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Expression of bluetongue virus rVP7 protein in prokaryotic expression system and evaluation of crude lysate for Dot-ELISA

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Abstract
Bluetongue (BT) is an arthropod-borne infectious, non-contagious viral disease of domestic and wild ruminants caused by the *Bluetongue virus* (BTV), which belongs to the genus *Orbivirus* and family *Reoviridae.* The diagnosis of Bluetongue, is based on serological testing as well as molecular techniques. The group specific VP7 protein is important for the detection of anti-BTV antibodies. Considering the recombinant VP7 ability for the detection of BTV antibodies, the study was planned to express the BTV partial VP7 gene in prokaryotic system and use it for detection of the anti-BTV antibodies. In the present study pET-32-a-BTV-trc.-VP7 recombinant clone in BL-21(DE3) was confirmed by the PCR. The recombinant clone was induced with 1mM IPTG at 30 °C at 220 rpm and rVP7 followed by the expression was confirmed by the 12.5% SDS-PAGE. The rVP7 protein was purified using denatured condition and affinity chromatography techniques and analyzed by SDS-PAGE analysis. The PCR revealed expected product of around 550bp. The expressed protein was extracted by sonication and freeze -thawed methods in denaturing lysis buffer, did not showed any rVP7 protein in supernatant, the rVP7 was found in the pellet might be due to the inclusion bodies. As the recombinant protein was expressed but remained in the pellet, therefore further attempts were made to use the crude lysate for the detection of antibodies in pre adsorbed serum with negative antigen against BTV using the Dot- ELISA. The Dot-ELISA revealed that all the positive serum samples were found to be positive, whereas the Bl-21 lysate negative antigen was very faintly visible, the positive sample can be easily differentiated based on the colour intensity. The four negative serum samples tested did not showed any positivity. Hence, the present study concludes that the Dot ELISA platform based on the crude lysate of rVP7 expressing clone can be used for the detection of the anti-BTV antibodies with additional steps of pre-adsorption of testing sera with crude lysate of the negative antigen (Bl- 21 *E. coli* cells).

Keywords: Bluetongue virus, prokaryotic expression, Dot- ELISA, recombinant protein

1. Introduction

Bluetongue (BT) is an arthropod-borne infectious, non-contagious viral disease of domestic and wild ruminants caused by the *Bluetongue virus* (BTV*)*, which belongs to the genus *Orbivirus* and family *Reoviridae* (Mertens *et al.* 1989 [1]; Patel and Roy, 2014 [2]. The *Bluetongue virus* spread naturally to susceptible hosts by the bite of blood sucking midges of genus *Culicoides* and family Ceratopogonidae (Ander *et al.* 2012; Benelli *et al.* 2017 [3, 4]. Bluetongue outbreaks are seasonal, occurring in the late summer and early fall. *Bluetongue virus* outbreaks occur in tropical, subtropical, and temperate regions around the world, anywhere a suitable vector population exists for virus transmission (Wilson and Mellor, 2009; Maheshwari *et al.*, 2012 [5, 6]. Domestic (sheep, goats and cattle) and wild (deer, pronghorn antelope and bighorn sheep) ruminants, camelids, elephants and wild carnivores are commonly affected by *Bluetongue virus* (Robinson *et al.* 1967; Alexander *et al.* 1994; Brown *et al.* 1996 [7, 8, 9]. Severe clinical disease is most commonly seen in sheep, whereas cattle, goats, and Camelids, have asymptomatic or subclinical illness (Backx *et al.* 2007; MacLachlan *et al.*, 2009 [10, 11]. Fever, oral erosions and ulcers, cyanosis of the tongue, lameness with Coronitis, serous to bloody nasal discharge, later Mucopurulent, Hyperaemia and Oedema of the lips, face, ears, and sub-maxillary region ('monkey-face' appearance), repiratory distress and muscular necrosis are all clinical signs of BT, which progress to debility and death (MacLachlan *et al.*, 1994, 2004; Backx *et al.* 2007 [12, 13, 10]. Bluetongue is currently rampant in India. India has reported 23 Bluetongue Virus serotypes (except 22-25-28) out of the total of 28 based on the presence of neutralizing antibodies and virus isolation (VI).

Virus isolation has discovered fifteen serotypes (BTV- 1-6, 9, 10, 12, 16-18, 21, 23, and 24), whereas, serological testing has identified 22 serotypes (BTV-1-20, 23 and 24) (Prasad *et al.* 1992; Sreenivasulu *et al.* 2004; Joardar *et al.,* 2009; Chauhan *et al.*, 2014; Ranjan *et al.* 2015) [14-18].
Bluetongue virus is a non-enveloped virus with a triple-layered icosahedral capsid and a ten-segmented double-stranded RNA (dsRNA) genome (Grimes *et al.*, 1998; Ratinier *et al.*, 2011 [19, 20]. The BTV genome codes for seven structural proteins (VP1-VP7) and five non- structural proteins (NS1-NS5), (Mertens and Diprose, 2004; Ratinier *et al.* 2011 [21, 20]. Major group-specific antigen determinant is the core VP7 protein of *Bluetongue virus*. The VP7 protein is found on the surface of core particles and is encoded by short RNA segment 7 (S7). The VP7 protein has molecular weight of 38.58 kDa and is found on the surface of core particles (Yu *et al.,* 1988; Oldfield *et al.,* 1990 [22, 23]. The VP7 protein is a key serogroup factor that separates the various phylogenetic groups (Ranjan *et al.* 2015 [18]. It is conserved throughout all BTV serotypes sequenced (Kowalik and Li *et al.*, 1991 [24]. Due to its highly conserved sequence and antigenicity across all serotypes, the *Bluetongue virus* VP7 protein is a favored choice for designing group-specific serological tests (Kowalik and Li *et al.,* 1991 [24]. To detect *Bluetongue Virus* group-specific antibodies, a number of enzyme immunoassays have been developed, which use cell-associated viral antigen or partially purified virus antigen (Afsar *et al.*, 1987; Drolet *et al.,* 1990 [25, 26]. The source of antigen in these antibody-detection tests is a concentrated viral stock. The purification of a similar amount of virus or its components takes much longer time, more difficult, and expensive than recombinant antigen (Wade-Evans *et al.*, 1993 [27]. The recombinant antigens should be used in ELISA assays for these reasons. Furthermore, recombinant antigens are stable with low batch-to-batch fluctuation and lack infectivity, making them ideal reagents for a wide range of ELISA kit applications.
The *Bluetongue virus* VP7 Protein has previously been expressed in yeast or *Baculovirus*. *Bluetongue virus* antibodies can be detected using both competitive and dot ELISA (Martyn *et al.*, 1990; Naresh and Prasad, 1995 [28 29]. It's also been expressed in vaccinia virus and used as a capture ELISA antigen (Cloete *et al.*, 1994 [30]. The expression of viral proteins in the baculovirus system is a commonly used approach that involves insect cell culture and is ideal for large-scale recombinant protein production (Yamakawa and Furuuchi, 2001 [31], However, insect cell cultivation is laborious and expensive, whereas producing large amounts of pure recombinant protein in a prokaryotic system is less expensive and easier. Hence, the study was planned to express the partial VP7 gene in prokaryotic expression system and use this expressed recombinant VP7 protein for detection of anti-BTV antibody.

2. Materials and Methods

2.1 Revival of the pET-32a-BTV- trc-VP7 clone and confirmation of BTV VP7 gene by Polymerase Chain Reaction

The recombinant clone BL-21 of pET-32-a-BTV- trc-VP7 Clone was created in previous study was revived from the -20 ºC by streaking onto LBAmp plates. The plates were incubated at 37 °C for approximately 16 hrs. Then the single colony obtained was inoculated in LB amp broth (Ampicillin @ 50 microgram /ml). From the overnight grown culture 100 ul of the culture was centrifuged at 5000 rpm for 5 min at table top centrifuge machine. The supernatant was discarded and the pellet was once washed with milliQ water and re-centrifuged again and the supernatant was discarded. To the pellet 100 microliter of MilliQ water was added and content was transferred to PCR tube and incubated in PCR Machine (Trio, PCR Machine, Himedia, India) at 95 0C for 4 minutes. The PCR was carried out using published primers (Pathak *et al.*, 2008 [32] and reaction was set as 25 µl 2X Master mix (Cat # M7128, GoTaq Green Mastermix Promega, USA) reagent, 5 µl of DNA (Supernatant from the above tube), and 2µl for each forward nstVP7 for primer (20pmol) and Rev nstVP7 primers (20pmol) and 16 µl Nuclease free water. The reaction was carried out in cyclic conditions as 95 0C for 2 min for initial denaturation followed by thirty five cycles as 95 0C for 30 sec (denaturation), 58 °C for 1 minutes (Annealing) and 72 °C for 1 minutes. The final extension at 72 0C for 10 minutes was incorporated followed by the hold at 40C. The amplicon of PCR was confirmed by tby agarose gel electrophoresis (AGE) using 1.0% agarose containing 0.5 ug/ml of ethidium bromide in 0.5X Tris-boret –EDTA buffer (TBE). The amplicons were visualized using the transiluminator and photographed in gel documentation system (Gel Documentation system, ProCCD 116, Biozen, Agra, India).

2.2 Expression and purification of rVP7 protein by Ni NTA batch chromatography and confirmation by SDS-PAGE

From the confirmed clones, recombinant clone harbouring pET-32-a-BTV- trc-VP7 plasmid was inoculated in 3ml LB Amp (@ 50 µg/ ml) for overnight at 37 °C and 220 rpm. From the overnight culture 500µl was inoculated into 50 ml of LBAmp broth in 1 lit flask and incubated at 37 °C and 220 rpm until the optical density (OD) reached 0.40-0.60. (At mid exponential phase) measured by Smart Spec Tm plus (Bio-Rad, USA). From the culture 1ml of the bacteria was removed and centrifuged, supernatant was discarded and pellet was saved at -80 °C. The culture was induced with a final concentration of 1mM IPTG and incubated in orbital shaker incubator at 37 °C and 220 rpm for 8 hrs. The induced bacterial 1ml at 6 hrs post induction at 8 hrs post induction suspensions were removed from the culture and centrifuged for two minutes at 10000 rpm. The pellets were kept, while the supernatants were discarded. The pellet was kept at -80 °C. AT 8 hrs post induction, the remaining culture was collected and centrifuged and pellet was saved.

2.2.1 Extraction of rVP7 expressed protein

The bacterial pellet was resuspended in 400 µl of binding buffer (6M urea, 10mM sodium dihydrogen phosphate, 10mM di-sodium hydrogen phosphate and 20mM Imidazole and 300mM sodium chloride) followed by six freeze-thaw cycles were performed on the bacterial pellet and exposed to three cycles of sonication with a 60 second break in between each cycle, at a 20 µm amplitude, for 10 second pulses each in an ice bath. The lysate was centrifuged at 10000 g for 10 minutes at 4 °C. The pellet was also kept together with the properly collected supernatant using Ni-NTA chromatography, the supernatant was subsequently was used for further purification.

2.2.2 Purification of BTV-VP7 protein affinity chromatography

A 300 µl Ni-NTA agarose beads (His-PurTm Ni-NTA Superflow agarose cat # 25214, Thermo Scientific, USA)

slurry was transferred into a 1.5 ml micro centrifuge tube for purification and centrifuged for 700 g for two minutes, and supernatant was carefully removed. The beads were equilibrated with 600 µl of equilibration buffer (20mM sodium phosphate, 300mM sodium chloride, 10mM imidazole pH 7.4). To the equilibrated Ni-NTA agarose beads pellet the cell supernatant were added mixed well by slowly in rocking motion in the hybridization oven for 45 minutes. The mixture was centrifuged at 700 g for two minutes. The supernatant was collected and stored at -20 °C as flow through (f1). The beads were washed thrice with 600 µl of washing buffer (10mM sodium dihydrogen phosphate, 10mM di-sodium hydrogen phosphate and 20mM Imidazole and 300mM sodium chloride and each time the supernatant was collected (wash 1, 2 and 3) and kept at -20 °C. To the washed beads, to elute the recombinant protein125 µl elution buffer was added and thoroughly mixed and kept in hybridization oven 37 °C for 10 min. The tube was centrifuged at 700 g for 2 minutes, and the eluted protein (E1) was collected in a micro centrifuge tube reextraction was done once again and eluted protein was kept at -80 °C. The BL-21 lysate, crude VP7 lysate were quantified using the standard Biuret test and reading were taken using the SmartSpecTm plus (Bio-Rad, USA) spectrophotometer.

2.2.3 Confirmation of Purified rVP7 protein by SDS-PAGE

The expression analysis of the purified rVP7 recombinant protein were confirmed by SDS-PAGE. The gel was analysed for Uninduced, induced flow through, washes, elutes were prepared concurrently for SDS-PAGE examination. The 10µl of each sample including dye were added to the corresponding wells. In addition, pre-stained protein marker (cat # MBT092-10LN Hi-media, India) was added. The gel was run until the dye reached the gel's bottom with at a steady voltages of 80V for 1.5–2 hours at room temperature. The gel was stained with Coomassie brilliant blue staining dyes as per standard protocol and documented.

2.2.4 Dot ELISA using the crude lysate of recombinant rVP7 expressing clone

The Dot ELISA was carried out as per Afshar *et al.*, (1987(33] with some modifications in steps. The PVDF membrane was cut into small strips and labelled properly, onto which 3µl (30µg of protein) of the Crude lysate of pET- 32a-BTV-trc-VP7 BL-21(DE3) (*E. coli*) cells (recombinant clone) was added as drop, similarly 3µl (30µg of protein) of the Crude lysate of BL-21(DE3) (*E. coli*) cells was also added as drop approximately (µg of protein). The loaded PVDF membrane strips were air dried for 30 min. After air drying the membranes was blocked with 3% skim milk powder in PBST (PBS+ 0.1% tween 20) and incubated for 1hrs in rockers of hybridization oven. The strips were washed once with PBST for 3 min and rinsed it. Meanwhile, 5µl of BL-21 cell lysate (approx. 300 µg) was taken into labeled tubes and in each tube 20µl of the clinical positive serum was added in respective tubes keeping separate tube for the known positive serum from the IVRI, Mukteshwar supplied Kit. This mixtures were incubated for 30 min at 37 °C. After incubation 975µl of the blocking buffer was added thus the serum was used at 1:50 dilution. The strips were after blocking and washed were transferred to the relabeled serum diluted micro centrifuge tubes and were incubated for 1 hrs. At 37 °C in hybridization oven rocker. After one hour the antibody were removed and three washing with PBST were given. The anti-sheep HRP conjugate 1:2000 in blocking buffer was added and incubated for 1 hrs. At 37 °C in hybridization oven. Then anti-sheep HRP conjugate suspension was removed and the strips were washed thrice with PBST and developer solution (3mg DAB in 5ml PBST and 5 µl H2O2. was added and incubated till the dot appeared (5-15 min). Then the strips were rinsed with milliQ water to stop the reaction for excess colour formation. The strips were dried and visually inspected for the brown colour development and documented.

3. Results and Discussion

In the present study BTV structural VP7 gene clone in prokaryotic system (pET-32a-BTV trc-VP7 clone in BL-23 (DE3) cells) created and available in virology laboratory of the Department of Veterinary Microbiology and Animal Biotechnology T & RC was revived showed as white colonies on LB Amp plate. The different colonies of clones were confirmed by polymerase chain reaction using the published primers targeting the region of 390 to 939 with expected product of around 550 bp. The PCR amplicons obtained, were analysed by agarose gel electrophoresis revealed a specific product around 550bp as depicted in the figure 1. The same primers have been employed in previous study also showed the expected amplicon of 550bp (Pathak *et al.*, 2008(32]. The PCR conducted on the clone under study confirms the presence of the structural vp7 gene in pET-32a-BTV trc-VP7 clone in BL-23 (DE3) cells. So the further studies can be carried out with confidence to having a colony with our gene of interest in the vector.

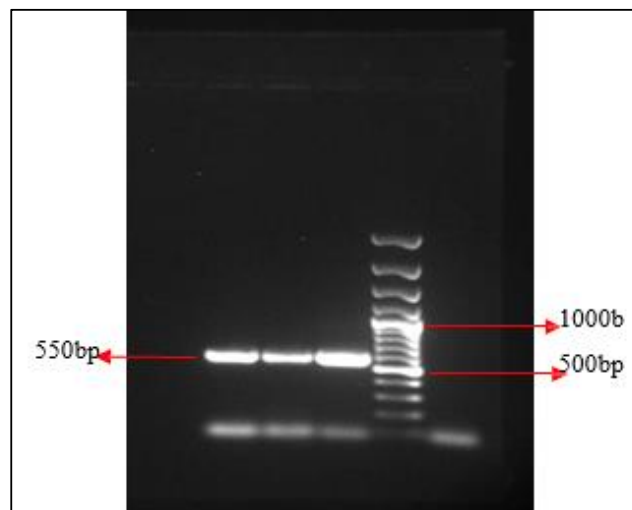

Fig 1: Confirmation of PCR amplicons of truncated VP7 gene insert in pET32a-BTV-VP7 in BL

The expression of vp7 gene in pET-32a-BTV trc-VP7 clone in BL-23 (DE3) was induced with 1mM IPTG at 30 degree celsius at 220 rpm in the orbital shaker incubator and induced cultures were collected at 6 and 8 hr and pelleted on SDS - PAGE analysis showed an expected band in the range of 36 kDa which was compared with the pre-stained protein marker as depicted in figure. 2. In the present study the expression of the VP7 at 6 and 8 hrs did not showed significant increased as per time frame (Fig. 2). Similarly, to induce the expression of the recombinant protein various researchers also used the varying degree of IPTG and different temperature. In the similar kind of work a BTV 23 Dehradun isolate truncated VP7 gene was cloned and expressed in the pET-32-a in

prokaryotic systems and was induced with IPTG showed an increased expression from 3 h, reached the peak at about 9 h and declined (Pathak *et al.*, 2008(32[]. The expression of the recombinant protein with increased induction time, an increase in the amount of VP7 was observed from 2 hours to 5 hours but from 5 to 16 hours, the amount of VP7 expressed did not increase and only number of other cellular proteins that pelleted along with BTV VP7 increased (Russell and Gildenhuys, 2018 [34]. In another study. it has been indicated that after induction with 1.0 mM IPTG at 370 C for 4h, the recombinant protein pET-28a/VP7 was expressed at a high level in the cells as inclusion bodies and the recombinant protein pET-28a/V7 were purified from their inclusion bodies by gel cutting purification (Wu *et al.*, 2015 [35].

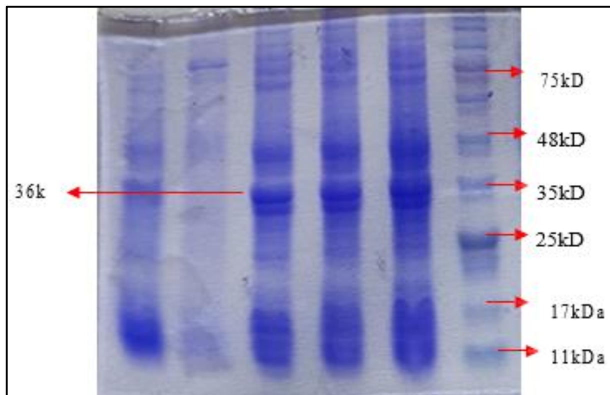

Fig 2: SDS-PAGE analysis of induced pET32a-BTV- VP7 clone showing expression of rVP7 protein

Lane 1:-pET32a-BTV- VP7 Uninduced; Lane 2:- BL-21 Cells lysate; Lane 3:-pET32a-BTV- VP7 induced 6hrs (5 µl); Lane 4:-pET32a-BTV- VP7 induced 8hrs (5 µl); Lane 5:-pET32a-BTV- VP7 induced 6hrs (7 µl); Lane 6:- Prestain Protein Ladder (Cat no MTB092 HiGenoMB, Himedia, India).

The bulk induced clone was purified using the denaturation condition using His tag- Ni-NTA agarose bead based affinity chromatography. Different fractions of protein obtained were analysed using SDS PAGE. Supernatant fraction did not showed expected protein but the recombinant VP7 was found in pellet (Fig-3).

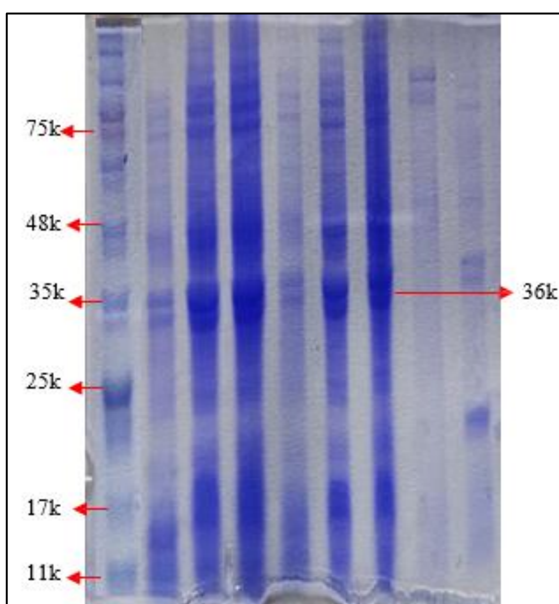

Fig 3: SDS PAGE analysis of purified protein by Ni

3.1 NTA Affinity chromatography

Lane 1:Pre-stained Protein Ladder (Cat no MTB092 HiGeno Hi-media, India); Lane 2: pET32a-BTV- VP7 recombinant bacterial cells uninduce clone; Lane 3: pET32a-BTV- VP7 recombinant bacterial cells induced- 6hrs; Lane 4: pET32a-BTV-VP7 recombinant bacterial cells crude Lysate; Lane 5: Recombinant Bacterial cells Supernatant After Freeze Thaw and Sonication; Lane 6: pET32a-BTV-VP7 recombinant bacterial cell Pellet I; Lane 7: pET32a-BTV-VP7 recombinant bacterial cells Pellet II; Lane 8: Purified BTV-rVP7 protein Elute I; Lane 9: Wash 1 after binding at rVP7 to Ni-NTA resin.

In the present study, the induction temperature was lower upto 30 degree celsius with intention to express protein in soluble form, but could not get the protein in the soluble form. The rVP7 protein might have been overexpressed and form inclusion bodies and which were unable to solublized in the current condition employed under present study. The other researchers have also shown the difficulty of the purification of recombinant VP7 in prokaryotic system (Reddy *et al.,* 2006; Wang *et al.,* 1994 [36, 37]. It has also been found that the high level of expression could result in the formation of insoluble protein (Wu *et al.,* 2015 [35]. It has also been reported that, lowering the temperature for cell growth can produce a recombinant protein in soluble fractions (Schein, 1989 [38]. In the present study the protein have been attempted to express at low temperature but unable to get the protein in supernatant. However, some of the researchers were able to express the the same size protein and using the denaturing conditions able to get the solublaized fraction of rVP7 (Pathak *et al.,* 2008, Venkatesh *et al.,* 2015 [32, 39]. In another study the induction 0.1mM IPTG at 37 degree celsius for 5 hrs and solubilization using urea, Triton-X and subsequent nickel chromatography resulted into the solubalization of inclusion bodies (Russell *et al.*, 2018 [34]. However, the recombinant VP7 protein expressed in different systems has shown the variable expression level and purification. Expression of full-length VP7, was achieved in insect cells and purified by affinity chromatography (Hosamani *et al.* 2011 [40]. BTV-VP7, has been expressed at high level in S. frugiperda cells using a recombinant baculovirus (Oldfield *et al.,* 1990 [23]. In another study, the recombinant capripox virus expressing the BTV genes has shown its suitability for immunization (Perrin *et al.,* 2007, Wade Evans *et al.,* 1996 [41, 42]. The baculovirus have been also used for the expression of BTV genes and formation of core like particles (Loudon and Roy, 1991 [43]. The VP7 gene was expressed in the yeast expression system and purification using sonication was carried out and it had shown good reactivity in ELISA (Martyn *et al.,* 1990 [28].

As the expressed protein was found to be in the pellet, with several attempts of modification of conditions for denaturation and purification but unable to get in supernatant. As a very scanty rVP7 in supernatant and not in the elute, hence, the attempts was made to use the crude lysate for detection of anti-BTV antibody by developing a platform of Dot-ELISA. The previously positive tested clinical serum samples confirmed to be positive by ELISA to have anti-BTV antibodies and known negative serum were used for Dot-ELISA. The crude protein of expressed rVP7 BL-21 DE3 cells and crude proteins of BL- 21 (without vector) were prepared using freeze- thaw and sonication. In the present study, rVP7 crude lysate (un- purified) is being attempted for detection of anti-BTV antibody detection, to avoid the reactivity with *E. coli* antigens an additional pre-adsorption of

antibodies to lysate of BL-21 cells was carried out. Out of the total 13 serum samples attempted for detection the anti-BTV antibody, 9 serum samples showed an intense dot of brown colour at rVP7 placed dot, whereas as at BL-21 crude lysate very very faint dot was visible. The colour intensity between rVP7 dot and negative control (BL-21 cell lysate) was highly differentiating. The result of the Dot-ELISA are depicted in figure 4. All negative samples tested were found negative. Thus all serum samples tested by Dot-ELISA were shown 100% positivity result with previously confirmed positive and negative serum samples. In the current study where known positive and known negative serum samples were tested with pre-adsorption of them with BL-21 crude lysate preparation indicated that the rVP7 in crude preparation can be use for the detection but with caution to have differentiating visibility in negative and positive dots.

The Dot-ELISA can be further optimized with the purified antigen. The rVP7 protein have been used by several researchers for the detection of the anti-BTV antibodies. In another study detection of anti-BTV antibodies in cattle and sheep serum was described utilising a modified solid phase blocking enzyme immunosorbent assay (ELISA) using a monoclonal antibody (Mab) against the group specific *Bluetongue virus* (BTV) antigen. The b-dot-ELISA test offers potential use as a quick, easy, and affordable diagnostic tool for seroepidemiological research unrelated to bluetongue (Afshar *et al.*, 1987) [33]. In another study, the ability of DIA and other test was evaluated, it showed that DIA detected BTV antibodies as early as 7 dpi in four animals while in three other animals DIA could detect antibodies only after 21 dpi (Gupta *et al.*, 1990 [44]. A blocking dot enzyme- linked immunosorbent assay, using a monoclonal antibody against a group specific antigen of *Bluetongue virus* for its application for testing of several hundreds of bovine field samples similar specificity found for the dipstick b-dot ELISA compared with that of the nc strip b-dot ELISA when both tests are compared with the c- ELISA (Afshar *et al.,* 1991 [45]. In another study, ELISA and DIA were tried to ascertain the suitability of these tests in the serodiagnosis of bluetongue disease, the relative specificity and sensitivity between ELISA and DIA were 75%, 87.6% and 100%, respectively (Chander *et al.*, 1991 [46]. For the detection of antibodies against BTV lateral flow assays was carried out using specific antibody (IgG) bound to the VP7 protein and formed a red band at the test region.

Sr. 1: Hyper immune sera against BTV- 23 Dehradun isolate. (Positive control); Sr. 2-9: Positive clinical serum samples;
Sr. 10: Known negative Serum (Negative control); 11-13: Negative serum samples

Fig 4: DoT-ELISA showing Anti BTV antibody detection using rVP7 protein

Thus the ICS developed is suitable for routine diagnostic testing and has the potential to replace other tests for the detection of anti-BTV group-specific antibodies, when it becomes commercially available in the future (Yang *et al.*, 2010 [47]. The BTV VP3 expressed in the prokaryotic system has found to be suitable for the detection of antibody in Dot-ELISA (Wang *et al.,* 2020 [48]. The recombinant protein has shown its ability to be use for the diagnosis of anti-BTV antibodies in serum samples by ELISA and lateral flow (Pathak *et al.,* 2008 [32], Venkatesh *et al.,* 2015 [39], Chand *et al.,* 2021 [49]. In the present study, the rVP7 was expressed in prokaryotic system and a Dot ELISA platform based on crude lysate was employed to confirm the utility of rVP7 protein for detection of anti-BTV antibodies. Based on the results obtained, the present study concludes that the Dot ELISA platform based on the crude lysate of rVP7 expressing bacterial clone can be used for the detection of the anti-BTV antibodies with additional steps of pre- adsorption of testing sera with crude lysate of the negative antigen (Bl-21 *E. coli* cells).

4. Conclusions

The present study has shown the feasibility of Dot-ELISA based on crude lysate of Bluetongue virus recombinant VP7 protein expressed in the prokaryotic system for detection of antibodies against blue tongue disease.

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