BOVINE VIRAL DIARRHEA AND PERSISTENTLY INFECTION OF CATTLE AT NINEVEH PROVINCE, IRAQ

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ABSTRACT

The current study was conducted to determine the prevalence of Bovine viral diarrhea and persistently infected (PI) animals at Nineveh province ,Iraq, using antigen capture enzyme linked immunosorbent assay (AC-ELISA) test and multiplex reverse transcriptase polymerase chain reaction (RT- PCR technique) as a diagnostic tool and to determine BVDV genotypes, However, investigation of some epidemiological risk factors associated with occurrence of disease was also encountered. The study was started from January to August, 2017. A total of 494 cattle ear notches (112 adult cows, 30 younger calves and 352 young beeflot calves) was sampled and tested. For investigating of the persistently infected animals, The positive cattle were screened for a second time after four weeks from the first sampling, Moreover, epidemiological data collected through interview with the farmer s' owner. Furthermore, The 5-Untranslated rejoin (5' UTR gene) of BVDV extracted from ear notch were used to determine the genotypes of the virus. Results indicated that the overall prevalence of BVD in Nineveh province based on AC-ELISA and multiplex PCR technique was 5.46% and 13.96%, respectively.Moreover, the prevalence of PI in cattle was 0.8% for each test. According to Kappa value (0.457), it has been found that, the multiplex PCR is highly efficient for diagnosis of BVDV in cattle than AC-ELISA test. It was indicated that both BVDV1 and BVDV2 were detected in Nineveh province at (12.95%, 1.01%) respectively. Results were also indicated that the cattle ages, genders, breeds, importation, large herd size, regions and the seasons were

significantly associated with higher prevalence of BVD. It has been concluded that BVD is an endemic disease in Nineveh province, However, at several factors could assist for its spreading. Moreover, two genotypes were detected, BVDV1 and BVDV2.Therefore, control programs as well as early detection of PI animals should advised, and have the priority.

INTRODUCTION

Bovine viral diarrhea (BVD) is an important worldwide disease in both domestic and wild ruminants, resulting in fundamental damages in infected herds as well as major economic losses in the beef and dairy cattle industry (1,2). Bovine viral diarrhea virus has been known as a potential cause of serious disease, including different degresof wastage, diarrhea, reproductive loss and death,However, it act as an essential threat to herd health (3).

The virus of BVD belongs to the family Flaviviridae, genus Pestivirus, and is classified into biotypes based on the presence or absence of observable cytopathology in infected cell cultures, as cytopathic (CP) or noncytopathic (NCP) (4). The predominant biotypes in clinically illness cattle are noncytopathic strains. Bovine viral diarrhea viruses are differentiated on the basis of genomic differences into 2 major genotypes BVDV1and BVDV2 (5).

The RNA genome of BVDV consists of a single-stranded, positive-sense, approximately 12.3 kb in length, and single open reading frame (ORF), flanked by 5'- and 3'- untranslated regions (5'-UTR, 3'-UTR) encodes a polyprotein of about 4,000 amino acids (6). The polyprotein is co- and post-translationally processed into 12 polypeptides in the following order: N-terminal autoprotease (Npro), capsid protein (C), envelope proteins (Erns, E1, and E2), p7, and non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (7).

The generation of persistent infection (PI) animals is more frequent when fetal infection occurs between 30 and 90 days of gestation, which can be established by NCP biotype of both BVDV-1 and BVDV-2 (8). The PI animal is considered to be the main source of BVD infection because it eliminates a large amount of virus in secretions and excretions along all their life (9).

The clinical manifestations of a BVD infection depend upon the viral strain, the animal's immune status and the reproductive position at the time of infection (10). Severe cases of the disease are characterized by fever, anorexia, depression, erosions and hemorrhages of the gastrointestinal tract, diarrhea, and dehydration, However, diarrhea might not be a prominent in

mild cases (11). Most BVD infections are subclinical, Nevertheless, the course of the disease might vary between 2-3 days up to 4 weeks. In addition, the diseased animals will suffer from high body temperature ranged between 40-41°C at early stages, but usually return to normal or below in 1-2 days and before diarrhea start (12). The prevalence of BVDV infection varies between different countries and regions within a same country, this finding may be related to differences in management, environmental variation, size of herds, and existence of PI animals in these herds (13,14).

Several different laboratory methods were used for diagnosis of BVD such as virus isolation, indirect enzyme linked immune assay (ELISA), serum neutralization (SN), antigen capture (AC-ELISA), immunohistochemistry (IHC) and reverse transcriptase polymerase chain reaction (RT-PCR) (15).

A preliminary study to investigate BVD in Nineveh province have been done before (19). However, The current study aimed to determine the prevalence of BVD and persistently infected animals and to investigate some of the epidemiological risk factors associated with the occurrence of the disease, Moreover to determine BVDV genotypes.

MATERIALS AND METHODS

Animals and study design :-

The study was conducted on 494 animals of both sexes and of different ages (112 adult cows, 30 younger calves and 352 young beeflot calves), Reared in the different parts of Nineveh province, (Gogjalee, Jelekhwan, Entisar, Bartilla and Kolantapa), Iraq. The study was started from January to August,2017. Complete clinical examinations have been done for all animals .

Sample collection :- A total of (494) ear notch samples (1 centimeter square) was collected from the external ear pinna using a sterile disposable surgical blades, the collected samples were placed in a sterile test tube and transported to the laboratory by using ice bag. The samples were then stored at -20 °C until tested by Antigen capture enzyme linked immunosorbent assay (AC-ELISA) and multiplex reverse transcriptase polymerase chain reaction RT-PCR.

Antigen capture enzyme linked immune assay (AC-ELISA):- Ear notch samples were analyzed using the commercial BVDV Antigen Kit (IDEXX Laboratories, Inc. USA). All animals show positive results were re-sampled four weeks after the first round of testing to differentiate transient and persistently infected animals (17). The procedure was carried out following the manufacturer's instruction of AC-ELISA kit. The (S/N) for each sample was determined as follows: - S/N = Sample - negative control mean.

Samples with S/N values ≤ 0.2 are negative. Samples with S-N values > 0.2, but less than or equal to 0.3 are suspect and Samples with S/N values > 0.3 are positive.

RNA extraction and amplification from cattle ear notches :- The RNA of BVDV was extracted from 494 ear notches employing the QIAamp® Viral RNA kit (RNA extraction from ear notches without purification) According to the manufacturer's instructions (ADIAGENE, BioX Diagnostic. France). Amplify the highly conserved region 5' UTR gene of BVDV from ear notch samples (n=494), as a target in multiplex PCR technique using QIAGEN OneStep RT-PCR Kit (Qiagen, Germany) for BVDV detection and determination of genotypes. The oligonucleotides of specific primers were designed by Gilbert *et al*, 1999 (21) which were provided by First BASE Laboratories Sdn. Bhd. Malaysia (Table 1). In this PCR process, Two positive cDNA derived from persistently infected calve was used as positive control. The GenBank accession numbers for cDNA positive controls were (MF347399, MF491394) for BVDV1 and BVDV2 respectively. Further, cDNA extracted from non-infected calve was used as negative control for each PCR amplification. In this study, all the animals tested positive were re-sampled four weeks after the first round of testing to differentiate persistently infected animals. Multiplex PCR reactions were conducted in a total volume of 50 µl, composed of 10µl 5XQaigen RT- PCR buffer, 3µl of each primer, 2µl of dNTP mix, 2µl of RT-PCR enzyme, 10µl of template (RNA sample) and 17µl dH2o. The mixture was briefly centrifuged and reverse transcription was done at 550C for 30 min in the thermo-cycler machine. This was followed initial denaturation at 95 ºC for 15 min, followed by 40 cycles of denaturation at 94 ºC for 20 s, annealing at 50 ºC for 30 s, extension at 72 ºC for 30 s and final Extension-72 ºC for 15 min, The samples were removed at the end of the program when the temperature on the screen showed 4ºC

Table 1: The Oligonucleotide primers used to amplify the 5'UTR gene.

Primer	Sequences 5'-3'	Amount of oligo (nMoles)	Size (bp)
BVD_F	TGG AGA TCT TTC ACA CAA TAG C	30.7	
V1-R	GGG AAC CTA AGA ACT AAA TC	33.7	360
V2-R	GCT GTT TCA CCC AGTT (A/G)TA CAT	27.8	604

Statistical analysis :- The difference in the prevalence between the various risk factors was assessed by using two-sided Chi-square and Fischer's exact test in IBM-SPSS statistics version19 program. The relative risk (RR) for the association between risk factors for BVD using 2 by 2 tables in Epi-Info™ 7 software (version 7). To compare between AC-ELISA and multiplex PCR for diagnosing BVDV using Kappa value in IBM SPSS statistics 19 (SPSS Inc.), if Kappa ≥1 means higher compatibility between the two tests.

RESULTS

In the current study, 494 ear notch samples were tested by AC-ELISA and multiplex PCR technique. The results showed that the overall prevalence of BVD in Nineveh province was 5.46% (27 out of 494) and 13.96% (69 out of 494) by the two tests used respectively (Table 2). Moreover, the prevalence of PI in cattle in Nineveh province by AC-ELISA and multiplex PCR technique was 0.8% for each test (Table 2).

The present study revealed that, no compatibility between AC-ELISA and multiplex PCR base on overall infection rates of BVD in cattle, as Kappa value was 0.457, which means that multiplex PCR is highly efficient for diagnosis of BVD in cattle (Tables 3).

This study revealed that the positive bands for BVDV1, were at approximately 360 bp (Fig. 1), for BVDV2, the positive bands were at approximately 604 bp (Fig. 1), with detection rate (12.95%, 1.01%) respectively (Table 4).

Based on multiplex PCR, the results of this study demonstrate significant differences ($P<0.05$) between the age of cattle. The prevalence of BVD disease was significantly higher in cattle aged between (>6 months-2years) (RR: 5.94 times, CI: 0.85 - 41.34) than cattle (>2 years) and young ages (<6 months) (Table 5). In the present study, the prevalence of BVD showed a significant difference between cattle gender. The prevalence showed a significant higher values in males

(RR: 5,32times, CI: 1.9-14.32) compared to female cattle. The study also revealed a significant difference between cattle breeds. The prevalence of disease was significantly higher in Iranian, Syrian and Turkey breeds (RR: 2.29. times, CI: 1.15 – 4.55), (RR: 3.29 times, CI: 1.60 – 6.78) and (RR: 2.42 times, CI: 1.32 - 4.42) respectively, compared with local breeds (P<0.05) (Table 5).The prevalence of BVD was significantly higher among imported cattle (RR: 2.51 times, CI: 1.44 – 4.40) compared with the native cattle (P<0.05) (Table 5).Our study has demonstrated that the prevalence of BVD disease was significantly higher in large size herds (RR: 7.59 times, CI: 3.11 – 18.53) compared to small herds (P< 0.05) (Table 5).

In general, the prevalence of BVD was significantly affected by geographical regions in Nineveh (P<0.05). As, Gogjalee region was showed higher prevalence of the disease (RR: 7.47 times, CI: 1.08-52.54) compared to other regions (Table 6). This study demonstrated that the prevalence of BVDwas also significantly affected by seasons (P<0.05). The prevalence of the disease was significantly higher in winter and spring seasons, which were 27.56 % and 12.12 % , respectively (RR: 7.94 and 3.49 times, respectively) compared to the summer season, which was 3.46% (Table 7).

Table 2: Prevalence of bovine viral diarrhea and Persistent infection using AC-ELISA and multiplex PCR.

Type of test	No of sample	Overall prevalence of BVD %	Prevalence of PI %
AC-ELISA	494	27 (5.46) [a]	4(0.8) [a]
Multiplex PCR		69 (13.96) [b]	4(0.8) [a]

Values significantly different (P < 0.05) between tests are labeled with the different letters (a, b or c).

Table 3: Comparison between AC-ELISA and multiplex PCR technique depending on the Kappa value for diagnosis of BVD.

	Multiplex PCR	
Infected	Uninfected	Total No.

AC-ELISA	Infected	24	3*	27
	Uninfected	45**	422	467
		69	425	494

*** Mean false negative** **** Mean false positive**

Table 4: Detection rate of BVDV genotypes in ear notch cattle samples using multiplex PCR techniques (n= 494).

Type of PCR	Primer	Genotypes detected	Product size (bp)	No. positive (%)
Multiplex PCR	Specific	BVDV1	360	64 (12.95)[a]
		BVDV2	604	5 (1.01)[b]
		Overall %		69 (13.96)

Value significantly different (P < 0.05) is labelled with the different letters (a, b or c).

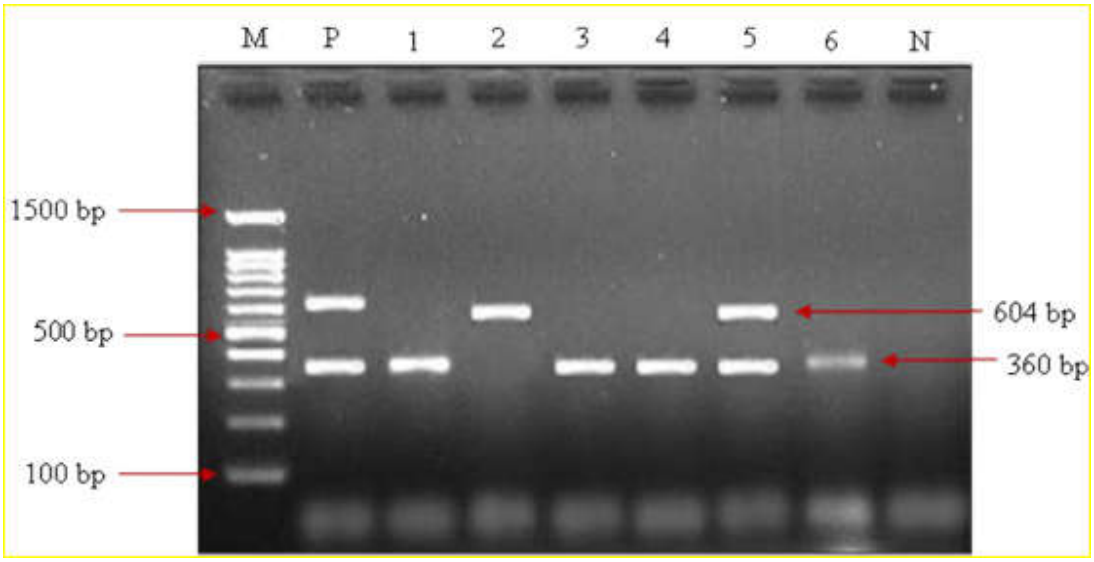

Figure 1: Gel electrophoresis image showing: lane M) Exact Mark 100-1500bp DNA ladder; Lane P) cDNA extracted from PI calf used as positive control for BVDV1 and BVDV1; Lane 1, 3, 4, 6) Multiplex PCR technique detected only BVDV1 in approximately band size 360bp; Lane 2) Multiplex PCR technique detected only BVDV2 in

approximately band size 604bp; Lane 5) Multiplex PCR technique detected both BVDV1 and BVDV2 in approximately band size 306 and 604bp respectively; Lane N) cDNA extracted from BVDV-free calf used as negative control.

Table 5: Relative risk of cattle factors associated with prevalence of bovine viral diarrhea based on PCR.

Factors	No. of cattle tested	PCR test			
		No of positive (%)	RR	95% CI	P
Type of cattle					
Young calves	30	1 (3.33) [a]	1		
Adults	112	3 (2.67) [a]	1.24	013 – 11.53	0.61
Younger feetlot calves	352	65 (18.46) [a]	5.53	0.79 – 38.53	0.02
Age					
< 6 months	30	1 (3.33) [a]	1		
>2 years	136	3 (2.20) [a]	0.66	0.07 – 6.14	0.71
>6 months- 2 years)	328	65(19.81) [b]	5.94	0.85 - 41.34	0.03
Gender					
Female	122	4 (3.27) [a]	1		
Male	372	65 (17.47) [b]	5.32	1.9 – 14.32	0.000
Adults and young calves breed					
Local breed	71	2 (2.81) [a]	1		
Turkish breed	35	1 (2.85) [a]	0.98	0.05 – 10.50	0.99
Iranian breed	36	1 (2.77) [a]	1.01	0.09 – 10.81	0.99
Youngers feedlots breed					
Local breed	122	12 (9.83) [a]	1		
Syrian breed	46	11 (23.91) [b c]	2.43	1.15 – 5.11	0.01
Turkey breed	130	28 (21.53) [b c]	2.18	1.16 - 4.10	0.01
Iranian breed	54	14 (25.92) [b c]	2.63	1.30 – 5.13	0.003
Origin					
Native	193	14(7.25) [a]	1		
Import	301	55(18.27) [b]	2.51	1.44 – 4.40	0.0005
Herd size					
Small ≤10	184	5(2.71) [a]	1		
Large ≤40	310	64(20.64) [b]	7.59	3.11 – 18.53	0.0001

Table 6: Relative risk of regions factors associated with prevalence of bovine viral diarrhea based on PCR.

Regions risk factors	No. of cattle tested	PCR test			
		No of positive (%)	RR	95% CI	P
Jelekhwan	39	1(2.56)a	1		
Entisar	28	1 (3.57) a,c	0.71	0.04-10.99	0.81
Bartilla	49	3 (6.12) a,c	0.44	0.04-4.11	0.46
Kolantapa	117	١٤ (١١.٩٦) a,c	0.21	0.02-1.57	0.08
Gogjalee	261	٥٠ (١٩.١٥) b	7.47	1.08-52.54	0.01

Values significantly different (P < 0.05) are labelled with the different letters (a, b or c).

Table 7: Relative risk of seasonal factors associated with prevalence of bovine viral diarrhea

Factors	No. of cattle tested	PCR test			
		No of positive (%)	RR	95% CI	P
Summer 2017 (Jun- July-Aug)	173	6 (3.46) a	1		
Spring 2017 (March-April-May)	165	20 (12.12) b	3.49	1.43-8.48	0.002
Winter 2017 (Jan-Feb)	156	43 (27.56) c	7.94	3.47 - 18.15	0.000

Values significantly different (P < 0.05) are labelled with the different letters ($^{a, b \text{ or } c}$).

DISCUSSION

It has been found that the overall prevalence of BVD in cattle at Nineveh using AC-ELISA was 5.46%. Lower and/or near similar prevalence have been reported in previous studies of the disease in Iraq and other countries. As (19,20,21) whom reported that the prevalence of BVD in cattle herds in Iraq, Iran and Turkey was 7.14%,4% and 0.03% respectively, using AC-ELISA. Moreover, the overall prevalence of BVD in Nineveh was 13.96%, which was higher compared to previous studies in Iraq and other countries that used the PCR technique to diagnose this disease. As the prevalence of BVD in cattle was ranged 6 -10% in Iraq (19,22).However, in Tunisia and Egypt was 2.65% and 10.4% respectively (23,24).

In the current study, It was the first time that the overall prevalence of PI (0. 8%) in cattle was registered at Nineveh province, These results are almost lower to those reported in Iran, Egypt, Pakistan and Japan are, 3.2% , 1.6%, 3.25% and 7% respectively (17,25,26,27). However, in most endemic countries with BVD, The rate of PI were ranged between 0.1-2% (28).

In general, the prevalence of BVD might differs from country to another and regions within the same country, which could be due to different management practices, a numbers of animal samples, types of sample, The sensitivity of the diagnostic test used, incidence of competent vectors, cattle activity, presence and sufficient control programs, the climatic variations, extensive cattle trade and uncontrolled animal movement, the variation in the cattle population size, biosecurity and presence of persistent infection of BVD, were all could be considered as the main causations (21,29).

It has been indicated that, ear-notch considered as reliable, given their high sensitivity and specificity tested by multiplex PCR technique for detection of PI cattle. This finding was in agreement with previous study (30).

The present study revealed that no compatibility between AC-ELISA and multiplex PCR base on overall infection rates of BVD in cattle. This finding that multiplex PCR is highly efficient for diagnosis of BVD in cattle. this finding agree with previous study (20).The reasons probably is due to the short course of acute infection resulting lower levels of virus expected to be present, Moreover the PCR technique is more sensitive and more accurate for detection the prevalence rate of clinically BVD infected cattle and it is preferable to use in the epidemiological and for screening studies. The same results were also confirmed by (31).

In this study, 5'UTR regions were used because it contains variable regions and it is easy to obtain a fragment by RT-PCR for sequencing, contains highly frequented sequences existing in diverse copies in the genome and the sequences of this gene are available in molecular databases, as well as, it is highly important in the epidemiological and phylogenetic studies of pestiviruses (32, 33).

For the first time, two genotypes were detected in this current study with prevalence rates 12.95%, 1.01% for BVDV1 and BVDV2 in Nineveh province respectively. This finding may be attribute to the fact that BVDV1and BVDV2 have worldwide distribution. This finding corresponds with other studies in different countries such as in Iran,Turkey and Tunisia (23,34,35). Meanwhile, this results inequality with previous document made in another province of Iraq, such as Jarullah, et al (19) who detect BVDV genotype 1 only in Basrah and Nassirya cities in Iraq.

It has been found that a significant difference in the prevalence of BVD among the age of infected cattle was indicated, Since, the lowest prevalence was recorded in young calves <6 months, which might due to passive immunity arising from the immunocompetent dam. This finding was in a sporting data with (36). In addition, the current study was also indicated a high prevalence recorded in age between >6 month- 2years, which agree with previous studies (37, 38). The reasons could be that the productivity concerning by producer, and herd size. Large herds more animals are the main source of production and are more encouraging for monetary benefit by the producers in Nineveh province. In contrast, Wilson, et al., (39) reported that animal ages have no effected values.

The current study showed a significant difference in the prevalence of BVD between male and female animals. This finding was also reported by (37). This may explain that the bovine males are more predominant than females in Nineveh province, especially as a feedlot type (meat production animals) which are on a maximal monetary account. In addition, it might also due to the age of animals imported from other countries where the disease is endemic (21, 40). In contrast, Haji et al., (41), reported higher prevalence in female cattle than males and attributed the reason to the age of animals. However, Wilson, et al.,(39) revealed no significant differences according to animal sex. Furthermore, Houe, et al. (28) revealed that both sexes play important role in transmission of disease either vertically or horizontally.

The results showed no significant difference in the prevalence of BVD between adult cow and young calves breed,Same results was mentioned by (42). However, Results were also indicated a significantly different among imported cattle than native animals and between feedlot breeds was reported. The prevalence of the disease was highly among Iranian, Syrian and Turkey breeds compare to native, due possibly to the fact that the existence of PI in these animals and immunosuppression , stress condition during transportation, most of beeflot calves in Nineveh province are imported from Iran, Syria and Turkey in which the disease is prevalent (43,44,45).

The study showed that the prevalence of BVD was significantly higher in large size herds compared to small size herds. This finding was consistent with (46,47). This disparity in the results may be due to the direct pasture contact, continual purchasing new animals, density, heavy production, calf mortality, use of natural breeding, a larger number of cattle could suggest a higher probability of exposure to infection. Furthermore, presence of other animal species such as sheep and goat. The same result was obtained by (48,49) as they found that BVD can infect a variety of other species that are in natural contact with cattle poses a risk for reinfection of pestivirus-susceptible cattle populations, and may lead to interspecies transmission and increase the incidence of Pestivirus infections.

The current work demonstrated that the prevalence of BVD infection was significantly affected by geographical regions in Nineveh, As, higher prevalence of the disease was detected at Gogjalee region compared to other regions. Regions differences could in part be explained by factors such as high cattle population density, close distances between animals, poor management, animal movements or livestock trade, largest animal markets, meeting between the owners and attenders, climatic factors and possible number of PI animals. These findings are in agreemet with previous studies (50,51).

The present study revealed that the prevalence of BVD disease was also significantly affected by the seasons. As, the prevalence of the disease was significantly higher in winter and spring compared to summer season. The same data were also indicated by (21,52). The reasons may be the stressor effects of cold weather in winter, heavy rainfall, high velocity wind, the difference between night and day temperature, overcrowding and sharing of cattle housing which lead to direct contact between the animals.

مرض الاسهال البقري الحموي والاصابات الدائمة في الابقار في محافظة نينوى ، العراق

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الخلاصة

اجريت الدراسة الحالية لتحديد نسبة حوث وانتشار مرض الاسهال البقري الحموي فضلاًعن الاصابات الدائمة في الابقار باستخدام فحص المقايسة المناعية مرتبطة المستضد وفحص سلسلة البلمرة المتعدد المعكوس كفحوصات تشخيصية فضلاً عن تحديد مورثات حمة مرض الاسهال البقري الحموي ، كما تم استسقاء بعض عوامل الخطر الوبائية والمرتبطة بحدوث المرض . امتدت الدراسة من شهركانون الثاني الى اب ٢٠١٧ ، أذ تم فيها فحص (٤٩٤) عينة من ثلمات الأذن الماخوذة من الحيوانات المشكوك بأصابتها والتي مثلت (ابقار بالغة العمر ١١٢،عجول صغيرة العمر ٣٠ وعجول فتية لاحمة التسمين٣٥٢) ، علماً ان الحيوانات موجبة نتيجة الفحص تم اعادة فحصها مرة ثانية بعد مرور أربعة اسابيع وذلك للكشف عن الاصابات الدائمة . استحصلت جميع البيانات الوبائية بوساطة سؤال مالكي الحيوانات. تم الكشف عن مورثات حمة الاسهال البقري الحموي بواسطة استخلاص المنطقة الخامسة غير مترجمة الانضمام 5UTR gene. اكدت نتائج الدراسة ان النسبة المؤوية للانتشار العام للمرض هي ٥.٤٦% و ١٣.٩٦% على التوالي ولكلا الفحصين التشخيصيين ،كما كانت النسبة المؤوية للاصابات الدائمة ٠.٠٨% لكلا الفحصين ايضاً وطبقاً لقيمة كابا ٠.٤٥٧ فأن اختبار سلسلة البلمرة المتعدد المعكوس كان اكثر كفاءة تشخيصية بالمقارنة مع فحص المقايسة المناعية مرتبطة المستضد . كما اوضحت نتائج الدراسة الكشف عن نوعي موروث مرض الاسهال البقري الحموي ١ و٢ وبنسب موؤية (١٢.٩٥% و ١.٠١%) على التوالي. كما تبين من نتائج الدراسة ان للمرض تاثيرات معنوية واضحة على عمر الحيوان المصاب ، الجنس ، السلالة ، عملية الاستيراد ، حجم القطيع ، منطقة تواجد الحيوان فضلا عن اختلاف فصول السنة الواحدة . استنتج من هذه الدراسة ان مرض الاسهال البقري الحموي مستوطن في محافظة نينوى بموروثيه ١و٢ وهناك عوامل وبائية متعددة قد تلعب دور مهم في انتشارة، عليه فأن تطبيق البرامج الوقائية والكشف المبكر عن الاصابات الدائمة يجب اعطائها الاولوية .

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