



## Prevalence of border disease virus in sheep and goats in Mosul, Iraq

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### Abstract

Globally, Border disease virus (BDV) has caused substantial economic losses among small ruminants (sheep and goats). This is the first molecular study carried out in Mosul city, Iraq. To determine the prevalence of Border Disease Virus and to examine problem of persistent infection (PI) using Reverse transcriptase polymerase chain reaction technique (RT-PCR) in female local breed of small ruminants. During the period between November 2018 to June 2019, 364 blood samples were collected from 264 local Awassi sheep and 100 local cross breed goats secure provided by private breeders. The animals were of  $\geq 1.5$  years old and the samples were obtained from various locations in Mosul city, with varying rearing methods and had not been vaccinated against BDV. This investigation indicated that the prevalence of BDV infection in sheep and goats were 15.9% (42/264) and 3% (3/100) respectively, whereas the occurrence of PI in sheep was 2.38% and in goats was 0%. Hence it was concluded that Border disease was circulating in small ruminants in Mosul city. This calls for a need to design programs to monitor and control the disease and eventually eradicate it is prevalence in Mosul city.

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### Introduction

Border disease virus (BDV), is an important infectious agent in sheep and goats causing serious economic losses (1). Four species of the genus *Pestivirus* within the family Flaviviridae have been recognized including BDV of sheep and goats, bovine viral diarrhoea virus 1 and 2 of cattle and classical swine fever virus of swine (2). Novel Pestivirus species, like Giraffe virus, Pronghorn virus, Bungowannah virus and HoBi-like virus were isolated (3,4). Currently, an increase has been noted in the number of unspecified atypical pestivirus species reported in Europe, the Americas, and Asia (5). A novel taxonomy was proposed for the genus *Pestivirus* (family Flaviviridae) in which all species were renamed as A to K Pestivirus (6). Economically, all pestiviruses are important pathogens implicating many post-natal and pre-natal infections with losses in cattle, swine and sheep populations (7,3).

The *Pestivirus* genome is about 12.3 kb, comprising one open reading frame between the 5-untranslated regions

(UTR) and 3-UTR encoding N-terminal autoprotease (Npro), capsid protein (C), Three envelope proteins (E<sup>ms</sup>, E1 and E2), p7, and six unstructured proteins (8,9). RNA is initiated internally by non-cap system via a type IV internal ribosomal entry area in the vicinity of the 5-noncoding region of the virus genomic RNA (10). Two biotypes of pestiviruses have been distinguished based on their propagation in the cell culture as cytopathic (CP) or non-cytopathic (NCP) (11). Border disease virus is in turn separated into four sub-groups according to its antigenic characteristics and host species (12). Other than these subgroups, two new sets were established as BDV5 and BDV6 based on the pestivirus isolates in France (13). The disease causes sterility in ewes, and is also responsible for abortion, stillbirth and lambs born weak and undersized, exhibiting tremor, uncharacteristic body shapes, hairy fleeces, and PIs of the young. BDV has also been reported to cause mucosal disease-like damage in sheep (14,15).

An infection that occurs between the 50<sup>th</sup> and 60<sup>th</sup> day of gestation may lead to birth of immunotolerant PI lambs

that will continue shedding the virus for their whole lifetime and be the most significant source of BDV among ruminants (14-17). It should be noted that as there is no proven effective BDV vaccine commercially available to date, detecting and removing of PI animals is critical for any BDV control approach to succeed (18). Serological investigations have revealed a global spread of BDV. The rates of seroprevalence in sheep are from 5 to 50%, based on country or territory of investigation (19). Serological investigations for BDV in Iraq have reported prevalence of 30.35% and 46.9% in sheep (20,21). Diagnostic work in the laboratory focuses on detecting BDV by isolating the virus and immunoassay inclusive of antigen capture ELISA, immunofluorescence or other immunohistochemical approaches (22,23). Numerous regions of the viral genome have been utilized for genetically typing pestiviruses. Currently, the 5' UTR, N<sup>pro</sup> and E<sup>2</sup> regions are most often utilized (24). Due to the 5-UTR being comparatively highly conserved, it is utilized for defining the pan-pestivirus reactive primer regions and is often employed to investigate genotypes (13). The RT-PCR has been successfully used as a molecular tool for researching and diagnosing BDV infections. This has made it possible to detect pestivirus RNA in a range of clinical samples including blood, tissues, serum and swabs (25).

No molecular report exists of ovine and caprine border disease linked to this virus in Mosul city. Therefore, the aims of the present investigation were to verify the prevalence of BDV and PI in local Iraqi sheep and goats in Mosul city by using reverse transcriptase polymerase chain reaction technique (RT-PCR).

## Material and methods

### Animals and samples collection

Samples for the current study were obtained from various parts of Mosul city, Iraq including Gogjalee, Abo jarboaa, Al- Hamdanyah, Bartilla, and Bashiqa with different management systems, represented by 20 local

Awassi sheep and local goat flocks ( $\geq 1.5$ -year-old) in the period between November 2018 to June 2019. Most of studied farms practiced the semi-intensive and intensive production system of sheep and goat, and the majority of these farms raised and reared their animals close to beef and dairy cattle farms. The flocks sizes ranged from 15 to  $\geq 40$  animals.

Altogether 364 blood samples were obtained from 264 local female Awassi sheep and 100 local female cross breed goats provided by private breeders, with 20% of the animals from each farm selected at random and marked. The animals were apparently healthy or had a history of reproductive issues, respiratory problems and/or diarrhea. Furthermore, it was verified that BDV vaccination had never been administered in any of these farms. Blood samples from each animal were obtained from the jugular vein in sterile vacutainers containing ethylene diamine tetra acetic acid (EDTA) using different needles and taken in ice to the laboratory. Samples were stored at  $-20^{\circ}\text{C}$  until laboratory assays.

### RNA extraction and RT-PCR amplification

The RNA was extracted from the 364 the whole blood samples using the abm ulRNA Column Purification Kit (Canada. No.G487). The procedure was performed according to the manufacturer's instructions. The RNA concentration and purity of samples were verified with the Nanophotometer (RNA- analyzer) (BioDrop. Germany).

The next step was the amplification of the extremely conserved area 5' UTR gene of BDV from blood samples (n=364), as a focus in the PCR process in which a positive cDNA was obtained from PI sheep following previous work which used them as positive control (21). Furthermore, cDNA obtained from healthy sheep was utilized as a negative control for amplifying every PCR.

In this investigation, the oligonucleotides of particular primers were designed by Vilcek and Paton (26), and sourced from First BASE Laboratories Sdn. Bhd. Malaysia (Table 1).

Table 1: Oligonucleotide primers utilized in the amplification of the 5'UTR gene

Primers	Sequences 5'-3'	Target gene	Expected size (bp)
BD-F	5'TCGTGGTGAGATCCCTGAG 3'	Specific primers BDV	225
BD-R	5'GCAGAGATTTTTTATACTAGCCAGCCTATRC 3'		

One-Step RT-PCR Kit (Canada V6V 2J5) was performed in the thermocycler machine (Optimus 96G - UK) in a last volume of 50  $\mu\text{L}$  with 25 $\mu\text{L}$  2X One-Step RT-PCR Buffer, 1  $\mu\text{L}$  OneScript® (200 U/ $\mu\text{L}$ ), 2  $\mu\text{L}$  Bestaq<sup>TM</sup> DNA Polymerase (5U/  $\mu\text{L}$ ), 2.5  $\mu\text{L}$  Forward Primer (10  $\mu\text{M}$ ), 2.5  $\mu\text{L}$  Reverse Primer (10  $\mu\text{M}$ ), 6 $\mu\text{L}$  (2 $\mu\text{g}$ ) of Template (RNA sample) and 11 $\mu\text{L}$  of Nuclease-Free H<sub>2</sub>O. The thermal cycling conditions for the PCR amplification are presented in Table 2.

The analysis of final PCR products was applied using agarose gel electrophoresis and observable on 1.5% agarose gel stained with ethidium bromide and compared with DNA markers (100 base pair ladder, Axon Scientific Sdn Bhd, Malaysia). The RT-PCR procedural stages stated above were preformed according to the manufacturer's instructions and the procedure of (27).

Table 2: Thermal Cycler Conditions for One-step RT-PCR

Step	Temp	Duration	Cycles
cDNA Synthesis	42°C	30mins	1
Initial Denaturation	94°C	3mins	1
Denaturation	94°C	30secs	
Annealing	55°C	45secs	36
Extension	72°C	45secs	
Final Extension	72°C	5mins	1
Holding	4°C	-	1

**Statistical analysis**

Variations in mean values of the groups were analyzed employing chi-square in IBM SPSS Statistics version 19.

Table 3: Prevalence of BDV and PI in sheep and goats infected with BDV

Test	Animal	Tested animals (n)	Positive animals (n)	First PCR round (%)	Second PCR round (%)
PCR	Sheep	264	42	15.9*	(1/42) 2.38
	Goats	100	3	3	(0/3) 0.0

\* Value was significant (P<0.05) between infected sheep and goats.

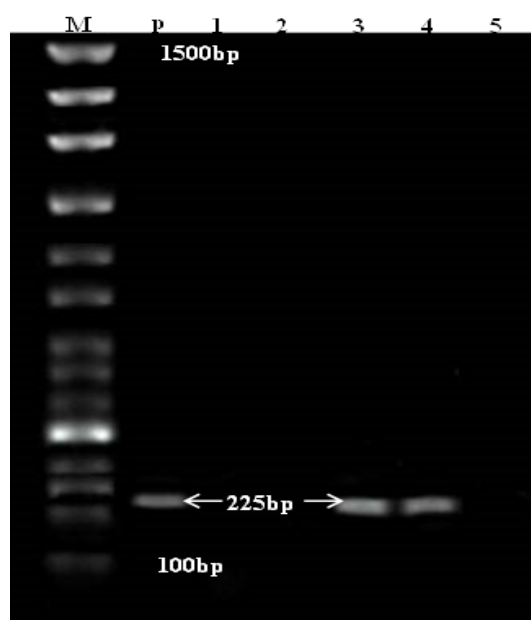


Figure1: Gel electrophoresis image indicating: lane M) Exact Mark 100-1500bp DNA ladder; Lane P) cDNA obtained from PI sheep utilized as positive control for; Lane 1, 4) BDV in approximately band size 225bp, (Lane 5) cDNA obtained from BDV-free animal utilized as negative control.

**Discussion**

This work is a pioneering molecular study carried out to find out the prevalence of BD and PI animals in local Iraq

**Results**

In total, 364 blood samples (264 from sheep and 100 from goats) were subjected to testing by RT-PCR technique for detection of BDV antigen, and 15.9% (42/264) and 3% (3/100) of the samples tested positive for BDV in sheep and goats, respectively (Table 3). To detect the possible existence of PI animals, all positive animals (42 sheep and 3 goats) were retested by RT-PCR after 21 days. The results showed that the prevalence of PI was (1/42) 2.38% in sheep whereas in goats was (0/3) 0.0% antigen positive (Table 3). For BDV, the positive bands were at nearly 225 bp (Figure 1).

sheep and goats in Mosul city employing RT-PCR technique. From the analysis of 364 blood samples, results indicated that the prevalence of border disease were 15.9% and 3% in sheep and goats, respectively and thus suggesting a high infection rate. This may be contributed to absence of vaccination and/or control programs in Mosul city, despite the occurrence of BDV being documented in previous studies (20,21). A novel introduction of BDV to the farms in Iraq is due to the presence of imported sheep, and goats from Iraq’s neighbors and other BDV-endemic countries such as Iran (27) and Turkey (28). This result agrees with what was mentioned by Hasan and Alsaad (29). Other possible causes also could be the presence of interspecies transmission between sheep, goats beef calves and dairy cows, which possibly plays a significant role as a contributor to the increasing occurrence of the disease. These findings correspond to the results of (30-33) who revealed that even though BDV is in general regarded as an agent for a sheep disease, but strictly speaking, it has no specific host and cross infection can take place between various domesticated animals and even non-domesticated species. Moreover, spread of BDV is also possible via the secretions and excretions of infected animals as well as through aborted fetuses, fetal membranes, and infected blood, sometimes the presence of PI animals in a herd considered as a factor in the spread of BDV. The various factors mentioned above have been the reason for the high prevalence of BDV reported in this study. The results mirror the findings of (28,34).

In this study, the prevalence of BDV in sheep was found to be significantly greater than in goats, this is likely due to the much larger sheep population than goat population in

the Mosul city. The findings of this study are supported in the literature by the reports from study by Albayrak *et al* (35) who finding indicated the seroprevalence of pestivirus were 3.31%, 2.46% and 6.40% in goats in various locations in Turkey and in Iran in sheep 79.4% and goats 70.9% respectively (36).

The outcomes from the current work indicated that the occurrence of BDV in Mosul was detected by utilizing a PCR technique are approximately the same or different from those indicated in other countries. In Turkey it was 22.2% (28), while, in Iran was 9% (27). In Spain the result ranged from 7.9-50% (37). The difference in the outcomes could be justified by several reasons such as varying production approaches, disparity in animal ages, the different methods of diagnosis employed, herd composition, existence and effectiveness of control initiatives, animal purchasing, the different climates, widespread trading and uncontrolled animal mobility, population size, and existence of PI animals. The outcomes of the present research match those of (38,39).

The outcomes of this investigation showed that the occurrence of PI in sheep and goats was 2.38% and 0%, respectively. In the majority of investigations done, the rates of PI sheep were from 0.3% to 20% (40). In contrast, Ouafaa *et al* (40) and Naouel Feknous1 *et al* (38) reported 0% PI percentage in both investigations on sheep to detect PI animals, attributing it to several factors such as small sample size besides numerous lambs being slaughtered young for economic reasons, lowering the possibility of detecting young PI animals when sampling. Lambs that are born immunotolerant have PI due to intrauterine infection in the first trimester of the gestation possess the BDV for life, are antigen positive and antibody negative, possess increased titer of BDV in their circulation and skin tissues that can be easily detected and are significant in maintaining and transmission of the causative agent between susceptible animal populations. This finding is consistent with Mao *et al* (41).

This study revealed low prevalence (3%) and no PI animal has been detected in goats. Probable reasons could be the small number of animals tested or the general fact that the BDV is rarely found in goats. These results agree with Oguzoglu *et al* (42). Previous studies have confirmed rare PI in goats and BDV infections in pregnant goats lead to abortions and malformations in fetuses and neonates (43-45).

The results of this study also indicate that the RT-PCR approach could be used to detect BDV and PI, through employing specific primers adjoining a 225 bp DNA fragment, selected from the 5' noncoding area of the pestivirus genome. This finding is in agreement with Vilcek and Paton (26). The study also revealed that the molecular technique is an accurate and sensitive method for detection and identification of the causative pathogen. This finding is consistent with what mention in literature of (46,47).

## Conclusion

As has been mentioned earlier, this is the first molecular report for detection of BDV in Mosul City, and from what has been mentioned above it can be concluded that this molecular study has verified that sheep and goats infected with BDV in Mosul, Iraq and probably endemic in the country. Monitoring and further research on BDV using epidemiology and phylogenetic analysis studies are required for the detection and isolation of the virus strains circulate in other Iraqi cities for understand the spread and effect of pestiviruses in the livestock.

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## Conflict of interest

Author declare no conflict of interests of the manuscript.

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تقنية تفاعل البلمرة المتسلسل العكسي في إناث السلالات المحلية من المجترات الصغيرة. خلال الفترة ما بين تشرين الثاني ٢٠١٨ وحزيران ٢٠١٩ ، تم جمع ٣٦٤ عينة دم من ٢٦٤ من الأغنام العواسية المحلية و ١٠٠ من الماعز المحلي تم تأمينها من حقول تربية القطاع الخاص من مواقع مختلفة في مدينة الموصل ومن حيوانات غير ملقحة ضد مرض الحدود. بلغ عمر الحيوانات  $\geq 1,5$  سنة. أظهرت نتائج الدراسة إلى أن نسبة انتشار المرض في الأغنام والماعز بلغت ١٥,٩% (٢٦٤/٤٢) و ٣% (١٠٠/٣) على التوالي، في حين كانت نسبة الإصابة الدائمة ٢,٣٨% في الأغنام و ٠% في الماعز. استنتجت الدراسة الحالية أن مرض الحدود منتشر في المجترات الصغيرة في مدينة الموصل. وهذا يستدعي الحاجة إلى تصميم برامج لمراقبة المرض والسيطرة عليه والقضاء في النهاية على انتشاره في مدينة الموصل.

## نسبة انتشار مرض الحدود في الأغنام والماعز في الموصل، العراق

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### الخلاصة

على الصعيد العالمي، يتسبب فيروس مرض الحدود في خسائر اقتصادية كبيرة في المجترات الصغيرة (الأغنام والماعز). يعد هذا التحري الجزئي الأول الذي يتم أجرأه للتحقق من نسبة انتشار فيروسي مرض الحدود والإصابة الدائمة في مدينة الموصل، العراق، باستخدام