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MOLECULAR AND SEROLOGICAL DETECTION OF BOVINE ADENOVIRUS TYPE-3 IN BASRA PROVINCE

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Key words: bovine adenovirus type 3,cattle, basra

ABSTRACT

Investigation of bovine adenovirus type 3 in symptomatic and asymptomatic cattle were carried out in this study ofdetecting circulating specific bovine adenovirus type 3 antibodies by Indirect ELISA and standard PCR technique. The study exhibited these virus have detected by ELISA more than PCR technique. The results were showed thatthe overall seropositivity ratio of bovine adenovirus antibodies in animals of Basra was (61.9%) and bovine fecal samples will tested by PCRwereonly seven fecal positive sample (6.1%) was found.

INTRODUCTION

Adenoviruses (AdVs) are double stranded DNA, non-enveloped viruses with icosahedral capsid symmetry composed of 252 capsomers, The size of genomes ranges between 24 and 45 kb (1,2). The virus can cause respiratory and enteric infections of calves. Bovine adenoviruses (BAVs) cause a variety of clinical signs including conjunctivitis, pneumonia, diarrhea, and polyarthritis and Bovine respiratory disease complex (BRDC) is a major problem for cattle and it continues to cause serious economic losses for cattle industry (3). Adenoviruses were first isolated from adenoid tissues by two independent groups attempting to identify the causative agents of acute respiratory infections(4,5).They have been isolated from mammals, birds, reptiles and other species (6,7). Their replication is mostly limited to single host species but it can present asymptomatically in species other than the natural host (8).

MATERIAL AND METHODS

The study included 322 symptomatic and asymptomatic sample from different region of Basra provincewith clinical symptoms of Bovine adenovirus represented by mild or severe diarrhea, nasal discharge and normal animal.Samples included 115 bovine fecal specimens, 92 bovine blood samples and 115 bovine nasal swab samples were collected from bovine with clinical signs from different regions (Abi-Elkhasib, Shutt-alarab, Basra center, Alqarma, Alzubair). Suspected animals were at age group ranged between2 month -five years of age in the city of Basra.Sample were collected in cool condition by ice box and used viral transport media (VTM) to keep the sample in moderate pH and inhibit bacteria, fungal growth until reach the samples to laboratory to be diagnosed. All samples were tested with standard PCR (AccuPower® PCR PreMix, Bioneer, Korea)and Indirect ELISA (BIO-X Adenovirus 3 bovine ELISA Kit 2*48 test, BIO K 063\2, made in Belgium).

Samples collection

Fecal samples and nasal swab samples were collected from 115 bovine of clinical sign including diarrhea, nasal discharge, fever, weight loss, in appetence. From diarrheic and non-diarrheic cattle's of different age and sex in Basra province (Abi-Elkhasib, Shutt-alarab, Basra center, Alqarma, Alzubair).Fecal samples were varied in amount from 0.2gm to 10gm per sample and collected in sterile disposable closed plastic containers containing viral transport media.The nasal sampleswere taken by sterile swabs. Blood sampleswere collected from the same animals in sterile 10 ml test tube. were transported under cold conditions to the laboratory where the necessary tests were performed or stored frozenuntil use.

ELISA Test

Indirect ELISA (Adenovirus 3 bovine ELISA Kit, BIO X, BIO K 063\2, Belgium) was performed for detection antibodies against Bovine adenovirus type 3 in serum samples of cows, according to the manufacturer's instructions. The signal read for each samples were divided corresponding positive control serum odd and multiply this result by 100 to express it as a percentage. Delta OD Sample

Val = _____*100

Delta OD positive

PCR Analysis

DNA extraction was performed by using Stool DNA Extraction Kit (Stool DNA extraction kit, LOT no.1302k, REF.K-3036, Bioneer, Made in Korea),for purification of viral DNA from stool, according to the manufacturer's instructions. The viral nucleic acid extraction kit III was designed for efficient purification of viral DNA from cell free samples, which uses the specific sequence of two pairs of primers, designed E2Afwd and E2Aseq1, which located in E2A region of BAV-3. The primers E2Afwd (5'-GAG ATG GAT GTG AAC AGC GA-3') and E2Aseq1 (5'-ACA TTC TGA TGC TGG TAC TG-3') amplified an approximately 644 bp product from the BAV-3 DNA.The reaction was heated in athermocycle for 3 min at 93°C and then submitted to 30 cycles of amplification. The conditions of amplification were 45 s at 95°C, 50 s at 55°C, and 1 min at 72°C. The final extension step was done at 72°C for 10 min.DNAconcentration was complete by Nano drop instrument, and the horizontal agarose gels was used for analysis of DNA virus amplification product size. The concentration of agarose used was 1.5%; gels were prepared as percentage weight / volume solutions.

RESULT

According to different regions of Basraprovince

Results show that out of (92)serum samples.Wereseropositivity ratio of bovine adenovirus antibodies in animals of Basra were (61.9%). Two regions out of five under survey recorded high seropositivity ratio including, Basra center (83.3%) and Shut-Alarab (71.4%). The other threeregions had a lower seropositivity ratio ,Qurma (50%), Abi-Elkhasib (56%) and Zubair (37.5%),compared to other districts (Table 1). The difference among these regions was considered to be statistically not significant ($P > 0.05$).

Table(1): Distribution of Bovine adenovirus infection according to ELISA results in bovine of different region of Basra province.

Regions	ELISA results				
	Examined n.(%).	Positive n.	%	Negative n.	%
Abi-Elkhasib	25(27.2)	14	56	11	44
Shut-Alarab	21(22.8)	15	71.4	6	28.6
Basra center	18(19.6)	15	83.3	3	16.7
Qurma	20(21.7)	10	50	10	50
Zubair	8(8.7)	3	37.5	5	62.5
Total	92	57	61.9	35	38.1
P>0.05 (X^2:5.14;p-value:0.273:DF:4)					

Animals clinical picture

Fifty eight (58) animals show clinical signs and 34 animal had been asymptomatic were investigated by ELISA and resultswererevealed that74.1% were seropositive for antibodies encountered in clinically diseased animals in Basra regions, followed by Shut-Alarab (87.5%) showed high rate of seropositivity. However 34 asymptomaticanimals were show 14 (41.2 %) seropositive samples. The high rate of seropositivity was observed in the animals of Basra center (70 %). The difference among animals in different Basra regions was considered to be not statistically significant (P > 0.05).

Table(2): Bovine adenovirus results in symptomatic animalsofdifferent region of Basra province based on ELISA.

Regions	ELISA results				
	Examined n.(%).	Positive n.	%	Negative n.	%
Abi-Elkhasib	17(29.3)	11	64.7	6	35.3
Shut-Alarab	16(27.6)	14	87.5	2	12.5
Basra center	8(13.8)	8	100	0	0
Qurma	14(24.1)	8	57.1	6	42.9
Zubair	3(5.2)	2	66.7	1	33.3
Total	58(100)	43	74.1	15	25.9
P>0.05 (X^2:4.301;p-value:0.3667:DF:4)					

Table(3): Bovine adenovirus results in asymptomatic animals ofdifferent region of Basra provincebased on ELISA

Regions	ELISA results				
	Examined n.(%).	Positive n.	%	Negative n.	%
Abi-Elkhasib	8(23.5)	3	37.5	5	62.5
Shut-Alarab	5(14.7)	1	20	4	80
Basra center	10(29.4)	7	70	3	30
Qurma	6(17.7)	2	33.3	4	66.7
Zubair	5(14.7)	1	20	4	80
Total	34(100)	14	41.2	20	58.8
P>0.05 (X^2:2.881;p-value:0.5779:DF:4)					

Determination of serum's positivity degree

Symptomatic bovine positive samples 14 (32.5%) were considered moderately positive where their serum's positivity degree values ranged between >56% -≤ 79 %, while the lowest positivity degree were detected in 2 cases (4.7%). Their positivity degree ranged between 10% -≤ 33 %, On the other hand the highly serum's positivity degree were detected in 16 positive samples (37.2%) where their positivity degree was >102 % (+++++).Most of asymptomatic bovine positive serum samples 7 (50%) showed lowest positivity degree ranged between 10 -≤ 33 % and the moderate positivity degree (>56 - ≤79 %) was observed in 2 cases (14.3%), while the highly serum's positivity degree were detected in 4 positive samples (28.6%).The difference between symptomatic and asymptomatic bovine serum's positivity degree was considered to be statistically highly significant($\mathbf{X^2}$:27.92;p-value:0.0009:DF:9).

PCR amplification results

The result of PCR amplification that performed on the extracted DNA was confirmed by electrophoresis as the strands of the DNA which are resulted from successful binding between primers and the extracted DNA. These successful binding appear as a single band under U.V illuminator using ethidium bromide as a specific DNA stain. Bands with expected size (644bp) were observed.

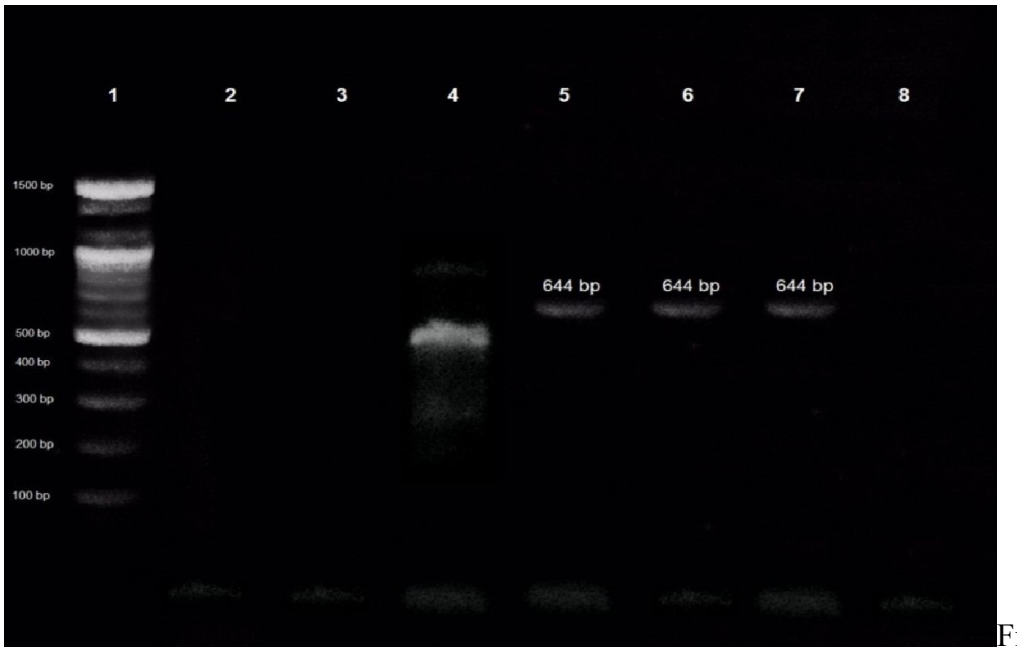

Figure.(1) Positive and negative results of PCR amplification ; Lane (1) laddermarker ; Lane

(5,6,7) positive BAV-3 specific gene (644bp); Lane (2,3,4,8) negative Broad-

Clinical samples

The PCR analysis was performed on (230) fecal and nasal swab samples (115 for each). Depending on the results of the PCR analysis only 7 (6.1%) fecal samples were PCR positive for BAV-3 specific gene, whereas 0 (0%) of nasal swab samples were negative for BAV-3 specific.

spectrum AtAdV on 1.5% agarose .

Table(4): Distribution of BAV-3 specific gene results according to different region of Basra based on PCR.

Basra regions	PCR results		
	Fecal samples		
	Tested samples n.(%).	Positive n.(%)	Negative n.(%).
Abi-Elkhasib	38(33.1)	1(2.6)	37(97.4)
Shut-Alarab	27(23.5)	1(3.7)	26(96.3)
Basra center	22(19.1)	5(22.7)	17(77.3)
Qurma	20(17.4)	0	20(100)
Zubair	8(6.9)	0	8(100)
Total	115	7(6.1)	108 (93.9)
X^2:13.533;p-value:0.001:DF:4			

DISCUSSION

Bovine respiratory disease complex (BRDC) is a major problem for cattle and it continues to cause serious economic losses for cattle industry. The causes of BRDC are multiple and complex, but the three factors of stress, viral infection and bacterial infection are almost always involved in cases of severe disease (9).This is the first study about the molecular and serological detection of BAV-3 in cattle Basra of

Iraq,will be a good beginning for further studies on BAV-3. On the basis of this work, a broad pathogen epidemiological investigation of BAV- 3 might be carried out and serological methods would be established for detecting antibodies against BAV-3 in Iraq. Vaccine for BAV-3 might be developed using the new identified BAV-3. All of these efforts would greatly improve the investigations on BRDC in Iraq, On the other hand, recombinant BAV-3 is being developed as a live vector for animal vaccination and for human gene therapy (10). Therefore the BAV-3 might be developed as an appropriate expression vector.

Results of the present study revealed that the seropositivity of BAV-3 was 61.9%, these results were lower than those reported by (11) and (12) whom detected antibodies against BAV-3 in tested (91.6 and100 % respectively) against BAV-3 in sera of young and adult cattle of Iran . Beside that serologic evidence in United States indicated high prevalence of BAV-3 in cattle. (13)determined antibodies in (81.38 %) of unvaccinated cattle using serum neutralization (SN) test.

The ubiquitous exposure to bovine adenoviruses makes determination of the specific type involved in an outbreak of respiratory or enteric disease, The critical assessment of BAV, broadly and by genotype, as indicators of BAV infection and environmental contamination from cattle manure requires improved knowledge of their prevalence, shedding, and genetic diversity. Many previously published PCR assays targeting BAV suffer from poor sensitivity, limited breadth of detection (14,15). In the present study the examination of bovine nasal and fecal samples using specific BadV-3, broad-spectrum PCR primer revealed the shedding of most prototype BAV(except BadV-3, -9,) by cattle, significantly expanding the genetic diversity of BAV detected to date by PCR (16). The current revealed that7(6.1%)out of 115 head of symptomatic cattle showed BAV 3 based PCR positive results but serologic data showing that 43(74.1%) BAV 3 based ELISA positive results and 14(41.2%) animals of asymptomatic cattle.

The current detection of BAV 3 in 7(6.1%) fecal samples only ,agreed with (17). who mentioned that frequencies determined by using PCR have a wide range, from 0% to 30% for samples collected from single animals whereas, it is disagreed with (18), who reported frequencies ranged from 50% to 75% for samples pooled

from multiple animals. The present finding of BAV3 in fecal samples and its negativity in PCR analysis of nasal swabs was disagreed with (19) who found positive PCR result where he used similar BAV3 having the same nucleotide sequence in the amplification DND previously extracted from nasal swabs collected from a group of feedlot cattle with acute respiratory disease.

التشخيص الجزيئي والمصلي لفايروس الغدي البقري-3 في محافظه البصرة

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الخلاصه

تم الكشف والتحقيق عن الفايروس الغدي البقري -3لعينات الابقار السريريه المصابه والغير مصابه في هذه الدراسه والتي تم فحصها بتقنيه الممتز المناعي المرتبط بالإنزيم وتقنيه تفاعل سلسلة البلمره، اظهرت هذه الدراسه بتشخيص الفايروس بتقنيه الممتز المناعي المرتبط بالانزيم اكثر من تقنيه تفاعل سلسلة البلمرهحيث لوحظ ان معدل الايجابيه للاجسام المضاده لفايروس البقري الغدي-3 في الابقار في محافظه البصرة كان(61.9%) وعينات البراز البقريه تم فحصها بواسطه تقنيه تفاعل سلسلة البلمره حيثما كانت فقط سبع عينات براز بقريه ايجابيه.

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