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Original article

Evaluation of commercial ELISA kits for the detection of antibodies against bluetongue virus

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Abstract

The aim of this study was to estimate the diagnostic value of different commercially available ELISA kits for the detection of bluetongue virus (BTV) antibodies in infected and vaccinated animals. The relative specificity of ELISA kits was evaluated using a panel of sera originating from healthy cattle, never vaccinated nor exposed to BTV. All ELISA kits applied had a high relative specificity (99.3 – 100%). The relative sensitivity of ELISA kits assessed using a panel of sera collected from BTV infected cattle was also high and similar for all the kits (97.3 – 100%). However, the relative sensitivity evaluated on the basis of testing vaccinated animals was different: the highest sensitivity was found for Ingenasa, PrioCHECK and ID VET ELISAs (96.5 – 98.3%). Slightly lower sensitivity was calculated for Pourquier and LSI kits (82.8% and 85.4%, respectively) and much lower sensitivity was found for VMRD ELISA kit (69.5%). The repeatability of BTV ELISA kits was expressed as a coefficient of variation (CV) of results of sera tested 5 times in the same day and in different days by the period of 2 months, by the same person, in the same conditions, and by using the same equipment. The CVs of sera tested in all ELISA kits ranged from 6.1 to 9.8% and were below 10% threshold adopted as a maximum for the acceptable repeatability of the method. In conclusion, it can be stated that the applied ELISA kits can be a valuable diagnostic tool for the serological monitoring studies in the BTV contaminated premises. All the methods are very specific and sensitive when testing BTV infected animals. Nevertheless, the Ingenasa and PrioCHECK can be the most useful in sero-surveillance of livestock following vaccination.

Key words: bluetongue, serology, ELISA kits, evaluation

Introduction

Bluetongue (BT) is an economically important viral disease of domestic and wild ruminants that induces variable clinical signs depending on the species and the breed (MacLachlan 1994). The disease is caused by the bluetongue virus (BTV) which is the member of the *Orbivirus* genus within the family *Reoviridae*. Twenty four immunologically distinct serotypes (BTV1 to BTV24) of the virus have been identified worldwide (Gorman 1990). In 2008 an additional putative BTV serotype 25 (Toggenburg virus) was iso-

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lated from goats in Switzerland (Hofmann et al. 2008) and recently a novel BTV serotype 26 was identified in Kuwait (Maan et al. 2011). The virus is transmitted by specific species of *Culicoides* midges, family *Ceratopogonidae* (MacLachlan 1994). An evidence for transplacental and contact transmission of BTV was also described (Menzies et al. 2008).

The disease reliance upon an arthropod vector that is normally exclusively found in tropical and temperate area restricted BT for a long time to America, Australia, Africa and some regions of Asia (Walton 2004). However, since 1998, probably due to climatic changes, BTV spread northwards into the Mediterranean Basin, where five BTV serotypes (1, 2, 4, 9 and 16) have been identified (Purse et al. 2005). In the summer of 2006, for the first time, the BTV has crossed latitude 50°N and BT outbreaks caused by BTV serotype 8 occurred in North-Western Europe: the Netherlands, Belgium, Germany, France, and Luxembourg (Wilson and Mellor 2008). In 2007-2008, the BT situation changed for the worse – virus serotype 8 spread to the other regions of Europe, the number of outbreaks increased rapidly (about 50000 cases of disease were reported) and new BTV serotypes (BTV1 and BTV6) were detected (Wilson and Mellor 2009). However, the implementation of BT compulsory vaccination programmes in Europe in spring 2008 resulted in reduction of BTV8 cases to 6 (five of them were reported in Italy and one in Spain) in the culicoides activity season 1st May 2010 – 1st July 2011 (http://eubtnet.izs.it/ btnet.reports/ BTV8.htlm).

According to the decision of the General Veterinary Inspectorate of the Ministry of Agriculture and Rural Development in Warsaw, Poland, all BTV susceptible animals imported to Poland after 15 June 2006 from BTV affected countries should be tested for the presence of specific BTV antibodies. Therefore, since October 2006 we introduced the competitive ELISA (c-ELISA) and agar-gel immunodiffusion (AGID) assays to determine seroprevalence of BTV-specific antibodies in serum samples collected from susceptible animals imported to Poland from EU countries (Niedbalski 2010). The c-ELISA is very rapid, specific, sensitive at detecting antibodies in BTV-infected animals and easy to use (Afshar et al. 1987), therefore was recommended for large-scale serological screening and international trade purposes (Anon 2008). However, our earlier studies (Niedbalski 2009) and results presented by the others (Eschbaumer et al. 2009, Oura et al. 2009) have shown that this ELISA is not sensitive enough to be used for the detection of post-vaccination antibodies. Currently, several commercial ELISA kits for BTV antibodies are available (Batten et al. 2008). The aim of this study was to evaluate the diagnostic value of different commercially available ELISA kits for the detection of BTV antibodies in infected and vaccinated animals.

Materials and Methods

Sera

A total of 455 samples of sera from Poland which originated from healthy cattle neither vaccinated nor exposed to BTV were used. These sera were supplied by the District Veterinary Inspectorates and tested as a part of the national serosurveillance program for BT. Besides, 460 "post-vaccination" sera of cattle vaccinated with BT serotype 8 vaccine imported to Poland from EU member countries were tested. Additionally, we analysed "post-infection" sera collected from BTV positive animals from Germany, and four panels of sera (each of 7-12 samples) from BTV-infected animals supplied within the ring trials for BTV (serotype 8) viral genome and antibody detection.

Testing

The sera were examined using six commercially available ELISA kits: Bluetongue Virus Antibody Test Kit, c-ELISA (VMRD Inc., Pullman, USA), Ingezim BTV DR 12.BTV.K0 Kit (Ingenasa, Spain), Bluetongue Virus Antibody Test Kit – LSIBT5 (Laboratoire Service International – LSI, Lissieu, France), ID Screen Blue Tongue Competition (ID VET, Montpellier, France), PrioCHECK BTV DR (Prionics AG, Schlieren-Zurich, Switzerland) and Pourquier Bluetongue Competition ELISA – detection of specific antibodies to bluetongue virus by ELISA (Inst. Pourquier, Montpellier, France). The first test was performed according to the procedure described previously (Niedbalski and Kęsy 2008). The Ingezim BTV DR and PrioCHECK BTV DR are based on a direct ELISA called double-recognition ELISA. A sample is considered to be positive if its OD value at 450 nm is higher than the cut-off (0.15 x mean positive control). 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 ID VET 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

lower or equal 35%. The last evaluated kit – Pourquier bluetongue ELISA is based on the competition of antibodies specific to VP7 protein presented in tested sera with the conjugate (anti-VP7 antibody coupled to the peroxidase) for the corresponding BTV epitop. According to the manufacturer's specifications, a sample is considered positive, if S/N percentage (ratio between the OD of analysed serum (S) and the OD of the negative (N) control) is lower or equal 70%.

Estimation of the relative specificity, sensitivity and repeatability

Comparative specificity of ELISA kits was estimated using negative sera originated 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 ELISA used was tested using sera collected from BTV infected cattle.

Results

The relative specificity of ELISA kits was estimated using a panel of sera originating from healthy cattle never vaccinated nor exposed to BTV. All the kits used had a high relative specificity (99.3 – 100 %) (Table 1).

The relative sensitivity of ELISA kits estimated using a panel of sera collected from BTV infected cattle was also high and similar for all the kits (97.3 – 100%). However, the relative sensitivity evaluated on the basis of testing the vaccinated animals was different for the kits (Table 2). When testing Ingenasa ELISA, it was found that among 460 "post-vaccination" sera only 8 samples were scored as negative (sensitivity of ELISA 98.3%). Similar results were obtained using PrioCHECK kit; of 283 tested sera, the antibodies were recorded in 279 samples. The high sensitivity (96.5%) was also found for ID VET ELISA. In comparison, in population of 425 sera tested in LSI and 298 in Pourquier ELISA kits, 62 (sensitivity 85.4%) and 51 (sensitivity 82.8%) were scored negative, respectively. The much lower sensitivity (69.5%) was found for VMRD kit; out of 467 sera, 142 gave negative reaction (Table 2).

The repeatability of ELISA kits was expressed as a coefficient of variation (CV) of results of sera tested 5 times in the same day and in different days by the period of 2 months, by the same person, in the same conditions, and by using the same equipment. The CVs of sera tested in all BTV ELISA kits ranged from 6.1 to 9.8% (Table 3).

Discussion

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. Due to the BT mass vaccination of all domestic ruminant species conducted in BTV-affected EU member states during 2008-2010, the ability to detection of both infected and vaccinated animals is important for export/import serological examination. The reliable and precise diagnostic tests are a major component of success in any surveillance system. 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 was to determine diagnostic specificity, sensitivity and repeatability of commercially available BTV ELISA kits, three were based on the principle of competition (VMRD, ID VET and Pourquier), Ingenasa, PrioCHECK and LSI kits were direct, double antigen and blocking ELISA, respectively. All ELISA kits used were easy and quick to perform, especially the VMRD, ID VET and Pourquier taking about 1 hour to complete. The relative specificity of ID VET kit was similar to its performance reported by Vandenbussche et al. (2008) and specificity of PrioCHECK and Pourquier kits was comparable to the earlier reported results (http://www.eavld2010.org, http://www.institut-pourquier.fr). Our results of analytical sensitivity of c-ELISAs evaluated on BTV

Table 1. Comparative specificity of the ELISA kits used.

Test	No. of sera examined	No. of negative sera	Specificity (%)
VMRD	455	452	99.3
Ingenasa	410	408	99.5
PrioCHECK	292	291	99.6
LSI	297	295	99.3
ID VET	312	310	99.3
Pourquier	276	276	100

Table 2. Comparison of the relative sensitivity of BTV ELISA kits.

Origin of sera	VMRD	Ingenasa	PrioCHECK	LSI	ID VET	Pourquier
Infected animals						
Positive results	73	75	75	74	74	72
Negative results	1	0	0	1	0	0
Sensitivity (%)	98.6	100	100	97.3	100	100
Vaccinated animals						
Positive results	325	452	279	363	417	247
Negative results	142	8	4	62	15	51
Sensitivity (%)	69.5	98.3	98.5	85.4	96.5	82.8

Table 3. Reproducibility of ELISA kits for the detection of BTV antibodies.

Test	Sera tested in the 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
PrioCHECK	1.586[b]	0.13	8.2	1.542[b]	0.13	8.4
	1.794	0.14	7.8	1.815	0.15	8.2
	1.710	0.13	7.6	1.748	0.15	8.6
	1.722	0.12	1.7	78	0.14	7.8
	1.887	0.16	8.4	1.842	0.16	8.7
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
Pourquier	18.4[d]	1.4	7.6	19.2[d]	1.6	8.3
	17.9	1.6	8.9	18.4	1.8	9.7
	16.7	1.4	8.3	16.9	1.5	8.9
	19.4	1.7	8.7	19.1	1.8	9.4
	16.8	1.2	7.1	17.3	1.4	8.1

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

"post-infection" sera correspond with the previously reported findings (Vandenbussche et al. 2008, http://www.institut-pourquier.fr). Moreover, the c-ELISA was a superior to the other ELISA assays in the detection of anti-BTV antibody in the sera samples from cattle and sheep early after infection with BTV (Afshar et al 1987). These results of analytical sensitivity examined on "post-vaccination" sera are in agreement with earlier studies on c-ELISA (Eschbaumer et al. 2009, Niedbalski 2009, Oura et al. 2009,) and obtained for validation of PrioCHECK (http://www.eavld2010.org) and Pourquier (http://www.institut-pourquier.fr) ELISA kits. If low levels of antibodies were found in the serum tested,

below the threshold of the detection of the VMRD ELISA, these vaccinated animals were scored as seronegative. However, according to the results obtained at the Bluetongue Community Reference Laboratory (CRL) in Pirbright (UK), for animals vaccinated with inactivated BTV8 vaccines on a single occasion, the absence of antibodies detected by the c-ELISA does not correlate with the lack of the protection (http://www.warmwell.com). The repeatability of all six BTV ELISA kits expressed as CV ranged from 6.1% to 9.8% (Table 3) and were below the 10% threshold adopted as a maximum for the acceptable repeatability of the method (Jacobson 1998). The CVs 6% for PrioCHECK and 5% for Pourquier kits were found by others (http://www.eavld2010.org, http://www.institut-pourquier.fr).

In conclusion, it can be stated that the ELISA kits applied can be a valuable tool for serological monitoring studies in BTV contaminated premises. All the methods used are very specific and sensitive when testing BTV infected animals. However, the sandwich ELISA assays (Ingenasa and PrioCHECK) have clear advantages and can be the most useful in sero-surveillance of livestock following vaccination. However, the recent studies of Eschbaumer et al. (2011) have revealed that if they are used exclusively, there is a risk of false-negative results for animals that were infected longer ago and might still be viraemic. So, it is recommended that samples should be tested with a c-ELISA first, and then, if the result is negative, a sandwich ELISA should be used for further analysis.

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