Detection of bovine viral diarrhea –mucosal disease (BVD-MD) in buffaloes and cows using ELISA

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Summary

Out of 210 tissue samples (lung and lymph nodes) collected from buffaloes examined by ELISA specific to(BVDV) Bovine Viral Diarrhea Virus antigen ten (10) samples were positive out of 160 lung tissue collected with (4.7%)and (3)positive sample were detected out of 50 lymph node tissue samples collected with (1.4%). Totally 13 positive samples with (6.1%) in buffaloes examined. The total positive tissue samples were divided to three age group. Group age one from (birth -6 month), group age two from (6-18 month) and group age three (18 month up). The positive samples were (1),(10) and (2) with (0.47%),(4.7%)and (0.95%)respectively that indicate the presence of BVD antigen in buffaloes (Bubals bubalis) .Out of (210) sera samples collected from buffaloes examined by ELISA specific to (BVDV) antibody (83) positive sera samples with (39.5%) in buffaloes. The positive serum samples were divided into three age groups, group age one from (birth -6 month), group age two from (6-18 month) and group age three (18 month up). The positive samples were (3), (60) and (20) with (1.4 %), (28.5%) and (9.5%) respectively in buffaloes. Out of (60) sera samples collected from cows examined by ELISA specific to (BVDV) antibody 21/60 positive samples with (35%) in cows.The positive serum samples divided to three age group ,group age one from (birth-6 month),group age two from(6-18 month) and group age three (18 month up). The positive samples (1), (14), and (6) with (1.6%), (23.3%) and (10%) respectively. The result above indicated the presence of the disease as persistent infection (PI) in group one, (MD) in group two and (BVD) in group age three.

Key words: (BVD) Bovine Viral Diarrhea, Buffaloes (Bubalus bubalis) , cow, ELISA, (MD) Mucosal Disease.

التحري عن وجود مرض الإسهال الفيروسي في الجاموس والأبقار باستخدام اختبار المقايسة المرتبطة بالإنزيم المناعي (الأليزا)

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الخلاصة

جمعت (210) عينة نسيج من الجاموس منها (160 عينة رئة و50 عينة عقدة لمفاوية) فحصت باختبار المقايسة المناعية المرتبطة بالإنزيم المناعي الخاصة بمستضد مرض الإسهال الفيروسي ألبقري ظهرت منها العينات في نسيج الرئة (10)عينات موجبة وبنسبة (4.7%) وفي نسيج العقد اللمفاوية 3 عينات موجبة وبنسبة(1.4%). بأجمالي (13) عينة موجبة وبنسبة (6.1%) في الجاموس (Bubalus bubalis) . قسمت العينات النسيجية الموجبة إلى ثلاث مجاميع اعتمادا على العمر المجموعة الأولى من (الولادة-6أشهر) والمجموعة الثانية من (6-18 شهر)والمجموعة الثالثة من (18 شهر فما فوق) ظهرت نتائج العينات الموجبة كالأتي {(1),(10),(2) وبنسب (0.47%) (4.7%) (0.95%)} على التوالي.
جمعت (210) عينه مصل الدم من الجاموس فحصت باختبار المقايسة المناعية المرتبطة بالإنزيم المناعي الخاصة بأضداد فيروس الإسهال الفيروسي ألبقري (BVDV) ظهرت (83) عينه موجبه بنسبة (39.5%) .قسمت عينات مصل الدم الموجبة إلى ثلاثة مجاميع اعتمادا على العمر المجموعة الأولى من(الولادة -6

اشهر) المجموعة الثانية من(6-18شهر) المجموعة الثالثة من (18 شهر فما فوق) فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي الخاص بأضداد المرض .ظهرت العينات الموجبة في المجاميع الثلاث (3) , (60) ,(20) بنسب ((1.4%),(28.5%),(9.5%)على التوالي مما يشير إلى وجود الأضداد في دم الجاموس (Bubalus Bubalis). جمعت 60 عينه من مصل دم الأبقار فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي الخاص بأضداد فيروس الإسهال الفيروسي ألبقري (BVDV) ظهرت (21) عينه موجبة بنسبة (35%) .قسمت عينات مصل الدم الموجبة اعتمادا على العمر المجموعة الأولى من (الولادة -6اشهر). المجموعة الثانية من (6-18 شهر)المجموعة الثالثة من (18 شهر فما فوق) فحصت باختبار المقايسة المناعية المرتبطة بالأنزيم المناعي ظهرت العينات الموجبة (1),(14),(6) وبنسب (1.66%)(23.33%),(10%) على التوالي ,تشير هذه النتائج لوجود أضداد فيروس الإسهال الفيروسي ألبقري . تشير هذه الدراسة وجود المرض بأشكاله الثلاث في المجموعات الثلاثة وكما يلي (PI) في المجموعة الأولى (MD) في المجموعة الثانية (BVD) في المجوعة الثالثة.

مفاتيح الكلمات: الإسهال الفيروسي , الجاموس ,الأبقار , الأليزا.

Introduction

Bovine viral diarrhea virus (BVDV) is an important economically disease causing severe economic looses . It is an RNA virus , member of the genus Pestivirus of the family Flaviviridae consist of two geno types, bovine viral diarrhea virus type 1 (BVDV1) and bovine viral type 2 (BVDV2).

BVDV infect mainly cattle and can infect sheep .goat and buffaloes (1 and 2). BVDV is a significant pathogen associated with gastrointestinal ,respiratory and reproductive disease (3) multiple clinical forms of the infection that vary from mild subclinical to fatal mucosal disease BVDV is a very common agent affecting livestock production throughout the world serological studies have shown that the presence of antibodies to BVDV in cattle is (60-70 %), (4), and (47%) in buffaloes(5). Fatal mucosal disease MD is caused by combination of cytopathic (cp) and non-cytopathic (ncp) biotypes of the virus (6). BVDV usually causes early embryonic death ,respiratory disease ,diarrhea ,congenital malformation , reproductive failures ,lameness , immunosuppression and (MD) mucosal disease (7) . Intrauterine infection with (ncp) biotype at early stage of gestation lead to birth of persistently infected (PI) calves , fatal infection with BVDV may result of calf immunotolerant to BVDV with an in apparent persistent infection which are serving as source of infection by shedding large quantities of virus life along with various body excretion .PI animals are difficult to identify , because of thrire normal appearance . ELISA test to detect viral RNA (Antigen) is becoming a popular screening method for detection of BVDV (3).

In IRAQ BVD virus in cow was proved by antibody detection by (8), later BVDV in cow was isolated by Al-Rodhan (9).

Materials and Methods

Samples collected from water buffalo (Bubalus bubalis) and cows from slaughter houses around Baghdad city (Abo-grab, Al-Shula, Al-Fudaiylia and khan-Tharey). Two hundred ten (210) tissue samples collected include 160 lung tissue samples and 50 lymph node tissue samples were taken to investigate the presence of the antigen in buffaloes (Bubalus bubalis) . Two hundred ten (210) blood samples were collected to detect the presence of antibody in buffaloes and 60 blood samples collected from cows. All samples were stored at (-20C)

ELISA Kits: Antigen capture ELISA (ACE) kits and Antibody ELISA kits . ELISA Kits purchased from belgium BIO-X diagnostics.

Method: ELISA procedure for Antigen and Antibody diagnosis of BVDV were performed according to instruction

Results and Discussion

In buffaloes ELISA antigen test was carried on (210) tissue samples collected (160- lung tissue and 50 lymph nodes tissue) positive samples were 13/210 with (6.1%).positive lung tissue samples were (10) with (4.7%) and positive lymph node tissue samples were (3) with (1.4%). the positive tissue samples were divided according to the age to three age groups. In group age one (birth-6 months) 1/210 one positive samples with (0.47%) in group are two (6-18 months)10/210 ten positive samples with(4.7%), in group age three 918 month up)2/210 two positive samples with (0.95%). The three age groups showed different clinical signs , group age one showed poor growth respiratory infection, diarrhea and death, group age two showed skin lesion blindness, depression, loss appetite and group age three showed mild or /no clinical finding or good health but shedding the virus (Table,1).

ELISA antibody test was carried on (210) sera samples in buffaloes (83) samples positive with (39.5%). The positive sera samples were divided to three age group according to the age ,group age one from (birth -6 months) 3/210 three positive samples with (1.4%), group age two from (6-18 months)60/210 sixty positive sera samples with (28.5%), group age three (18 months up)20/210 twenty samples with (9.5%). According to antibody validity these age groups classified as low or no antibody titer in group age one while high antibody titer in group age two and medium antibody titer in group age three tables 2. Out of the total serum samples collected from cows examined by ELISA 21/60 twenty one positive samples with (35%), these positive sera samples were divided into three groups ,group age one from (birth-6 months) 1/60 one positive samples with (1.6%), group age two (6-18 month) 14/60 fourteen positive samples with (23.3%), group age three (18 months up) 6/60 six positive samples with (10%) . According to antibody validity and age these three age groups classified as low antibody titer or non in group age one high antibody titer in group age two and medium antibody titer in group age three (Table,3). The proposed clinical diagnosis for the antibody result of buffaloes and cows was (PI) for age one. (MD) for age group two and (BVD) for age group three.

Table (1): Bovine viral diarrhea Antigen detection by ELISA according to age and type of tissue and the main clinical signs in buffaloes.

No. of tissue samples collected	Type of tissue samples	No. Of Collected samples	Pos.	%	Total positive samples	%	The age groups	Pos.	%	Main clinical signs
210	Lung	160	10	4.7	13	6.1	One day -6 months	1	0.47	Poor growth Respiratory signs Diarrhea death
	Lymph node	50	3	1.4			6-18 months	10	4.7	Skin lesion Blindness Depression Loss apatite Death
							18 months up	2	0.95	No symptom may be in good health Chronic case

Table 2: Bovine viral diarrhea antibody detection by ELISA according to age with validity and the proposed diagnosis in buffaloes.

No. of tissue samples collected	Positive samples	Per. %	Age	Pos.	Per %	Antibody titer and validity	Pr0p0sed diagnosis
210	83	39.5	One day-6 month	3	104	Low or/ no antibody titer	PI
			6-18 month	60	28.5	High antibody titer	MD
			18 month up	20	9.5	Medium antibody titer	BVD

Table 3: Bovine viral diarrhea antibody detection by ELISA according to age with validity and the proposed diagnosis in cows.

No. of tissue samples collected	Positive samples	Per.%	Age	Pos.	Per%	Antibody titer and validity	Proposed diagnosis
60	21	35	One day -6 months	1	1.6	Low or/no antibody titer	PI
			6-18 months	14	23.3	High antibody titer	MD
			18 months up	6	10	Medium antibody titer	BVD

Antigen and antibody detection of BVD-MD by ELISA in buffaloes and cows indicated the presence of the disease in IRAQ and in more than one form as can be seen from the tables of the results. This study detects the presence of BVD-MD virus antigen in lung and lymph node tissue with 6.1%. BVD-MD virus was present in lung tissue (4.7%) and lymph node tissue (1.4%) in this investigation, and was detected in similar tissue by (10 and11). Dividing the thirteen 13 positive cases of the antigen detection according to the age group revealed that the second age group (6-18 months) was the mostly affected such result agree with (12). The clinical finding of the three age group were not similar but different according to the form of the disease affecting the buffaloes, first and second age groups showed the most sever signs while many of the third age group were symptomless ,some in good health such finding also were mentioned and agree with (13).

In India (14) detected a total of 23.3%positive antibody cases by ELISA kits while it was 39.5 in this investigation and was (52.5%) in the investigating conducted by (15) the 6-18 months age group was the highest in the percentage, such deferens's in results could occur taking in to consideration the previous

disease situation and outbreaks of the disease if occurred, which could affect the total result of such investigations.

The validity were expected since the first age group were mostly infected during pregnancy and yielding low titer or no antibody, high antibody titer were found in the second age group, while the third age group were grown animals whom have experienced the disease and react to it in a mild form or chronic with titer in between the first and second age group such results agree with (16 and 17). Correlating between the clinical finding and antibody titer mentioned a proposed clinical diagnosis was made to identify the forms of the disease uncounted during this investigation. It was found that three forms were uncounted and could be suspected no similar researchers were found to be compared with using ELISA kits to detect antibodies in cows the second age group result (18) with23.3%was the highest percentage this result agree with (19).

The validity titer manifested in cows the second age group (6-18 months) being the highest no similar researchers were found to be compared with.

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