Preparation of latex agglutination reagents for rapid detection of viral antigen of bovine viral diarrhea in field samples.

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Abstract

BVDV antiserum was sensitized with latex particles for detection of BVDV antigen in field samples. A total of 50 samples (20 serum, 17 nasal swabs and 13 buccal swabs) were collected from animals suffered from respiratory manifestation and examined by prepared latex. The results showed that the positive percents in serum, nasal and buccal swabs were (40%, 11.8% and 30.8% respectively). Comparison between results of two tests (latex agglutination and Fluorescent antibody technique) for detection of BVDV antigen in collected samples showed that the percentage of positive reactors obtained by Fluorescent antibody technique in serum, nasal were (45%% and 17.6% respectively) but it was the same percentage in buccal swabs (30.7%).This means that Fluorescent antibody technique is more sensitive than latex agglutination while latex agglutination is more rapid and simple than Fluorescent antibody technique.

Key words: *BVDV , buccal swabs, Fluorescent antibody technique, latex agglutination .*

Introduction

Bovine viral diarrhea is a viral disease caused by BVDV which is a pestivirus in the family Flaviviridae. It is closely related to classical swine fever and ovine Border disease viruses. BVDV occurs in two forms, non cytopathogenic and cytopathogenic. There are two antigenically distinct genotypes (types 1 and2), and virus isolates within these groups exhibit considerable biological and antigenic diversity **(Belknap *et al.,* 2000).**

BVD is manifested clinically by diarrhea, respiratory affection, oral lesions, ocular symptoms and abortion (**Carman *et al.,* 2005)**. Severity of clinical affection depended upon multiple factors including immune status of animals, pregnancy state, strains of virus and environmental stress. BVD infection raises the incidence of retained placenta, silent heat and mastitis **(Goyal *et al.,* 2002).**

Initially BVDV was discovered in North America from cattle with gastroenteritis, diarrhea, and abortion **(Pellerin *et al.,* 1994),** but later it has also been isolated from cattle in many countries like. Germany, Belgium, in most central European countries, North America and Japan **(Mahony *et al.,* 2005).**

In Egypt the disease was firstly diagnosed by **(Hafez, 1972)** and the virus was isolated from animals suffered from pneumoenteritis **(Baz, 1975).** Persistently viraemic healthy animals resulting from congenital infection can be readily identified by isolation of non cytopathogenic virus in cell cultures from blood or serum.

The latex immuno-agglutination assay is a test used to detect the presence of antibody or antigen in a variety of body fluids including urine, saliva, blood, or cerebrospinal fluid. Antibodies to a given target antigen or antigens to a given target antibody are passively adsorbed or covalently bonded onto the surface of latex particles. These particles are then mixed with the sample fluid in question, and, if the antigen or antibody is present in the fluid, the particles form larger aggregates due to antibody-antigen interactions **(Brian *et al.*, 2008).** Because of the economic importance of BVD disease, preparing of latex kits for detection of BVD antigen act as good and rapid step for the disease diagnosis.

Aim of work:

Preparation of of latex reagent for detection of BVDV antigen in field samples land and com-

paring the results with FAT .

Materials and methods:-

1- Swabs: 17 nasal and 13 buccal swabs were collected from animals showing respiratory manifestation (Table 1) and prepared using phosphate buffer saline (PBS) then centrifuged at 3000 rpm for 15 minutes, clear supernatant fluids were kept at -20^{o}C for BVDV antigen detection, according to **OIE (2008).**

2- Blood samples

20 blood samples without anticoagulant were collected from animals showing respiratory manifestation. For serum separation, for BVDV antigen detection **OIE, (2010)**

3- Cell culture:

Median Darby Bovine kidney cells (MDBK) free from non-cytopathic strains of BVDV obtained from virology department Animal Health Research Institute (AHRI), grown in Eagles MEM supplemented with 10% fetal calf serum. The cell culture was used for isolation and propagation of BVD virus suspended samples for FA detection according to **(Fernelius, 1969a).**

4- BVD antiserum:

BVD polyclonal antibodies, National Veterinary Service Laboratory (NVSL) mes, Iowa, USA. It was kindly supplied from Viral Strain Bank and ELISA Research Unit, Animal Health Research Institute (AHRI), Agriculture Research Center.

5- BVDV(NADL strain):

It was obtained from Veterinary Serum and Vaccine Research Institute, Abbassia. It was used for examination of sensitized latex with BVDV antiserum.

6-Fluorescent conjugate anti-BVD antibodies:

Anti-BVDV fluorescent conjugate prepared in swine, kindly supplied by Institute of Virology, Tierartzliche Hochschule (TIHO), Hanover, Germany. The conjugate was used in direct FAT for detection of BVDV in collected samples. The working dilution was 1:20 in PBS.

7-Latex beads:- latex beeds:

Polystyrene particle diameter 510nm Sigma Aldrish Co.

8- Sensitization of Latex beads with BVDV reference antiserum :

Sensitized latex beads with reference BVDV antiserum for detection of BVDV antigen in field samples according to **(Nakamura, *et al.*, (1993):**

Sensitized polystyrene blue latex beads (diameter, 0.510 nm; Sigma, St Louis, M0) were prepared. Latex beads were washed with 0.05 M bicarbonate buffer, pH 9.6. Beads were centrifuged. The latex beads were suspended in 25 ml of carbonate bicarbonate buffer. Diluted antibodies were mixed with suspended beads. The mixture was shaked on a shaker for 6 hours then centrifuged . The pellet was resuspended in PBS containing 0.5% bovine serum albumin (BSA) and the mixture was incubated overnight at 37^{0}C in an incubator. After centrifugation the pellet was collected and resuspended in 6 mL PBS containing BSA + sodium azide.

9- Confirmation of sensitized beads with BVD antiserum:

This done by using of reference BVDV strain (NADL) by latex agglutination test**.**

10- Detction of BVDV antigen in felid samples

1-Using Latex agglutination test:-

A total of 50 samples (Serum, nasal swabs and buccal swabs) were prepared, the supernatant tested using the prepared sensitized beads with BVD antiserum and the results of flucks of latex agglutination were observed and recorded.

2- Direct fluorescent antibody (DFAT), technique:-

Results

The supernatants of the prepared (Serum, nasal swabs and buccal swabs) were inoculated into MDBK cells prepared on cover slip on Leighton tubes, incubated at 37^{o}C for four days. The cover slips then removed, washed with 0.01M phosphate buffered saline Ph 7.2 and distilled water and fixed with cold acetone, then air dried and stored at -20c till stained **(Gerada *et al.*, 1971)**. Fluroescin – conjugated 1/100 dilution anti BVDV IgG (Eappel organon, Teckni-

ka Crop West Chester) was added, followed by incubation at 37°C in humid box for 45 minutes. Examination with fluorescent microscope was done (**Fernelius, 1969a).**

1-Confirmation of sensitized beads:

The sensitized beads were reacted with the reference BVDV (NADL) strain. Showing flacks of latex agglutination figure (1).

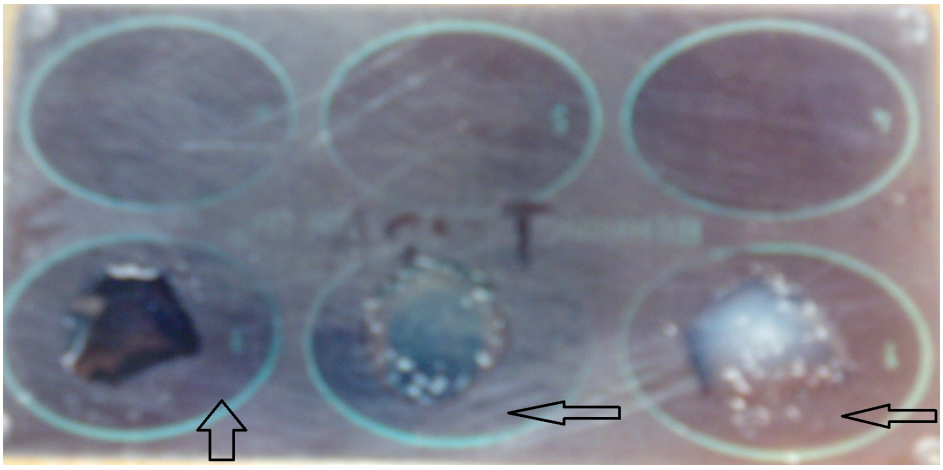

Figure (1). Showing flacks of latex agglutination
-Horizontal arrows pointed to positive latex
-Vertical arrow pointed to negative latex

2-Detection of BVDV antigen in field samples:

By Latex agglutination test:-

20 serum samples, 17 nasal swabs and 13 buccal swabs were tested by latex agglutination test, revealing that the viral antigen was detected with a positive percentage 40, 11.7 and 30.7 in the tested samples respectively. The total percent of positivity was 28 % **(table 2)**.

By direct fluorescent antibody (IFA), technique

20 serum samples, 17 nasal swabs and 13 buccal swabs were tested by the immunofluorescent antibody technique (IFA), revealing that the BVD viral antigen was detected in a positive percentage 45, 17.6 and 30.7 respectively, with a total percentage, 32 **(table 3)**

3-Comparing between results of the latex agglutination test and the immnofluorescent antibody technique:

The detection of BVDV antigen in tested field samples by direct fluorescent antibody technique and latex agglutination test revealed that the highest overall percentage (32%) was obtained with the immunofluorescent antibody technique, followed by latex agglutination (28%) **(Table 4).**

Table (1). Showing results of detection of BVDV by latex agglutination test.

Samples	No of samples	No. of +ve	No. of -ve	% of +ve
serum samples	20	8	12	40
nasal swabs	17	2	15	11.8
buccal swabs	13	4	9	30.8
total	50	14	36	28

Table (2). Results for detection of BVDV antigen by fluorescent antibody technique

Samples	No. of +ve	No. of -ve	% of +ve
Serum samples	9	11	45
Nasal swabs	3	14	17.6
Buccal swabs	4	9	30.7
Total	16	34	32

Table (3). Showing comparison between result of latex agglutination test and direct flurescent antibody technique.

samples	Tests			
	Latex agglutination		Fluorescent antibody technique	
	No. of +ve	% of +ve	No. of +ve	% of +ve
Serum samples	8	40	9	45
Nasal samples	2	11.8	3	17.6
Buccal samplea	4	30.8	4	30.7
Total	14	28	16	32

Determination of the sensitivity, specificity and correlation percentage:

The detection of BVDV antigen by latex agglutination test in comparison to IFA technique, the results revealed that the sensitivity, specificity, and correlation percentage of latex agglutination test were 64, 80 and 76 respectively **(table 5).**

Table (4). Determination of sensitivity, specificity, and correlation of detection of BVDV antigen by latex agglutination test in comparison to FA.

Fluorescent antibody technique	Latex agglutination test		
	Positive	Negative	Total
Positive	9a	7b	16
Negative	5c	29d	34
Total	14	36	50

a=True positive b=False negative

c=Fals positive d=True negative

Sensitivity= a/a+c =64% Specificity =d/b+d = 80%

Correlation= (a+d) / (a+b+c+d) =76%

Discussion

Bovine viral diarrhea is an infectious disease its signs range from subclinical to the fulminating fatal condition called mucosal disease. Acute infections may result in transient diarrhea or pneumonia, usually in the form of group outbreaks with high mortality. Acute forms of the disease associated with high mortality have also been described, often, but not always, associated with a haemorrhagic syndrome. However, most infections in the young calf are mild and go unrecognized clinically but act as a source of infection. The virus spreads mainly by contact between cattle. Vertical transmission plays an important role in its epidemiology and pathogenesis. Infections of the bovine fetus may result in abortions, stillbirths, teratogenic effects or persistent infection in the neonatal calf. Persistently viraemic animals may be born as weak, unthrifty calves

or may appear as normal healthy calves and be unrecognised clinically. Some of these animals may later develop mucosal disease with anorexia, gastrointestinal erosions, and profuse diarrhoea, leading invariably to death. Mucosal disease can arise only in persistently infected animals (**Baker. 1995).**

BVDV is classified within the family Flaviviridae and is a member of the genus Pestivirus **(Underdahl *et al.,* 1957)** .

The diagnosis of the disease depends upon detection of BVD antigen by FAT and immunoperoxidase , isolation of the virus through tissue culture. Other antigen detection tests are those that capture antigen on some type of solid support (antigen-capture ELISA or ACE tests) (**Bezek, 1995).**

In Egypt, bovine virus diarrhea, mucosal disease (BVD-MD) was initially detected as the result of a serological survey of cattle and sheep, using the serum neutralisation test. In 1970, the causal pestivirus was isolated from bovine calves and buffalo calves with pneumonia and enteritis. Mixed viral infections were also prevalent. Cases of immune tolerance were identified. The Egyptian industry had complained of unthrifty cattle and high death rates of bovine calves and buffalo calves.

A rinderpest outbreak in 1982 involved some cattle and buffalo which had been vaccinated against the disease, and such animals were positive to the gel diffusion test for BVD MD pestivirus. Extensive immunosuppression due to BVD-MD virus was suspected, because the attenuated cell-culture vaccine against rinderpest, issued in Egypt since 1965, had not been tested for freedom from non-cytopathic BVD-MD virus (**Baz, 1992)**.

Our study aimed to highlight on rapid, accurate and specific diagnosis through introduction of a new technique called latex agglutination test. For applying this technique reference antisera was sensitized with latex particles for detection of BVD antigen for detection of BVD antigen **Brian, *et al.*, (2008)** and comparing the traditional technique (FAT) with newly introduced technique.

Sensitized latex particles with reference BVDV antisera were tested against reference BVDV antigen and the obtained result showed clear flacks photo (1).

Concerning the detection of BVDV antigens in field samples ,latex agglutination test was used as (a kit containing sensitized latex particles with reference antisera , buffer, positive and negative antigens). The viral antigen in Serum, nasal swabs and buccal swabs of infected cattle was detected (table 2). Data presented in this table revealed that the BVDv antigen was detected in serum samples, nasal swabs and buccal swabs with a percentage 40%, 11.7% and 30.7% respectively. The results showed that the high number of positive were found in serum samples and this agreed with OIE Terrestrial Manual (2008) which stated that the serum samples are suitable samples for isolation of the virus from live animals.

On using of FAT for the same purpose, the obtained results varies to some extent from those obtained by latex agglutination (table 3). Data presented in this table revealed that the BVDv antigen was detected in serum samples, nasal swabs and buccal swabs with a percentage 45%, 17.6% and 30.7% respectively (**Fernelius, (1969a)**.

On comparing between results of the two tests (latex agglutination and fluorescent antibody technique) for detection of BVDV antigen in tested samples as showed in(table 4). The result revealed that the highest overall percentage (32%) was obtained with fluorescent antibody technique, followed by latex agglutination (24%).

Regarding to the results of calculation of the sensitivity and specificity of latex agglutination test (**Table 5**), it was found that the sensitivity percent was (64%) and specificity percent was (80%). From this table there were some samples positive by latex and negative by FA and vise versa. Fluorescent antibody technique is more sensitive but it takes longer time than latex agglutination and this regard with **(Laamann *et al.,* 1997)** who said that the time factor which requires several days to complete the test was the main disadvantage of this FA procedure beside the presence of NCP BVDV

strains contaminating the MDBK cells or serum used in the test .

As a conclusion latex agglutination is simple and rapid test for detection of BVDV antigen in the field samples.

References

Baker J.C. (1995). The clinical manifestations of bovine viral diarrhea infection. Vet. Clin. North. Am. – Food Animal Practice, 11, 425–445.

Baz, T. (1975). Isolation, characterization and serological studies on BVD-MD virus in Egypt Ph.D Vet. Theses, Microbiology, Cairo University, Cairo.

Baz, T.I. (1992). Bovine virus diarrhoea-mucosal disease and Border disease in Egypt. Rev. sa. tech. Off. int. Epiz., 1992, 11 (3), 867 -872

Belknap, E.; Collins, J.K. and Larsen, R.S. (2000): Bovine viral diarrhea in New World camelids. J Vet Diagn Invest 12: 468-570.

Bezek DM. (1995): Bovine Virus Diarrhea Virus Infection: Individual and Herd Diagnosis. The Compendium, August 1995: s57-s62.

Brian C. Heinze, Jae-Young Songb, Jin-Hee Han A. and Jeong-Yeol Yoona (2008): Latex Immunoagglutination Assay for Bovine Viral Diarrhea Virus Utilizing Forward Light Scattering in a Microfluidic Device. Proc. of SPIE Vol. 68, 86.

Carman S, Carr N, DeLay J et al: (2005): Bovine viral diarrhea virus in alpaca: abortion and persistent infection. J Vet Diagn Invest 17:589-593.

Fernelius, A.L.; Lambert, C. and Hemmes, G.J. (1969-a). BVDV-host cell interactions: adaptation and growth of virus in cell lines. Ibid. 1561-1572.

Fernelius, A.L.; Lambert, C. and Packer, R.A. (1969-b). BVDV-host cell interactions: adaptation propagation, modification and detection of virus in rabbits. Amer. J. Vet. Res., 30:1541-1550.

Gerada,M.R. Uckerbauer: A. Girad, G.L. Bannister and P. Boulanger (1971). Studies on Bovine viral diarrhea serum nutralization-comlement fixation and immunofluorescence. Can. J. Comp. Med., July. 35 (3), 230-235.

Goyal, S.M., Boulijihad, M. and Haugerud, S. (2002): Isolation of bovine virdiarrhea virus from an alpaca. J Vet Diagn Invest 14:523 -525.

Hafez, S. (1972). Preliminary studies on bovine viral diarrhea mucosal disease (BVD MD) and infectious bovine rhinotracheitis (IBR) in Egypt. Proceeding of the 10 thArab Veterinary Congress, Cairo, Egypt.

Laamanen UI; Neuvonen EP; Yliviuhkola EM and Veijalainen PM. (1997): Comparison of RT-PCR assay and virus isolation in cell cultures for the detection of bovine viral diarrhea virus (BVDV) in field samples. Res Vet Sci. 63(3): 199-203.

Mahony, T. J., McCarthy, F. M., Gravel, J. L., Corney, B., Young, P. L., and Vilcek, S. (2005). Genetic analysis of bovine viral diarrhoea viruses from Australia. Vet Microbiol 106, 1-6.

Nakamura, T.; kato, A.; Lin, Z.; Hiraga, M.; Numoya, T.; Otaki, Y. and Veda, S. (1993a). A rapid quantitative method for detecting infectious bursal disease virus using polystyrene latex microspheres. J. Virol. Meth. 43, 123 - 130.

OIE Terrestrial Manual (2008). Bovine viral diarrhoea chapter, 2 .4 .8, 698.

OIE Terrestrial Manual (2010).

Pellerin C., Hurk J.V.D., Lecomte J., and Tijssen P. (1994). Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreak and high mortalities. Virology 203, 260-268.

Underdahl NR, Grace OP, Hoerlein AB, (1957). Cultivation in tissue-culture of cytopathic agent from bovine mucosal disease. Proc Soc Exp Biol Med 94:795-797.

تحضير مشخصات لاختبار التلزن للكشف السريع عن أنتيجين فيروس الاسهال الفيروسى البقرى فى العينات الحقليه

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الملخص العربى
تم تحميل الأجسام المناعيه لمرض الأسهال البقرى الفيروسى على حبيبات اللاتكس وذلك لاستخدامها فى الكشف عن وجود انتيجين فيروس الأسهال البقرى المعدى من العينات المجمعه من الحقل , كما تم تجميع 50 عينه (20عينه سيرم و17 مسحات أنفيه و13 مسحات فميه) من حيوانات عليها أعراض تنفسيه وتم فحصها باختبار التلزن المحضر ضد انتيجين الاسهال الفيروسى البقرى المعدى وكانت النسبه المئويه للعينات الايجابيه في عينات السيرم والمسحات الأنفيه والفميه 40% -11.8% و 30% على التوالى. بمقارنه نتائج فحص عينات السيرم والمسحات الأنفيه والفميه باختبارى التلزن والفلورسنت المشع كانت النسبه الايجابيه بالفلورسنت المشع هى 45%- - 30.7% على التوالى ولكن النسبه متساويه فى المسحات الفميه (17.6%) . من هذه النتائج يتضح أن اختبار الفلورسنت المشع أكثر حساسيه من اختبار التلزن . ووجد ايضا أن اختبار التلزن سريع وبسيط فى الأداءمن اختبار الفلورسنت المشع.