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# Immunological Characterization of an SDS/KCL Isolated Total Protein Antigen of Bluetongue Virus Serotype 13

Vicki L. Shore
*Utah State University*

# IMMUNOLOGICAL CHARACTERIZATION OF AN SDS/KCl ISOLATED TOTAL PROTEIN ANTIGEN OF BLUETONGUE VIRUS SEROTYPE 13

by

Vicki L. Shore

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## ABSTRACT

Because of the damaging effects of bluetongue disease on the livestock industry, interest exists in developing a means of inducing passive immunity in infected animals. Antibodies against VP2, the polypeptide responsible for immunoantigenicity in bluetongue virus (BTV), have been found to elicit neutralizing antibodies; however, production of this purified antigen is very costly and thus impractical for commercial purposes. Total viral protein, prepared by a simple, rapid, highly reproducible SDS/KCl method, was injected into rabbits and found to produce antibodies which are useful in immunoblots. However, plaque neutralization assays failed to detect any neutralization activity by these antibodies. Denaturation of the antigenic determinants of the viral polypeptide during antigen preparation is suggested as one of the potential problems. BTV protein may be restored to its native state by a simple procedure involving urea exchange and a separative column. Further investigation is being conducted to determine the accuracy of the proposed hypothesis.

## INTRODUCTION

Bluetongue virus (BTV) belongs to the family Reoviridae and the genus Orbivirus. Twenty-four serotypes of BTV have been isolated in various world regions, five of which (BTV-2, 10, 11, 13, and 17) have been found in the United States (Mecham et al., 1986). BTV causes a degenerative disease in sheep, goats, cattle, and other non-domesticated ungulates, which is sometimes fatal (for

review, see Gorman et al., 1983). Infected animals are found to have viremia 1 to 31 days post-infection (Leudke, 1969) and later may develop myofibrile degeneration, edema, fever, and vomiting. Death appears to be due to cardiac failure and subsequent pulmonary congestion (Mahrt and Osburn, 1986).

The detrimental effects of this disease on the livestock industry have initiated interest in development of an effective vaccine. Inactivated or subunit vaccines are preferred over attenuated vaccines, such as the one currently in use in South Africa, due to several complications with attenuated vaccines (Stott et al., 1985), especially viral genetic reassortment resulting in loss of immunogenicity (Appleton and Letchworth, 1983).

Bluetongue virus consists of seven structural viral proteins. Five of these (VP1, VP3, VP4, VP6, and VP7) compose an inner core; the remaining two (VP2 and VP5) make up an outer capsid (Voerwoerd et al., 1972). VP2 is considered to be the serotype-specific polypeptide responsible for eliciting neutralization (Huismans and Erasmus, 1981; Kahlon et al., 1983). Attempts to produce antibodies against the subunit antigen of VP2 have elicited a serotype-specific neutralization response (Huismans et al., 1987; Appleton and Letchworth, 1983; Inumaru and Roy, 1987), yet the production of these antigens is very time-consuming and costly making them unfeasible in a commercial setting. Also, the second outer capsid protein, VP5, has been implicated as a minor determinant in viral neutralization (Mertens et al., 1986). Hence,

total viral protein, which could be prepared quickly and easily, was sought as a subunit antigen. This study examines an attempt to produce neutralizing polyclonal antibodies against such an antigen.

## MATERIALS AND METHODS

### Virus production

United States prototype BTV-13 was obtained from Arthropod-Borne Animal Diseases Research Laboratory. The virus was plaque purified three times in mouse L-929 cells.

Monolayer baby hamster kidney cells (BHK-21) were used for virus production. The BHK-21 cells were maintained in Eagle's minimum essential media (MEM) supplemented with 5% calf serum (Hyclone Laboratories) in a humidified 5% $CO_2$ atmosphere at 37° C. BHK-21 cells were infected with BTV-13 at a multiplicity of infection (MOI) of 1.0 PFU and incubated for 60-72 hours at which point the cells were harvested and pelleted. After sonifying the cell pellet, the viral-containing supernatant was titered by plaque assay in L cells (Howell et al., 1967) with the exception that Sephadex G-25 was used as an overlay in place of agarose. The virus was stored at 4° C.

### Antibody production

Polyclonal antibodies were produced by multiple intramuscular inoculations of total viral protein from BTV-13, obtained by the method of Li et al. (1989). Serum was collected biweekly and screened for the presence of polyclonal antibodies by immunoblotting. The serum was stored at -20° C until use.

Antibody sterilization

Anti-serum was thawed and diluted 1:10 in phosphate buffered saline (PBS). The serum was sterilized with a 0.22$\mu$ Millex-GV syringe filter in a hood.

Neutralization Assay

Monolayer L cells were seeded onto six-well plates and grown to confluency under the same growth conditions as BHK-21 cells. In sterile tubes, two-fold serial dilutions of antibody were made in Eagle's MEM which had not been supplemented with calf serum. Equal volumes of BTV-13 which had been previously titered and diluted were added. A tube at each viral dilution which did not contain antibody was prepared as a positive control. All tubes were incubated at 37$^{o}$ C for 1 hr with periodic agitation. After incubation, the contents of each tube were added to the appropriate plate. The plates were incubated at 37$^{o}$ C in a humidified 5% $CO_2$ environment for 1 hr. After the absorption period, the wells were overlayed with Sephadex G-25 in Eagle's MEM containing calf serum. The plates were incubated at 37$^{o}$ C for six days as for plaque assay.

## RESULTS

Determination of polyclonal antibody production

Serum from rabbits injected with the SDS/KCl isolated BTV-13 protein antigen reacted positively with all BTV protein bands in immunoblots, as shown in Figure 1. The antibodies recognized not only the purified protein from BTV-13, but also the proteins from

the four other U.S. serotypes (2, 10, 11, and 17). These results confirm the successful production of polyclonal antibodies against the SDS/KCl isolated subunit antigen. It is also interesting to note that the antibodies, which were made against BTV-13 protein, strongly cross-reacted with VP2 of BTV-13 and 11 but not with VP2 of the other serotypes. Since VP2 is the serotype-specific polypeptide, this suggests some homology exists between the antigenic epitopes of BTV-13 and 11.

Neutralizing effects of polyclonal antibodies

Several neutralization assays were performed using the procedure described under Materials and Methods. The viral dilutions which gave the most readable number of plaques (50-100 plaques per well) were chosen for use in the neutralization assays. Since the anti-serum recognized VP2 of BTV-13 and 11, both serotypes were tested in the neutralization assays. The first trial used a viral dilution of $10^{-7}$ with two-fold antibody dilutions ranging from 1:25 through 1:2000. No neutralization was detected for either serotype. The experiment was repeated using a different preparation of virus at the same dilution. Similar results were again obtained. The assay was repeated several times varying both the viral preparations and the antibody dilutions used. Antibody dilutions tested ranged from 1:10 through 1:2000. No neutralizing activity was detected in any of the assays performed, suggesting that no neutralizing antibodies were present in the serum tested. Typical results are shown in Figure 2.

## DISCUSSION

The procedure used to produce the total protein antigen used in this study was developed by Li et al. (1989). Briefly, BTV-13 was purified using a modified version of the technique developed by Mertens et al.(1987). The viral pellet was then treated with sodium dodecyl sulfate (SDS) at a final concentration of 1%. The SDS complexes with the viral protein but not the viral genome. The SDS-bound proteins can then be precipitated with ice-cold KCl. This technique is simple, quick, highly reproducible, and recovery is greater than 95%, making it valuable as a means of producing the desired antigen. The total viral protein obtained by this method produced antibodies useful in diagnostic immunoblots, but did not induce production of neutralizing antibodies in rabbits. This could very likely be due to the comlexing of SDS with the viral protein. The binding of SDS may promote conformational changes in the proteins causing an alteration in the antigenic site or sites on VP2. Antibodies made against such a protein would not necessarily neutraize virus whose protein is in its native state. Should this be the case, neutralization assays would give negative results, as found in this experiment.

If the above suspicions are true, a method of renaturing the SDS/KCl separated viral protein to its native configuration prior to injection into experimental animals could eliminate this potential source of error. A procedure using urea exchange and a purification column may prove to be the solution. If the denaturation is reversible, the urea will exchange with the SDS,

allowing the protein to renature since urea induces few conformational changes when bound to protein as does SDS. The column will then be used to remove the SDS and excess urea, permitting the urea-protein complex to elute out. The eluate will be injected into the rabbits, their anti-sera collected, and the neutralization assay repeated. Work is currently in progress toward this end. Should the aforementioned hypothesis and proposed solution prove to be accurate, and neutralization result, this method will provide advancement toward the development of a subunit vaccine against BTV.

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**Figure 1.** Immunoblot of the seven BTV viral proteins of the five U.S. serotypes (2, 10, 11, 13, and 17) probed with anti-serum from rabbits injected with SDS/KCl isolated BTV-13 total protein.

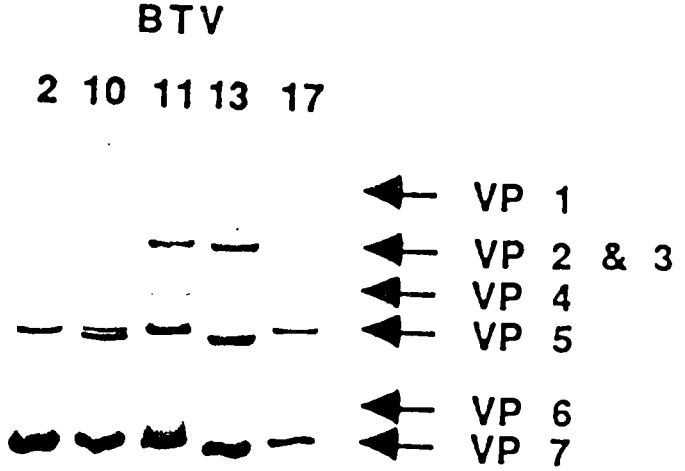
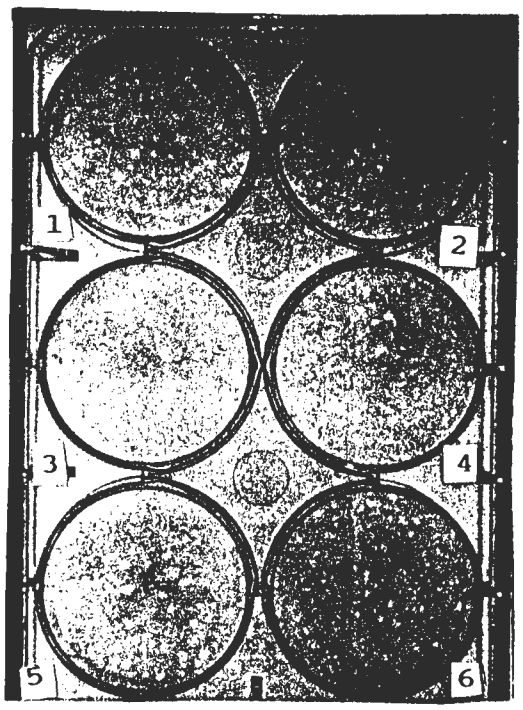
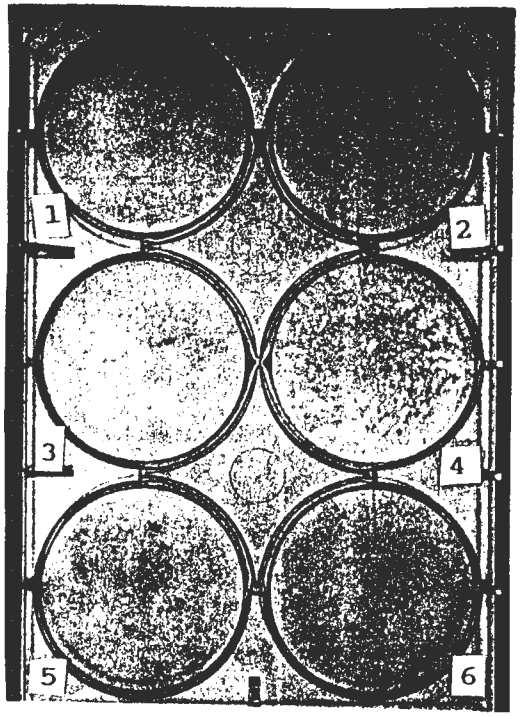

BTV
2 10 11 13 17
VP 1
VP 2 & 3
VP 4
VP 5
VP 6
VP 7

Figure 2. Neutralization assays performed using BTV-13. (A) Viral dilution=$10^{-5}$; Two-fold antibody dilutions range from 1:20 through 1:640 beginning with well #1. (B) Control plate. Wells 1 & 2 are negative controls; well 3 is a positive control of viral dilution=$10^{-5}$; well 4 is a positive control of viral dilution=$10^{-4}$; wells five and six are double negative controls of antibody dilution=1:100. (C) Viral dilution=$10^{-4}$. Two-fold antibody dilutions range from 1:20 through 1:640 beginning with well #1.

(A)




(B)




(C)