated nor exposed to BT virus. All ELISA kits had a high relative specificity (99.2-99.4%). The relative sensitivity of ELISAs estimated using a panel of sera collected from BTV infected cattle was also high and similar for all kits (97.4-100%). However, the relative sensitivity evaluated on the basis of testing of vaccinated animals was different for the used ELISAs: the LSI, ID VET and Ingenasa kits had a high sensitivity (85.2-98.2%) but the sensitivity of VMRD ELISA was much lower (68.6%). The repeatability of ELISAs was expressed as a coefficient of variation (CV) of results of sera tested 5 times in the same day and 10 times in different days through the period of 2 months, by the same person, in the same conditions, and by using of the same equipment. The CVs of sera tested in Ingenasa and ID VET kits ranged from 6.1 to 9.8% and were below the 10% threshold adopted as a maximal for the acceptable repeatability of a method. In conclusion, it can be stated that the applied ELISAs can be a valuable diagnostic tool for the serological monitoring studies in the BTV infected premises. Nevertheless, the Ingenasa and ID VET ELISAs can be the most useful in sero--surveillance of livestock following vaccination.

Keywords: bluetongue, serology, ELISA kits, comparison

Bluetongue (BT) is a vector-borne viral disease of ruminants, including sheep, goats and cattle that induces variable clinical signs depending on the species and the breed (13). The disease is caused by the bluetongue virus (BTV) which is a member of the *Orbivirus* genus within the family *Reoviridae*. Twenty four immunologically distinct serotypes (BTV1 to BTV24) of the virus have been identified worldwide (3). The virus is transmitted by a specific species of *Culicoides*, the family *Ceratopogonidae* (5). Recently, evidence for transplacental and contact transmission of BTV was presented (9).

The distribution of BT is determined by the geographical distribution of the arthropod vector and includes Africa, southern Asia, Australia, the Middle East, and the Americas (16). Historically Europe has experienced only sporadic incursions of BT, involving a single-virus serotype on each occasion. However, since 1998, BT outbreaks have occurred annually, involving strains from six distinct BTV serotypes – BTV-1, 2, 4, 8, 9, and 16 (8). Since August 2006, for the first time the BTV has crossed the 50°N latitude and BT outbreaks have occurred in the northern part of Europe: the Netherlands, Belgium, Germany, France, and Luxembourg (17). In 2007-2008, BTV-8 spread to the other regions of Europe where the disease had never been observed before: the United Kingdom, Denmark, Norway, Switzerland, Czech Republic, Sweden, Spain, Hungary, and Austria. According to the latest epidemiological data, from May 1, 2008 to September 1, 2009, a total of 30692 BTV-8 outbreaks were reported (http://eubtnet.izs.it/btnet.reports/BTV8.htlm).

An introduction of BTV in livestock causes substantial economic losses due to the disease itself and, more substantially, to the complete trade block between infected and non-infected areas (6). The occurrence of BT in the bordering countries suggests that Poland is now at high risk for BTV epizootic infection. Recently observed animal movements, including international trade and import of BTV susceptible animals into Polish territory, support the necessity of serological surveillance of BTV-specific antibodies in the population of imported animals. That is why since October 2006 we have introduced the competitive ELISA (c-ELISA) and agar-gel immunodiffusion (AGID)

assays to determine of seroprevalence of BTV-specific antibodies in serum samples collected from susceptible animals imported to Poland from EU countries after 15 June 2006 (11, 12). The c-ELISA is very rapid, specific, sensitive at detecting antibodies in BTV-infected animals, and easy to use (2, 13), therefore it has been recommended for large-scale serological screening and international trade purposes (7). However, our recent studies have indicated that this ELISA is not sensitive enough to be used for the detection of post-vaccination antibodies (10).

Currently, several commercial ELISAs for BTV antibodies are available. The aim of this study was the comparison of the diagnostic value of different ELISA kits for the detection of BTV antibodies in infected and vaccinated animals.

Material and methods

Sera. The following sera were examined:

– negative sera: a total of 420 samples of sera from Poland which originated from healthy cattle neither vaccinated nor exposed to BT virus. These sera were supplied by the District Veterinary Inspectorates and tested as a part of the national serosurveillance program for BT.

– positive sera: 398 „post-vaccination" sera of cattle imported from EU member countries vaccinated in 2008 with BT serotype 8 vaccine. Moreover, 39 „post-infection" sera collected from BTV positive animals from Germany, the Netherlands and a calf born in Poland from a German origin BT positive dam were analysed. In addition, three panels of sera from BTV-infected animals supplied within the ring trial for BTV (serotype 8) viral genome and antibody detection 2007, 2008 and 2009 were tested.

Testing. The sera were examined using four commercially available ELISA kits:

Bluetongue Virus Antibody Test Kit, c-ELISA (VMRD Inc., Pullman, USA), Ingezim BTV DR 1.2.BTV.K.O Kit (Ingenasa, Spain), Bluetongue Virus Antibody Test Kit – LSIBT5 (Laboratoire Service International – LSI, Lissieu, France) and ID Screen Blue Tongue Competition (ID VET, Montpellier, France). The first two tests were performed according to the procedures described previously (10, 12). The LSI kit is based on the principle of blocking ELISA. According to the manufacturer's specifications, a sample is considered positive if its percent of inhibition (% Inh.) of HRP-labelled conjugate binding is lower than 45%. The IDVET kit is based on the detection of antibodies specific to the VP7 protein of BTV and is designed to detect antibodies during infection by any type of BTV and/or post-vaccination antibodies induced by any vaccine presenting the VP7 antigen. A positive reaction is scored when the percent of OD sample/OD negative control (S/N%) is ≤ 35.

Estimation of the relative specificity, sensitivity and repeatability. Comparative specificity of ELISA kits was estimated using negative sera originating from healthy cattle. For the purpose of relative sensitivity of ELISA kits the available „post-infection" and „post-vaccination" sera collected from BTV infected and vaccinated animals were applied. The repeatability of used ELISA was tested using five sera samples collected from BTV infected cattle.

Results and discussion

The rapid spread of BTV-8 outbreaks in North-Western Europe during the last three years has highlighted that all diagnostic laboratories dealing with BTV must establish a level of preparedness that shall enable them to the rapid and reliable detection of this pathogen. Diagnostic tests are a major component of success in any surveillance system. Due to the BT mass vaccination of all domestic ruminant species conducted in BTV-affected EU member states in 2008, the ability to detect both infected and vaccinated animals is important for export/import serological examination. Therefore, considerable efforts should be directed towards the validation of available diagnostic kits for the detection of antibodies to BTV.

The aim of this study has been to determine of diagnostic specificity, sensitivity and repeatability of four commercially available ELISA kits: two were based on the principle of competition (VMRD and ID VET), Ingenasa and LSI kits were direct and blocking ELISA, respectively. All used ELISAs were easy and quick to perform, especially the VMRD and LSI kits, which can be performed within about 1 hour. The relative specificity of ELISAs was estimated using a panel of sera originated from healthy cattle never vaccinated nor exposed to BT virus (tab. 1). All ELISA kits had a high relative specificity (99.2-99.4%). The specificity of ID VET kit was similar to its performance reported recently by Vandenbussche et al. (14). The relative sensitivity of ELISAs (tab. 2) estimated using a panel of sera collected from BTV infected cattle was also high and similar for all kits (97.4-100%). The author's results of analytical sensitivity of c-ELISAs evaluated on BTV „post-infection" sera support the

Tab. 1. Comparative specificity of used ELISAs

Test	No of sera examined	No of negative sera	Specificity (%)
VMRD	420	417	99.2
Ingenasa	360	358	99.4
LSI	272	270	99.2
ID VET	285	283	99.3

Tab. 2. Comparison of relative sensitivity of ELISA kits

Origin of sera	VMRD	Ingenasa	LSI	ID VET
Infected animals				
Positive results	77	78	76	74
Negative results	1	0	2	0
Sensitivity (%)	98.7	100	97.4	100
Vaccinated animals				
Positive results	273	392	339	385
Negative results	125	7	59	13
Sensitivity (%)	68.6	98.2	85.2	96.7

Tab. 3. Reproducibility of ELISAs for the detection of BTV antibodies

Test	Sera tested in this same day			Sera tested in different days		
	Result	SD	CV	Result	SD	CV
VMRD	0.320[a]	0.027	8.4	0.342[a]	0.030	8.8
	0.286	0.021	7.3	0.271	0.028	9.6
	0.313	0.019	6.1	0.338	0.031	9.2
	0.427	0.037	8.6	0.412	0.033	8.0
	0.289	0.024	8.3	0.318	0.029	9.2
Ingenasa	1.685[b]	0.14	8.3	1.624[b]	0.14	8.6
	1.842	0.11	6.1	1.925	0.16	8.3
	1.690	0.12	7.1	1.762	0.17	9.6
	1.747	0.12	6.7	1.681	0.16	8.3
	1.862	0.15	8.0	1.937	0.18	9.3
LSI	63[c]	5.8	9.2	67[c]	6.6	9.8
	72	6.1	8.4	68	6.5	9.5
	64	5.7	8.9	61	5.1	8.3
	68	5.4	7.9	72	5.8	8.0
	73	6.4	8.7	77	7.4	9.6
ID VET	23.3[d]	1.8	7.7	21.9[d]	1.9	8.6
	17.9	1.2	6.7	18.6	1.6	8.1
	23.7	2.1	8.8	22.3	2.1	9.4
	19.6	1.6	8.1	20.7	1.6	7.7
	18.2	1.4	7.7	19.5	1.8	9.2

Explanations: a – OD_{620}; b – OD_{450}; c – % Inh.; d – S/N%; SD – standard deviation; CV – coefficient of variation

previous reports (2, 14). Moreover, the c-ELISA was superior to the other ELISA assay in the detection of anti-BTV antibody in the sera samples from cattle and sheep early after infection with BTV (1). However, the relative sensitivity evaluated on the basis of testing of vaccinated animals was different for the used ELISAs (tab. 2). When testing in ID VET and Ingenasa ELISAs, out of about 390 „post-vaccination" sera, only some samples scored negative (sensitivity of ELISAs 96.7-98.2%). In comparison, in a population of 339 sera tested in LSI kit, 59 scored negative (sensitivity 85.2%) (tab. 2). The much lower sensitivity (68.6%) was found for VMRD kit; out of 273 sera, 125 gave a negative reaction (tab. 2). These results are in agreement with our earlier studies (10) and presented by others (http://www.warmwell.com). It was found that if low levels of antibodies were present in the tested serum, below the threshold of the detection of the c-ELISA, this vaccinated animal was scored as seronegative. However, according to the results obtained at the Bluetongue CRL in Pirbright (UK), for animals vaccinated with inactivated BTV-8 vaccines on a single occasion, the absence of antibodies detected by the c-ELISA does not correlate with the lack of protection (http://www.farmtalking.com). The repeatability of ELISAs was expressed as a coefficient of variation (CV) of results of sera tested 5 times in the same day, and in different days 10 times within the period of 2 months, by the same person, in the same conditions, and by using of this same equipment (tab. 3). The CVs of sera tested in Ingenasa and ID VET kits ranged from 6.1 to 9.8% (tab. 3) and were below the 10% threshold adopted as a maximal for the acceptable repeatability of method (4).

In conclusion, it can be stated that the applied ELISAs can be a valuable tool for the serological monitoring studies in the BTV infected premises. However, the Ingenasa and ID VET ELISAs can be the most useful in sero-surveillance of livestock following vaccination.

References

1. *Afshar A., Thomas F. C., Wright P. F., Shapiro J. L., Shettigara P. T., Anderson J.*: Comparison of competitive and indirect enzyme-linked immunosorbent assays for the detection of bluetongue virus antibodies in serum and whole blood. J. Clin. Microbiol. 1987, 25, 1705-1710.
2. *Afshar A., Trotter H. C., Dulac G. C., Reddington J. J.*: Evaluation of a commercial competitive ELISA test kit for the detection of group-specific antibodies to bluetongue virus. J. Vet. Invest. 1993, 5, 336-340.
3. *Gorman B. M.*: The bluetongue virus. Curr. Top. Microbiol. Immunol. 1990, 162, 1-19.
4. *Jacobson R. H.*: Validation of serological assays for diagnosis of infectious diseases. Rev. Sci. Tech. Off. Int. Epiz. 1998, 17, 469-486.
5. *MacLachlan N. J.*: The pathogenesis and immunology of bluetongue virus infection of ruminants. Comp. Immunol. Microbiol. Infect. Dis. 1994, 17, 197-206.
6. *MacLachlan N. J., Osburn B. T.*: Impact of bluetongue virus infection on the international movement and trade of ruminants. J. Am. Vet. Med. Assoc. 2006, 228, 1346-1349.
7. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.3 – Bluetongue, OIE, Paris 2008.
8. *Mellor P. S., Wittmann E. J.*: Bluetongue virus in the Mediterranean Basin 1998-2001. Vet. J. 2002, 164, 20-37.
9. *Menzies F. D., McCullough S. J., McKeown I. M., Forster J. L., Jess S., Batten C., Murchie A. K., Gloster J., Fallows J. G., Pelgrim W., Mellor P. S., Oura C. A. L.*: Evidence for transplacental and contact transmission of bluetongue virus in cattle. Vet. Rec. 2008, 163, 203-209.
10. *Niedbalski W.*: Prevalence of the bluetongue virus antibodies in ruminants imported to Poland in 2008. Bull. Vet. Inst. Pulawy 2009, 53, 175-178.
11. *Niedbalski W., Kęsy A.*: Seroprevalence of antibodies against bluetongue virus in animals imported to Poland from the EU countries. Polish J. Vet. Sci. 2008, 11, 205-208.
12. *Niedbalski W., Kęsy A.*: Występowanie przeciwciał przeciwko wirusowi choroby niebieskiego języka w surowicy zwierząt sprowadzonych do Polski. Medycyna Wet. 2008, 64, 76-79.
13. *Paton J. F., Work T. M., Jessup D. A., Hietala S. K., Oliver M. N., Mac Lachlan N. J.*: Serologic detection of bluetongue virus infection of black-tailed deer: comparison of serum neutralization agar gel immunodiffusion, and competitive ELISA assays. J. Wild. Dis. 1994, 30, 99-102.
14. *Vandenbussche F., Vanbinst T., Verheiden B., Van Dessel W., Demeestere L., Houdart P., Bertels G., Praet N., Berkvens D., Mintiens K., Goris N., De Clercq K.*: Evaluation of antibody-ELISA and real-time RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during the epidemic in Belgium in 2006. Vet. Microbiol. 2008, 129, 15-27.
15. *Verwoerd D. W., Erasmus B. J.*: Bluetongue, [w:] Gard S., Coetzer J. A. W., Tustin R. C. (eds.): Infection diseases of livestock. Oxford University Press, New York 2004, 1201-1220.
16. *Walton T. E.*: The history of bluetongue and a current global overview. Vet. Ital. 2003, 40, 31-38.
17. *Wijaszka T., Truszczyński M.*: Występowanie na świecie w latach 2006-2007 ważnych epizootii, według danych OIE. Medycyna Wet. 2007, 63, 1270--1272.

Author's address: doc. dr hab. Wiesław Niedbalski, ul. Zielona 48/4, 98-220 Zduńska Wola; e-mail: wieslaw.niedbalski@piwzp.pl