Onderstepoort J. vet. Res., 59, 119–122 (1992)

SEROLOGICAL DIFFERENTIATION OF FIVE BLUETONGUE VIRUS SEROTYPES IN INDIRECT ELISA

D. H. DU PLESSIS, Biochemistry Section, Veterinary Research Institute, Ondertepoort, 0110

ABSTRACT

DU PLESSIS, D. H., 1992. Serological differentiation of five bluetongue virus serotypes in indirect ELISA. *Onderstepoort Journal of Veterinary Research* 59, 119–122 (1992)

The serological reactivity in indirect ELISA of five different bluetongue virus (BTV) serotypes (4, 10, 15, 16 & 20) was compared using polyclonal antisera raised against virus particles and an outer structural protein, VP2. Rabbit and sheep antisera against BTV-10 produced higher ELISA values with their homologous antigens than with heterologous serotypes. A hyperimmune rabbit serum specific for virus particles was able to distinguish heterologous serotypes from each other, but a sheep serum from an infected animal was not. An antiserum directed against VP2, the protein resposible for serotype specificity in neutralization tests, was not serotype-specific in ELISA and cross-reacted with other serotypes. The discriminatory ability of a BTV-4 antiserum was improved by cross-absorption with heterologous antigens. This greatly reduced the ELISA signals with heterologous serotypes and produced an antiserum that was effectively serotype-specific.

INTRODUCTION

The capsid of bluetongue virus (BTV), the type member of the Orbivirus genus, family Reoviridae, has a double-shelled, icosahedrally symmetrical capsid comprised of seven different structural proteins. An inner core of five proteins is surrounded by a diffuse layer formed by two polypeptides designated VP2 and VP5 (Huismans & Van Dijk, 1990). Orbiviruses have been assigned to serogroups on the basis of complement fixation (CF) tests (Borden, Shope & Murphy, 1971) while the 24 serotypes within the BTV group are distinguished in vitro by the neutralization of infectivity with serotype-specific antisera (Haig, McKercher & Alexander, 1956; Howell, 1960; Howell, Kümm & Botha, 1970; Gorman, 1979; Verwoerd, Huismans & Erasmus, 1979; Jeggo Gumm & Taylor, 1983). VP2, one of the two outer polypeptides, has been identified as an important determinant of serotype specificity in immunoprecipitation (Huismans & Erasmus, 1981) and in neutralization tests (Kahlon, Sugiyama & Roy, 1983; Huismans, Van der Walt, Cloete & Erasmus, 1987; Inumaru & Roy, 1987).

For laboratory diagnosis of bluetongue, indirect or competition enzyme-linked immunosorbent assays (ELISAs) have been used to detect antibodies to viruses in the BTV serogroup (Hübschle, Lorenz & Matheka, 1981; Anderson, 1984; Afshar, Thomas, Wright, Shapiro, Shattigara & Anderson, 1987; Lunt, White & Blacksell, 1988; Afshar, Thomas, Wright, Shapiro & Anderson, 1989; House, House & Berninger, 1989; Reddington, Reddington & MacLachlan, 1991). These assays are generally more rapid and simpler to perform than classical tests such as complement fixation, but they have not yet been used for typing unknown isolates or antibodies. So far, only the polymerase chain reaction using serotype-specific oligonucleotides has shown potential as a rapid serotyping method (McColl & Gould, 1991).

As part of an investigation into the feasibility of developing enzyme-immunoassays to identify serotypes, this study compares the serological cross-reactivity of five different BTV serotypes in indirect ELISA. The serotypes were the putative ancestral serotype 4, serotypes 15 and 16 which are distantly related to it, as well as the two closely related serotypes 10 and 20 (Eramsus, 1990). Comparisons were made using antisera to BTV virions and a polyclonal antiserum specific for VP2. Absorption with heterologous antigens was used to remove cross-reacting antibodies from a BTV antiserum and thereby improve its ability to distinguish serotypes.

Received 11 February 1992 — Editor

MATERIALS AND METHODS

Virus propagation and purification

BTV serotypes were propagated in baby hamster kidney cells and purified by the method of Huismans *et al.*, (1987). The concentration of purified virus preparations was determined spectrophotometrically with an absorbance value at 260 nm of 5,42 corresponding to 1 mg/mℓ virus (Smith, Zweerink & Joklik, 1969).

Antiserum production

Hyperimmune antisera to BTV virions were raised in rabbits by injection of purified virus particles emulsified in Freund's complete adjuvant, followed by a series of injections in incomplete adjuvant. VP2 for antiserum production was isolated by treatment of BTV virions with $MgCl_2$ at pH 5,0 followed by addition of an anti-core serum to remove traces of core proteins, in particular VP7 (Huismans *et al.*, 1987). Sheep antiserum was from an animal that had been inoculated with the vaccine strain of BTV serotype 10 (BTV-10).

ELISA

Indirect ELISA was performed essentially as described by Voller, Bidwell & Bartlett (1976). The surfaces of the wells of polystyrene microtitre plates (Nunc Polysorp, Denmark) were coated overnight with 100 µℓ of purified virus at a concentration of 5 µg/mℓ in phosphate buffered saline (PBS). Bovine serum albumin, or non-fat milk powder (5 % m/v) in PBS was used for blocking and antisera were diluted in PBS containing 0,05 % (v/v) Tween-20 and

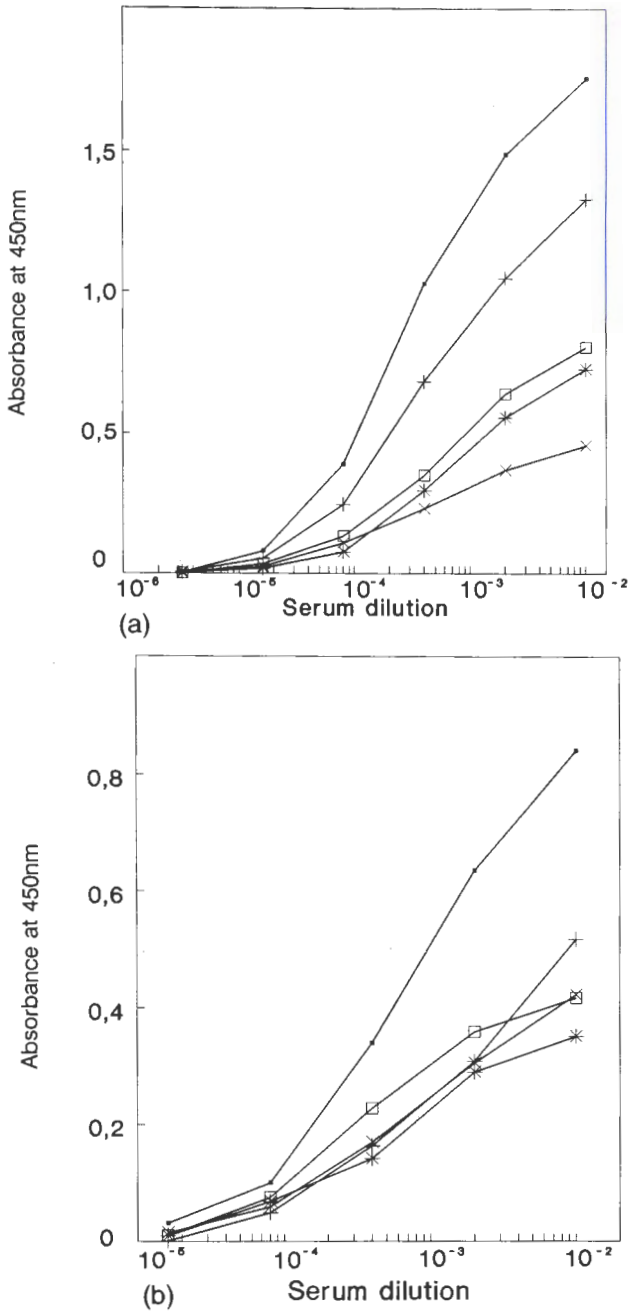

FIG. 1 Comparison of serological cross-reactivity of five BTV serotypes in indirect ELISA utilising: a) a rabbit antiserum to purified BTV-10 virus particles, b) a sheep antiserum from an animal infected with BTV-10. Immobilised antigens were: BTV-10 —●—; BTV-20 —+—; BTV-4 —□—; BTV-16 —✱—; BTV-15 —×—.

0,5 % (m/v) albumin or milk powder (Elite). Antibody was detected with horseradish peroxidase-conjugated protein A (Sigma) or anti-sheep immunoglobulin (Dako, Denmark). The substrate was o-phenylene diamine and colour development was monitored at 450 nm using an automatic plate reader. All comparisons were done in duplicate on one microtitre plate and were replicated on another. The values presented represent averages from a single microtitre plate.

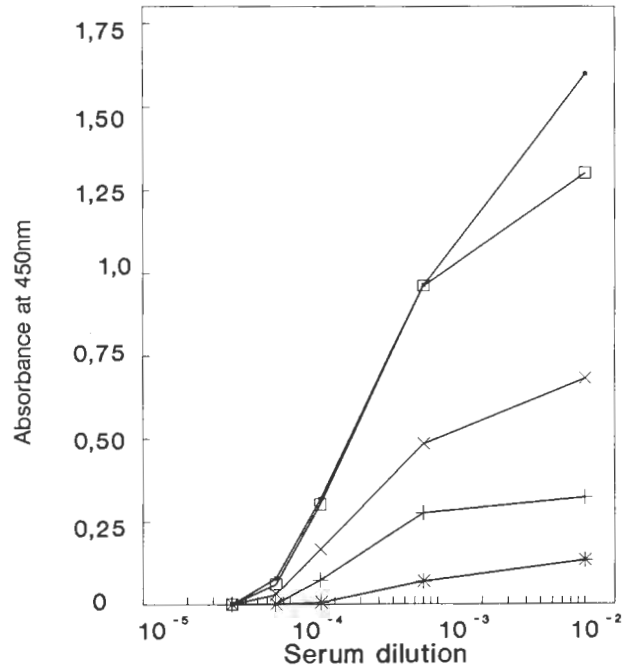

FIG. 2 Serological reactivity of five BTV serotypes in indirect ELISA with a rabbit antiserum specific for the outer structural polypeptide VP2 of BTV-10. Antigens were: BTV-10 —●—; BTV-4 —□—; BTV-15 —×—; BTV-20 —+—; BTV-16 —✱—.

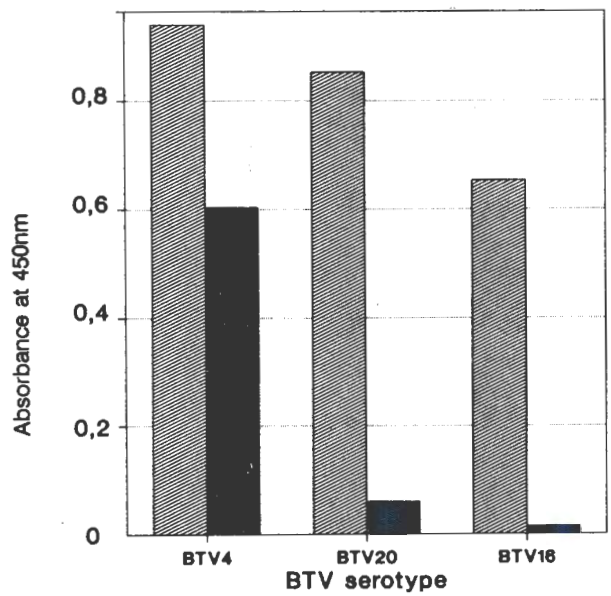

FIG. 3 Reaction of a BTV-4 rabbit antiserum diluted 1/2000 with homologous and heterologous BTV serotypes in ELISA using unabsorbed serum ▨; serum cross-absorbed with BTV-16 and BTV-20 virus particles ■

Cross-absorption of antiserum

BTV serotype 4 (BTV-4) antiserum was diluted 1/2000 in PBS and an amount of 50 µg of each of the purified serotypes BTV-16 and BTV-20 was added to 1 mℓ of the diluted antiserum. After 4 h at 37 °C the serum was centrifuged at 11000 rpm in a microfuge for 30 min. The supernatant serum was recovered, a further 50 µg of each of the heterologous serotypes was added and the suspension was

incubated overnight at 4 °C. The centrifugation step was repeated before the absorbed antiserum was tested in ELISA.

RESULTS

Cross-reactivity of BTV serotypes with virion-specific antisera

To ascertain whether the antigenic properties of five BTV serotypes diverged sufficiently to be distinguishable by an antiserum against an individual serotype, purified virions were adsorbed to the surface of plastic microtitre plate wells for assay in ELISA. Before adsorption, all virions were adjusted spectrophotometrically to a concentration of 5 µg/mℓ. A rabbit antiserum, raised against purified serotype 10 virus particles, reacted serologically with both the homologous and heterologous antigens in the ELISA, producing the highest absorbance values with BTV-10. Of the heterologous serotypes, BTV-20 showed the greatest degree of cross-reactivity and BTV-15 the least. BTV-16 and BTV-4 were not clearly distinguished from each other (Fig. 1a). In other determinations with BTV-10 antisera from different animals, the homologous ELISA values were consistently highest, but the degree of discrimination depended upon the serum. For example, the reaction of a sheep antiserum with its homologous antigen, BTV-10, was distinguishable from the heterologous reactions, but it did not conclusively differentiate between any of the other antigens (Fig. 1b). In the above and in subsequent experiments, there was no difference in the extent of cross-reactivity whether milk powder or BSA was used for blocking and in the diluents.

Comparison of serotypes with VP2-specific antiserum

VP2, an outer capsid protein has been identified as a determinant of serotype-specificity in immunoprecipitation assays. It was therefore of interest to ascertain whether an antiserum against this protein would recognise immobilised virus particles in a serotype-specific manner in ELISA. A rabbit antiserum directed against VP2 of BTV-10 was allowed to react with the five different BTV serotypes. BTV-10, the homologous serotype was recognised, but the antiserum also cross-reacted to some extent with each of the remaining four serotypes. At a 1/100 dilution, it yielded the highest ELISA absorbance values with the homologous antigen followed by the serotypes BTV-4, BTV-15, BTV-20 and BTV-16 respectively. At higher dilutions, however, BTV-4 and BTV-10 could not be distinguished (Fig. 2). The reactivity of the virus serotypes in this experiment was thus similar to that obtained with antisera to BTV virions. Homologous and heterologous serological reactions were distinguishable, but the antigens were not recognised in a strictly serotype-specific manner.

Discrimination of serotypes by cross-absorbed antiserum

In the studies described above, neither the virion, nor the VP2-specific antisera were serotype-specific. With the objective of improving the discriminatory ability of an antiserum, an attempt was made to remove the antibodies responsible for inter-serotype cross-reactivity. A rabbit antiserum directed against purified BTV-4 was used in this experiment. Fig. 3 shows that at a dilution of 1/2000, the unabsorbed antiserum reacted with the homologous antigen, BTV-4, as well as with BTV-16 and BTV-20. The two heterologous serotypes produced lower ELISA values, with the reading produced by BTV-16, reaching approximately 70 % of that of BTV-4. After cross-absorption with BTV-20 and BTV-16 antigens, ELISA readings with the heterologous serotypes were greatly reduced. For instance, the absorbance that BTV-20 produced was decreased from 0,85 to a value of 0,06, which is not significantly above background. Similarly, the reading resulting from reaction with BTV-16 was reduced from 0,654 to 0,015. This represents only 0,02 % of the homologous value. The cross-absorption procedure also reduced the signal with BTV-4 by approximately 30 %, but despite this, it greatly increased the differences between homologous and heterologous reactivities, producing what was effectively a serotype-specific antiserum.

DISCUSSION

The main objective of this study was to determine whether the antigenic properties of five BTV serotypes differed sufficiently to allow them to be distinguished in ELISA. Antigens were compared using antisera directed against BTV particles and against VP2, the structural polypeptide that has been identified as representing an important determinant of serotype-specificity.

A virus-specific rabbit antiserum raised by immunisation with BTV-10 virions did not react identically with all five serotypes. The homologous serotype yielded the highest ELISA values while those of the heterologous serotypes were lower. Comparison of the ELISA absorbance values suggests that BTV-20 is serologically closer to BTV-10 than BTV-15 and BTV-16 (Fig. 1a). While this is in broad agreement with neutralization and cross-protection data (Erasmus, 1990), these apparent relationships should be treated with reservation since they may depend upon the serum. For example, a sheep antiserum specific for BTV-10 yielded the highest ELISA values with that serotype, but unlike the rabbit antiserum, it was unable to reveal significant differences between the heterologous serotypes (Fig. 1b). When compared in ELISA using a VP2 antiserum, BTV-15 was serologically closer to BTV-10 than was BTV-20 (Fig. 2). This contradicts neutralization data (Erasmus, 1990) which show BTV-10 and BTV-20 to be closely related and BTV-15 as being distant. Neutralization of BTV can result from the recognition of a single epitope on VP2 (Gould, Hyatt & Eaton, 1988) while the ELISA compares overall serological reactivity. It may therefore not always be possible to correlate ostensible serological distances in ELISA with those suggested by neutralization and cross-protection assays. Nevertheless, from a practical point of view, a comparative ELISA based on a series of immobilised BTV serotypes could possibly allow the serotype against which an unknown serum is directed to be identified. It would need to be established, however, that BTV

antisera as a rule produce the highest ELISA readings with their homologous serotypes.

The VP2-specific antiserum yielded its maximum ELISA values with the homologous serotype, BTV-10, but like the virus antisera, it was not strictly serotype-specific. This was not unexpected since VP2-specific monoclonal antibodies may also recognise other serotypes (Ristow, Leendersten, Gorham & Yilma, 1988; White & Eaton, 1990) and VP2 may be immunoprecipitated by heterologous antisera (Huismans & Bremer, 1981; Huismans & Erasmus, 1981). The nucleotide sequences of different VP2 genes are not highly conserved, yet significant regions of similarity are evident (reviewed by Roy, 1989). Serological cross-reactivity of the protein is probably a reflection of these likenesses and has implications for the development of rapid serotyping methods. For example, VP2 produced by recombinant DNA technology may not prove to be an absolutely type-specific immunodiagnostic reagent.

Although neither the virion, nor the VP2 antisera uniquely recognised a particular BTV serotype, the discriminatory ability of a BTV antiserum could be improved by cross-absorption with heterologous antigens. Removal of cross-reacting antibodies was manifested by a significant reduction in the ELISA signals produced by the heterologous serotypes (Fig. 3). The multiplicity of BTV serotypes and the large amount of antigen required would probably limit its applicability, but in principle, a panel of serotype-specific antisera could be produced by judicious cross-absorption with heterologous antigens.

ACKNOWLEDGEMENTS

I thank Dr Nico van der Walt, Errol Boekhoven and Dr Albie van Dijk for antisera and antigens. The technical assistance of Elise Snyman and Frances Kamp is gratefully acknowledged.

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