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Rapid Method for Detection of Bluetongue Virus Infection Using Recombinant cDNA Probes

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Title

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cDNA probes.

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ABSTRACT:

Recombinant cDNA probes from genome segments 5 (S5), 7 (S7), 9 (S9) and 10 (S10) from USA bluetongue virus (BTV) 11-UC8 strain were developed and used to study the genetic relatedness and the feasibility of using recombinant cDNA probes for the identification of Israeli field isolates and for the developing of rapid BTV diagnostic tests. All the ³²P labeled probes were hybridized to the Northern blotted viral dsRNA from the USA BTV 2, 10, 11, 13 and 17 prototypes and Israeli BTV prototypes 2, 4, 6 and 16 Israeli BTV field isolates. Clone S5 was also hybridized to USA BTV field isolates from serotypes 10, 11, 13 and 17. Clone S5 hybridized only to USA and Israeli prototype 10 and some isolates from USA BTV 10 and 11. It did not hybridize with any of the Israeli BTV field isolates tested. Clone S7, S9 and S10 recognized all the USA and Israeli prototypes and Israeli field isolates tested showing S7 the highest degree of variation in the hybridization signal. Clones S9 and S10 are the most suitable candidates for BTV serogroup specific identification of Israeli isolates.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL:

Genome segments from USA and Israel bluetongue virus (BTV) serotypes, containing sequences that code for group or serotype specific antigens, will be cloned using relatively new procedures in recombinant technology. The clones will be used as probes against Northern blots of isolates of BTV from the USA and Israel to test the reliability of the probes and to compare the degree of genetic variability that may exist between field isolates within a defined taxonomic group. The library of BTV clones will be comprised of cDNA sequences for general diagnosis of BTV infection and a battery of probes for clasifying the serotypes of BTV isolates. Adaptation of hybridization protocols will allow rapid diagnosis of BTV infection and development of an efficient screening method for certifying animals and germ plasm free of BTV for export.

TITLE:

Rapid method for detection of bluetongue virus infection using recombinant cDNA probes.

DESCRIPTION OF THE RESEARCH

Introduction

Bluetongue (BT) is an insect borne disease that affects wild and domestic ruminants. It is caused by bluetongue virus (BTV), the prototype of the genus Orbivirus of the Reoviridae family. The viral genome consists of 10 double stranded RNA (dsRNA) segments (1) which code for at least 10 viral polypeptides (2), three of which are nonstructural (NS) proteins (NS1, NS2, NS3) and 7 structural proteins. The 7 structural proteins are arranged in an outer capsid (VP2 and VP5) and inner core (VP1, VP3, VP4, VP6 and VP7 (3). The proteins coded for by each BTV genome segment were determined by in vitro translation (4,5). Genome segment 2 codes for VP2, the outer capsid polypeptide responsible for the serotype specificity (6). Genome segment 5 codes for VP5, the second outer capsid protein, that appears to play a significant role in the adsorption of BTV to cells (7), and to be involved in some form of protective immunity (8) and serotype determination (9). Genetic studies have demonstrated that genome segments 2 and 5 are the least conserved among all BTV genome segments (10,11,12,13).

Genome segment 7 codes for VP7, an inner capsid protein that carries the group specific antigen determinant. This genome segment was found to be the least conserved among the dsRNA segments coding for BTV inner capsid and NS proteins (11,12,13,14). The BTV genome segment 9 codes for VP6 an inner core protein with the property of binding virion dsRNA, ssRNA and other nucleic acids species (15). The BTV genome segment 10 product is the NS3 protein, its function is unknown, but it was proposed that NS3 could play a role in BTV

replication and morphogenesis (16). Bluetongue virus genome segment 9 and 10 have been determined to be highly conserved among all BTV serotypes (12,13,17).

One important characteristic of the BTV serogroup is its genetic diversity that is reflected in the presence of 24 serotypes distributed in Africa, Asia, Australia, Middle East and North, Central and South America. Five BTV serotypes are present in the USA: 2, 10, 11, 13 and 17. In Israel, BT was first suspected in the winter of 1943/44 (18) and its presence was finally confirmed by the isolation and identification of BTV serotype 4 in 1950 (19,20). Since then, BTV serotypes 2, 6, 10 and 16 have also been isolated in that country (21). In Israel BT has been observed in sheep, cattle (18) and mountain gazelle (*Gazella gazella*) (22), and serologic evidence of BTV infection has also been detected in camels (23,24).

The segmented nature of BTV genome and the existence of multiple serotypes and strains, facilitate genome segment reassortment which contribute to the genetic diversity of viruses within BTV serogroup (25,-27). This genetic diversity is also reflected in variations in virulence and biological behavior of different BTV strains. Recently, UC8 strain from USA BTV serotype 11, isolated from a deer in California, was shown to be more neurovirulent for newborn mice (28,29) and fetal cattle (30) when compared to another USA BTV 11 strain, UC2. Bluetongue virus 11 UC8 strain was shown to be a reassortant between USA BTV 10 and USA BTV 11 (31).

The genetic relatedness between different BTV serotypes and strains from the same or different countries and continents has been studied (11-13,19,27,32-37) and variations in the hybridization had been observed between strains of the same serotype isolated in different countries (12,37). This genetic diversity may create some difficulties in the identification of BTV isolates from one country utilizing cloned genetic probes produced from strains present in another country. It has also been proposed that BTV isolates may be geographically grouped according to hybridization analyses (37).

The objectives of this study were the development of BTV cloned genetic probes from USA BTV 11 UC8 strain genome segments 5, 7, 9 and 10 and to test the reliability of the probes to identify BTV samples and to compare the degree of genetic variability that may exist between field isolates within a defined taxonomic group for the development of rapid diagnostic tests for BTV by hybridization techniques. This is the first report in which the genetic characteristics of BTV genome segments from prototypes and field isolates from Israel were studied.

MATERIALS AND METHODS

Virus and cells

The USA BTV prototype strains from BTV 2, 10, 11, 13 and 17 and the epizootic hemorrhagic disease 1 (EHDV 1) prototype strain were obtained from the Arthropod-Borne Animal Diseases Research Laboratory, Denver, Colorado. The EHDV 2 field isolate studied was obtained from the Washington Animal Disease Diagnostic Laboratory, Pullman, Washington. The 65 USA BTV field isolates studied by hybridization

with UC8 genome segment 5 were obtained from cattle, goats and sheep from different counties in California, during the years 1979, 1980 and 1981: there were 14 isolates of BTV 10, 17 of BTV 11, 17 of BTV 13 and 17 of BTV 17. All the USA BTV samples were replicated and stored as described (38). Israeli BTV prototypes 2, 4, 6, 10 and 16 and 8 field isolates from the same serotypes were isolated and propagated in Hamster lung and Bovine embryonic lung cells at the Kimron Veterinary Institute, Bet-Dagan, Israel. The only BTV 2 field isolate available was isolated from Culicoides using cell culture followed by two passages in embryonated chicken eggs. Three field isolates of BTV 4, 2 of BTV 6 and 1 of BTV 10 were isolated from goats and sheep.

dsRNA Extraction

The viral dsRNA from USA prototypes and field isolates and the Israeli BTV samples was extracted and processed as previously described (17). The two phenol extractions and the ethanol precipitations of the Israeli prototypes and field isolates were done at the Kimron Veterinary Institute. The samples were freighted to USA in this condition and the viral dsRNA was further purified by Lithium chloride precipitation.

Molecular cloning

Bluetongue virus 11 UC8 strain genome segments 5, 7, 9 and 10 were cloned into the SmaI site of the pUC13 plasmid.

Recombinant DNA probes

The purified plasmids were cut with EcoRI and BamHI restriction enzymes, electrophoresed on a 1% agarose gel and the inserts were obtained by electroelution. The cloned genes were labeled by a standard nick translation reaction using 50uCi of ^{32}P dCTP with a specific activity of $>5 \times 10^7$ cpm/ug.

Northern blotting

Five hundred nanograms of purified dsRNA from the USA and Israeli samples were electrophoretically fractionated in 10% polyacrylamide gels (39). After the electrophoresis, the gels were stained with ethidium bromide to determine, by visual comparison, that the samples were essentially in equimolar ratios (13). The electrophoresed dsRNA was denatured with 0.1N NaOH and transferred to a nylon membrane according to manufacturer's protocol (Zeta-Probe nylon membranes, BioRad, Richmond, California, USA).

Hybridization conditions

The membranes were prehybridized for 3 hours and hybridized as described (40). The cloned genetic probes labeled with ^{32}P dCTP, with a specific activity of $>5 \times 10^7$ cpm/ug and 5×10^6 cpm were used for each 14cmx18cm filter. The hybridization reaction were incubated for 16 hours at 42C with continuous shaking. The post hybridization washes were done as described (40). The membrane was autoradiograph at -70C for 16 hours.

RESULTS

Molecular cloning

Bluetongue virus 11 UC8 strain genome segments 5, 7, 9 and 10 were cloned into the SmaI site of pUC13 plasmid. The sizes of the obtained clones were first determined in 1% agarose gel electrophoresis using Lambda DNA/HindIII fragments and 1Kb DNA Ladder as molecular weight markers (41,42). The cloned genes were further characterized by sequencing of their 5' and 3' ends. The cloned BTV 11 UC8 genome segment 5 (S5), 7 (S7), 9 (S9) and 10 (S10) were found to represent a 95% copy (from position 82 to 1638), 47% copy (from position 612 to 1156), a 96% copy (from position 38 to 1046) and 98% copy (from position 1 to 806) of the respective full length genome segments (43-46) (Figs.: 1,2[A,B,C])

Hybridization of BTV 11 UC8 genome segment 5 probe with USA and Israeli BTV isolates

Our cloned genetic probe hybridized with USA BTV prototype 10 but not with USA prototypes 2, 11, 13, 17, the avirulent BTV 11 UC2 strain (Fig.: 1) and only with the Israeli prototype 10 (Fig.: 3). When the probe was hybridized to field isolates from USA serotypes it hybridized to 12 out of 14 BTV 10 isolates (Fig.: 4), 4 out of 17 BTV 11 samples (Fig.: 5) and with none of the BTV 13 (Fig.: 6) and BTV 17 (Fig.: 7). No hybridization was observed with the Israeli field isolates studied (Fig.: 3). Interestingly, the electrophoretic migration pattern of genome segment 5 of the samples that did hybridized to clone S5 was similar to that of BTV 11 UC8 strain and all the negative samples presented a faster migrating segment 5 (Figs.: 8,9).

Hybridization of UC8 clones S7, S9 and S10 with USA and Israeli BTV isolates

The radioactively labeled S7, S9 and S10 cloned genetic probes from BTV 11 UC8 strain were hybridized to Northern blotted viral dsRNA from the USA BTV and EHDV prototypes. The Three clones tested hybridized to all the USA prototypes at the stringency conditions used in this study. Clone S7 hybridized equally well with prototypes 11 and 17, showed a weaker hybridization signal with prototypes 2 and 10 and hybridized very poorly with BTV 13 (Fig.: 2A). The S9 clone showed the same degree of hybridization with the USA prototypes 10, 11, 13 and 17, but a lower level of hybridization with USA BTV 2 (Fig.: 2B). The cloned genetic probe S10 hybridized strongly with BTV prototype 2 and 10 and had a slightly lower hybridization signal with prototypes 11, 13 and 17 (Fig.: 2C). None of the three probes studied hybridized with either, the EHDV 1 prototype or the EHDV 2 field isolate.

Our clone S7 hybridized weakly to the BTV 2, 6 and 16 Israeli prototype strains, to one of BTV 2 field isolates and to all BTV 4 and BTV 6 Israeli field samples tested. The S7 probe showed a strong signal with one BTV 2 field isolate, the prototype strains of BTV 4 and BTV 10 and with the only BTV 10 sample available (Fig.: 10A).

The S9 clone hybridized to all the Israeli samples tested showing minor differences in the hybridization signal of one BTV 4 and one BTV 6 field isolates (Fig.: 10B). The S10 cDNA probe also recognized all the Israeli BTV samples tested exhibiting some differences in the degree of hybridization, and one BTV 4 isolate showed a weaker signal when compared with the rest (Fig.: 10C).

DISCUSSION

Genome segment 5 of BTV 11 strain UC8 was cloned and hybridized to the viral dsRNA extracted from USA and Israeli BTV prototypes and field isolates and their genetic relatedness was studied.

Previous reports about the genetic relationships among the 24 BTV serotypes, indicated that genome segment 5 from USA BTV 10 prototype strain is more closely related to USA BTV prototypes 11 and 17, and South African prototype 4 (13). Other studies about the genetic variation between strains from USA BTV 11, using a cDNA probe from genome segment 5 from USA BTV prototype 11 (Station strain), found that this probe hybridized weakly with the strains that showed a slow electrophoretic migrating genome segment 5, one of these being UC8 (10). Conversely, our clone from UC8 segment 5 did not hybridized with BTV 11 Station strain or other BTV 11 field isolates with a faster migrating segment 5 than UC8. The differences between our results and these previous studies may be attributed to differences in the BTV strains tested, in the amount of dsRNA Northern blotted and differences in the hybridization conditions employed, such as amount of radioactive probe used, temperature of hybridization and post hybridization washing conditions.

Our results indicated that genome segment 5 of BTV 11 UC8 strain is closely related to USA and Israeli BTV 10 prototype strains but not to the Israeli BTV 10 field isolate. We have confirmed that UC8 genome segment 2 belonged to serotype 11 by hybridizing it with a partial segment 2 clone from the prototype BTV 11 Station strain. This clone proved to be BTV 11 serotype specific by hybridization against 90 field isolates from the USA serotypes (paper in preparation). Our S5 clone has been sequenced and it showed a 98.76% homology with the previously reported sequence of BTV prototype 10 genome segment 5 (46). The results reported here demonstrated that a reassortment event occurred between a strain USA BTV 10 and USA BTV 11 that originated the BTV 11 UC8 strain. The occurrence of natural and experimentally produced reassortments between strains from different serotypes and strains from the same serotype from different parts of the world has been reported (26,27,47,48). The fact that S5 hybridized to the Israeli prototype BTV 10 without hybridizing to the Israeli BTV 10 field isolate and the different electrophoretic migration of genome segment 5 between these two strains suggested that the Israeli field isolate is genetically different than the prototype and most U.S. BTV 10 strains. This is the first evidence of the occurrence of natural reassortment events between Israeli BTV isolates.

United States BTV 11 UC8 strain genome segments 7, 9 and 10 have been cloned and hybridized to USA BTV prototypes 2, 10, 11, 13, 17 and Israeli BTV prototypes 2, 4, 6, 10 and 16 and field isolates from the

serotypes 2, 4, 6 and 10. The prototype strain of EHDV 1 and one field isolate from USA EHDV 2 were also studied.

The S7 clone cross-hybridized with all the USA BTV prototypes and Israeli BTV samples tested, showing a high degree of variation in the hybridization signals. Although a partial clone (45%) was used in this study, our results agree with previous published data that found BTV genome segment 7 to be least conserved among the segments coding for BTV inner capsid and nonstructural proteins (11-14). In the case of Israeli BTV serotype 2 samples, the prototype strain (Fig.: 10A, Lane B) and the field samples isolated directly from *Culicoides* (Fig.: 10A, Lane N) showed a weak hybridization signal. However, when this same BTV isolate was passaged twice through embryonated chicken eggs, a strong hybridization signal was obtained. This could reflect the fact that the genetic characteristics of a non plaque cloned BTV field isolate can be altered after successive passages in the embryonated chicken eggs or cell culture as it was previously described (49). It was also proposed that these variations could be the result of in vitro/in vivo mutations or genetic reassortment, multiple field BTV strains simultaneously infecting an animal, or combinations of these possibilities (49). The marked genetic diversity that characterizes the BTV serogroup is reflected in differences in the electropherotypes between and within the BTV serotypes. This electropherotypic variations has been described for BTV isolates from different parts of the world (33,50,51) and they are also present in the Israeli samples tested in this study as can be appreciated in the different migration patterns of genome segments 7, 9 and 10 illustrated in Fig.: 9. However a similar migration in polyacrylamide gels electrophoresis of equivalent genome segments does not necessarily reflect a similarity in their nucleotide sequences. The Israeli BTV 4 samples tested showed not only variations in their electrophoretic pattern, but also in the hybridization signal observed with our S7 clone. These findings could be the result of genetic drift, reassortment events and/or immunological pressure exerted on VP7 during a period of more than 40 years since the introduction of BTV 4 in Israel, as was previously postulated to occur with USA BTV 11 during a period of 12 years (52). The S7 cloned genetic probe appeared to be highly homologous to the Israeli BTV 10 prototype and the only field isolate from that serotype, but was more distantly related to BTV prototypes 6 and 16 and isolates of serotype 6.

The results presented here indicated that genome segment 9 is conserved between the USA and Israeli samples tested. Previous studies of the genetic relatedness of BTV genome segment 9 from different countries also showed this genome segment to be conserved among BTV serotypes (12,13). The S9 probe hybridized equally well with USA BTV prototypes 10, 11, 13 and 17 while exhibiting a weak hybridization signal with USA BTV 2. The hybridization pattern showed by the S9 probe with the 5 USA prototype strains was similar to that previously obtained using genome segment 9 of the USA prototype strain of BTV 11 (53). Interestingly, our S9 probe exhibited a strong signal with the Israeli BTV 2 samples indicating that USA BTV 2 genome segment 9 is not very closely related to the homologous genome segment of Israeli BTV 2. Our hybridization results with S9 showed that BTV genome

segment 9 from USA BTV 11 UC8 is more closely related to segment 9 of Israeli BTV 16, than genome segment 9 from USA and South African BTV 10 is to BTV 16 from Pakistan (12,13). As segment 9 from USA BTV 11 UC8 hybridized strongly with USA BTV 10, probably the differences in the hybridization with BTV 16 mentioned above, could be due to differences between the BTV 16 strains from Israel and Pakistan.

The genetic relatedness of S10 clone with the USA BTV prototypes and field isolates showed that this probe recognized a wide variety of BTV isolates, albeit variations in the hybridization signals were found among isolates of the same serotype (17). Similar differences in the hybridization signals with the Israeli samples were obtained indicating that S10 is also related to Israeli BTV isolates.

None of the BTV 11 derived tested showed cross-hybridization with EHDV prototype 1 and the EHDV 2 isolate.

In summary, the three cDNA probes recognized all the samples tested, genome segment 7 showed the highest degree of variation in the hybridization reaction and clones S9 and S10 are suitable candidates for BTV serogroup specific identification of Israeli BTV isolates.

17 13 11 10 2 UC2 UC8 C

Fig. 1: Hybridization of ^{32}P labeled clone S5 to Northern blotted viral dsRNA from USA BTV prototypes and BTV 11 strains. C: negative control. UC2 and UC8: BTV 11 strains. 2, 10, 11, 13 and 17: USA BTV prototype strains. The position of the 10 dsRNA segments of BTV 11 UC8 strain are indicated at the left as point of reference.

Fig. 2: Hybridization of USA BTV and EHDV 1 prototypes and EHDV 2 field isolate with (A): BTV 11-UC8 S7; (B): BTV 11-UC8 S9; (C): BTV 11-UC8 S10. C: Uninfected negative VERO cell control. U8: BTV 11-UC8 strain. The numbers: 2, 10, 11, 13 and 17 indicate the USA BTV prototypes. E1: EHDV prototype. E2: EHDV 2 field isolate.

ISRAELI PROTOTYPES AND FIELD ISOLATES

PROBE: BTV 11-UC8 GENOME SEGMENT 5

Fig. 3: Hybridization of ^{32}P labeled clone S5 to Northern blotted viral dsRNA from Israeli prototypes and field isolates. A: BTV 11-UC8 strain. B: BTV prototype 2. C: BTV prototype 6. D: BTV prototype 10. E and H: negative cell controls. F: BTV 4. G: BTV prototype 16. I, J and M: BTV 4 field isolates. K and L: BTV 6 field isolates. N and O: BTV 2 field isolates. P: BTV 10 field isolate. The position of the 10 dsRNA segments of BTV 11 UC8 strain are indicated at the right as a point of reference.

BTV 10 US FIELD ISOLATES

Fig. 4: Hybridization of ^{32}P labeled clone S5 to Northern blotted viral dsRNA from USA BTV 10 field isolates. A: BTV 11-UC8 strain. B: BTV prototype 10. C to P: USA BTV 10 field isolates. The position of the 10 dsRNA segments of BTV 11 UC8 strain are indicated at the right as a point of reference.

BTV 11 US FIELD ISOLATES

PROBE: BTV 11-UC8 GENOME SEGMENT 5

Fig. 5: Hybridization of ^{332}P labeled clone S5 to Northern blotted viral dsRNA from USA BTV 11 field isolates. A; BTV 11-UC8 strain. B: USA BTV prototype 11. C to S: USA BTV 11 field isolates. The position of the 10 dsRNA segments of BTV 11 UC8 strain are indicated at the right as a point of reference.

BTB 13 US FIELD ISOLATES

Fig. 6: Hybridization of ^{32}P labeled clone S5 to Northern blotted viral dsRNA from USA BTB 13 field isolates. A: BTB 11-UC8. B: USA BTB prototype 13. C to S: USA BTB 13 field isolates. The position of the 10 dsRNA segments of BTB 11-UC8 strain are indicated at the right as a point of reference.

PROBE: BTV 11-UC8 GENOME SEGMENT 5

Fig. 7: Hybridization of ^{32}P labeled clone S5 to Northern blotted viral dsRNA from USA BTV 17 isolates. A: BTV 11-UC8. B: USA BTV prototype 17. C to S: USA BTV 17 field isolates. The position of the 10 dsRNA segments of BTV 11-UC8 strain are indicated at the right as a point of reference.

Fig. 8: Electropherotypes of USA BTV prototypes and examples of USA BTV field isolates that hybridized and did not hybridized to clone S5. 2, 10, 11, 13 and 17: BTV prototypes. UC2 and UC8 strains. A and C: BTV 10 field isolates. B and D: BTV 11 field isolates. E: BTV 13 field isolate. F: BTV 17 field isolate. Lanes 10 to B: samples that hybridized with clone S5. Lanes 2 to F: samples that did not hybridized with clone S5.

ISRAELI PROTOTYPES AND FIELD ISOLATES

Fig. 9: Electropherotypes of Israeli BTV prototypes and field isolates. C: negative cell controls. UC8: USA BTV 11-UC8 strain. 2, 4, 10 and 16: BTV prototype strains. F2: BTV 2 field isolates. F4: BTV 4 field isolates. F6: BTV 6 field isolates. F10: BTV 10 field isolate. Only Lane 10 showed hybridization with clone S5.

Fig. 10: Hybridization of Israeli prototypes and field isolates with (A): BTV 11-UC8 S7; (B): BTV 11-UC8 S9; (C): BTV 11-UC8 S10. A: BTV 11-UC8 strain. B: BTV prototype 2. C: BTV prototype 6. D: BTV prototype 10. E: Uninfected Hamster lung cells. F: BTV prototype 4. G: BTV prototype 16. H: Uninfected Bovine embryonic cells. I, J and M: BTV 4 field isolates. K and L: BTV 6 field isolates. N and O: BTV 2 field isolates. P: BTV 10 field isolate.

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DESCRIPTION OF COOPERATION

This proposal was executed by the following parties:

a. Dr. Bennie I. Osburn
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b. Dr. Marius Ianconescu
Dept. of Virology
Kimron Veterinary Institute
Bet-Dagan, Israel

The laboratory at the University of California, Davis (UCD), under the direction of Dr. Osburn, was responsible for development, characterization and supply of the genetic probes to be employed in the aforementioned procedures. The techniques for genetic cloning and nucleic acid hybridization have been adapted successfully for the study of BTV. This laboratory has a well-developed background and many resources for the study of BTV. The initial cross-hybridization assays and the cloning procedures were executed at the UCD laboratory. Genetic probes and protocols were developed at UCD and transferred to the collaborating laboratory in Israel.

The laboratory at the Kimron Veterinary Institute, was responsible for acquiring domestic viral specimens and isolating nucleic acids components from the sample. This phase familiarized the personnel with the basic procedures for processing nucleic acid specimens. Nucleic acid hybridization technology was established at UCD for the study of BTV. Necessary equipment and supplies for nucleic acid handling and hybridization assays were defined and acquired. Genetic probes and hybridization protocols were acquired from the collaborating USA laboratory. Hybridization techniques were demonstrated on site by personnel from UCD. The screening of genetic probes, developed under this project and acquired from the collaborating USA laboratory, are being executed at the Kimron Veterinary Institute against domestic BTV isolates.

EVALUATION

The main objectives proposed in this project have been successfully achieved. The feasibility of using cDNA cloned genetic probes developed from USA BTV isolates in the rapid identification of Israeli BTV samples by hybridization techniques has been demonstrated.

The developed recombinant probes were evaluated as diagnostic reagents against Israeli and USA BTV isolates, establishing that there is a high degree of group specific genetic homology, in some BTV genome segments, between BTV isolates from USA and Israel. These renders them excellent candidates for BTV group specific

identification by hybridization techniques and may serve as base for the future development of inexpensive group specific antigenic reagents for serologic tests.

During the development of this proposal the cooperating laboratory at the University of California, Davis was able to improve its knowledge on the practical application of nucleic acid hybridization techniques for the diagnosis of viral diseases, specifically BTV, extending its diagnostic capabilities to the recognition of BTV isolates that have not yet been identified in the USA. The Kimron Veterinary Institute was able to establish a special laboratory and train personnel to apply and manipulate the techniques of nucleic acid hybridization for diagnosis of BTV.

The easy manipulation and inexpensive production of these cDNA probes and the easy adaptation and standardization of the hybridization protocols make possible the rapid diagnosis of BTV infection and may serve as base for the development of more sensitive and efficient diagnostic methods such as the polymerase chain reaction (PCR), in the certification of animals and germ plasm free of BTV for export. This will greatly contribute to the improvement of the commercial trade between countries with the consequent economic advantages for them.

LIST OF PUBLICATIONS

1. de Mattos CA, de Mattos CC, Dangler CA, Osburn BI, Ianconescu M, Kaufman R. Recombinant cDNA probes from United States bluetongue virus 11-UC8 for serogroup identification of Israeli strains of bluetongue virus. Archives of Virol 1990 (Submitted).
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