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Molecular investigation of BVDV in cattle in Thi-Qar Province, Iraq.

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***Abstract*— Bovine viral diarrhea virus (BVDV) is a complicated ruminant pathogen that naturally infects cattle, goats, sheep, buffalo, pigs, camelids, and wild ruminants. BVDV infection causes serious financial losses, directly due to the high mortality and morbidity rates caused by fatal mucosal disease and immunosuppression. The BVDV can be divided, based on antigenic and genetic differences, into three genotypes: Bovine viral diarrhea virus 1 (BVDV-1), Bovine viral diarrhea virus 2 (BVDV-2), and Bovine viral diarrhea virus 3 (BVDV-3). From September 2022 to May 2023, blood samples (225) were randomly collected from unvaccinated cattle from various regions within Thi-Qar province, Iraq. By-nested RT-PCR results showed that the dissemination of the bovine viral diarrhea virus in Thi-Qar province was 19.11% (43 out of 225). The infection rate of BVDV genotype I was 17.33%, while that of BVDV genotype II was 1.78%.**

Keywords— Bovine viral diarrhea virus, Nested PCR, Cattle, RNA.

I. INTRODUCTION

Bovine viral diarrhea (BVD) is one of the most serious infectious diseases of cattle, globally-distributed [1]. The BVDV belongs to the Flaviviridae family under the genus Pestivirus, which also contains the border disease virus of sheep, the classical swine fever virus of pigs, and several newly found atypical pestiviruses [2&3]. The BVDV virions exhibit small, nearly spherical shapes with a diameter ranging from 40 to 60 nm. The particles consist of an outer bilipid layer envelope with no discernible spikes surrounding an electron-dense capsid. The envelope glycoprotein includes envelope 1 (E1), envelope 2 (E2), and envelope ribonuclease (Erns) [4&5]. The genome has a single positive sense strand of RNA that is 12.3 kb long and enciphers an open reading frame (ORF) surrounded by un-translated regions (5′ and 3′ UTRs) [6]. The ORF codes a polyprotein composed of around 4000 amino acids that are co- and post-translationally cleaved by cellular and viral proteases to four structural (capsid protein C, Erns, E1, E2) and eight nonstructural proteins (N-terminal protease (Npro), protein 7 (P7), nonstructural protein 2 (NS2), nonstructural protein 3 (NS3), nonstructural protein 4A (NS4A), nonstructural protein 4B (NS4B), nonstructural protein 5A (NS5A), nonstructural protein 5B (NS4B) [7&8].

The BVDV can be divided, based on antigenic and genetic differences, into three genotypes: Bovine viral diarrhea virus 1 (BVDV-1), Bovine viral diarrhea virus 2 (BVDV-2), and HoBi-like pestivirus (BVDV-3), an atypical ruminant pestivirus [9&10]. In cell culture, the BVDV can have two biotypes either cytopathic (cp) or non-cytopathic (ncp). Furthermore, non-cp biotypes do not produce cell lysis while cp biotypes cause apoptosis in cultured cells [11, 12 &13]. Noncytopathic BVDV can cause persistent infection (PI) in calves after infecting the fetus between 40 and 120 days during pregnancy and always involved in severe clinical acute infections. Cytopathic BVDV strains are uncommon and frequently linked to mucosal disease outbreaks [14&15]. BVDV infection results in a wide variety of clinical manifestations such as gastrointestinal, respiratory, and reproductive diseases in cattle [16, 17, 18 & 19]. Also, congenital malformations include cerebellar hypoplasia, hydrocephalus, ocular degeneration, skeletal malformations, and growth retardation [20, 21, 22 & 23]. Immune dysfunction may also result from BVDV infections, which puts cattle at risk for additional diseases that reduce reproduction and general health [24 &25].

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II. Materials and Methods

A. *Samples collection:*

A total of 225 blood samples were randomly collected from unvaccinated cattle from various regions within Thi-Qar province [Al-Nassiriya, Al-Shatra, Al-Rifai, Al-Gharaf, Al-Nasr, Al-Fahud, and Al-Dawaya], Iraq, extending from September 2022 to May 2023, as shown in table [1]. The ages of the cattle ranged from 1 month to 10 years, both sexes, and different clinical signs.

Table (1): Number of samples from different regions during study months

Month	Nassiriya	Shatra	Rifai	Gharaf	Nasr	Fahud	Diwaya	Total
September	4	5	2	3	3	2	3	22
October	5	4	3	2	3	3	3	23
November	6	6	3	3	2	3	4	27
December	5	7	3	4	3	4	2	28
January	8	5	4	3	3	3	3	29
February	7	6	3	4	3	3	3	29
March	6	9	3	3	4	4	4	33
April	9	8	4	3	4	3	3	34
Total	50	50	25	25	25	25	25	225

B. *Viral RNA extraction:*

The RNA of BVDV was extracted using the EasyPure® Viral DNA/RNA Kit (Transgen, Biotech, China). The procedure was performed according to the company's instructions. Figure 1 represents the RNA that was extracted from blood samples.

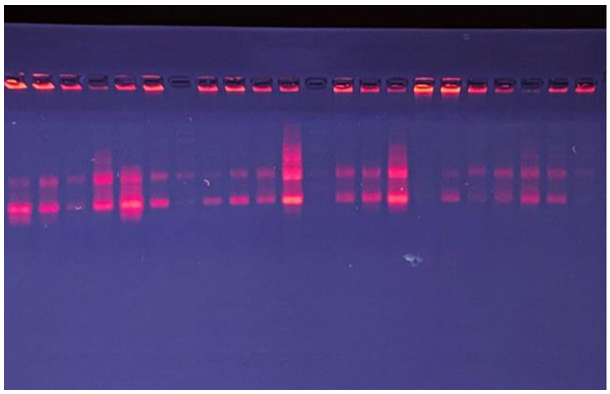

Figure (1): Agarose gel electrophoresis (Agarose 3 %, at 110 voltage for 40 min) stained with Ethidium Bromide dye showed a clear bands which represent DNA and RNA molecules that extracted from whole blood samples.

C. *RT-Nested-PCR(RT-nPCR):*

The primers were synthesis by Alpha ADN, Canada, for primary RT-PCR, were the external primers, 5'-AAGATCCACCCTTATGAGC-3' and 5'-AAGAAGCCATCATCACCCACA-3', which came from nucleotides 10385 to 10404 and 11528 to 11547, respectively [26]. For secondary PCR, the nested PCR primers were 5'TGGAGATCTTTCACACAATAGC-3' (BVDV-1specific),5'GGGAACCTAAGAACTAAATC-3' (BVDV-2 specific), and 5'-GCTGTTTCACCCAGTTAGTACAT-3', which came from nucleotides 10758 to 10779, 10514 to 10533, and 11096 to 11117, respectively [27]. A two-round, rapid-cycle RT-nPCR assay was performed according to instruction of company manufacturer by using TransScript® II One-Step RT-PCR SuperMix kit (TransGen Biotech, China).

Reverse transcription was carried out at 45°C for 30 minutes, followed by denaturation at 94°C for 3 minutes. The primary PCR reactions were cycled 40 times at 94°C for 40 seconds, annealing of primers at 57°C for 30 seconds and extension at 72°C for 90 seconds with final extension at 72°C for 10 minutes. The products were then used as template in a secondary PCR for 40 cycles. This was performed in the same manner as the primary PCR, but with multiple primers and without reverse transcriptase, RNase inhibitor and external primers. The amplified products were electrophoresed on a 2% agarose gel and stained with ethidium bromide then visualized by using ultraviolet transilluminator.

III. Results and Discussion:

Nested RT-PCR were done for (225) blood samples. Totally, 43 (19.11%) out of 225 samples revealed positive results for BVDV. The first product of PCR revealed that the positive bands were at 615 base pair (bp) [Fig. 2], while the second product was at 360 base pair (bp) [Fig. 3]. The detection rate of BVDV genotype 1 was 17.33%, while that of BVDV genotype 2 was 1.78%.

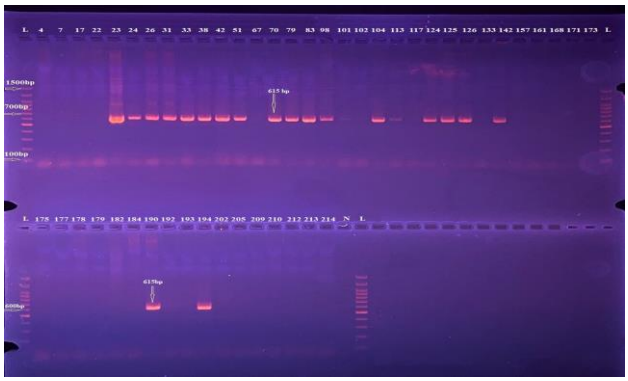

Figure (2) :- Agarose gel electrophoresis for PCR product of nested external primers (F –R) showing the bands at 615 base pair, Lane L : DNA ladder (100-1500bp), Lanes (11-27,38-44,55,57,62-67,71,126 and 142) represented positive results, Lanes (4-7, 31,45,51, 59,70,79-85, 102-125,138,140 156-212) represented negative results and lane N represent negative control .

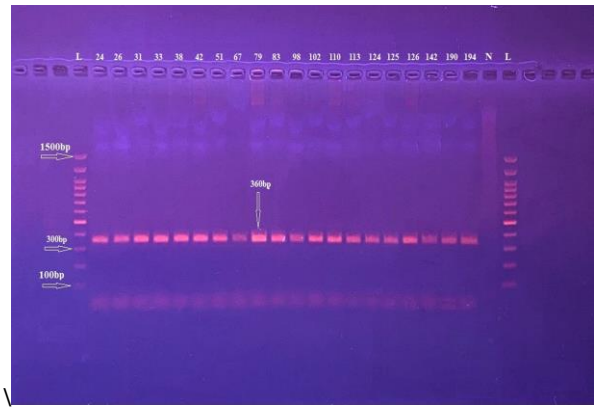

Figure (3) :- Agarose gel electrophoresis for PCR product of nested multiplex primers sets including BVDV-1 specific, BVDV-2 specific and secondary primer showing the bands at 360 base pair, Lane L: DNA ladder (100-1500bp), Lanes (24-194) represented positive results, and lane N represent negative control.

The bovine viral diarrhea virus (BVDV) has been linked to decreased meat and milk production and reproductive efficiency in cattle all around the world. BVDV-1 and BVDV-2 are the two kinds of BVDV that have been discovered globally. To determine the genetic diversity of the viruses, molecular epidemiology investigations should be carried out in every nation in the world [28]. The present study results revealed that the overall prevalence of BVDV was 19.11% in cattle herds in Thi-Qar province, Iraq, in contrast with earlier studies conducted in Iraq and other countries using the PCR method to identify this illness. Cattle in the areas around Baghdad had a 6% prevalence [29], and the frequency of BVDV was 10% in the Iraqi cities of Nasiriya and Basrah [30], while the prevalence of BVD in Nineveh province was 13.96 percent, as indicated by Sadam and Alsaad [31]. However, it was shown that different countries around the world had varying prevalence rates: in China, 40.72% [32], in the United States, 8.0% [33], and in Indonesia, 11.74% [34]. As well as from neighboring countries like Iran 18.49% [35], Turkey 11.4% [36], and Egypt 17.2% [37]. This may be caused by various management strategies used in various regions of the same country, sample size, mixed breeding strategies for animals, particularly those that serve as sources of BVDV infection, climatic variations, the persistence of BVDV, biosecurity, the efficiency of vaccination programs, and the sensitivity and specificity of diagnostic techniques.

The present investigation identified two genotypes, with prevalence rates for BVDV1 of 17.33% and BVDV2 of 1.78%. The global dissemination of both genotypes and the predominance of BVDV1 strains in the majority of world populations are potential contributory causes [38]. This result is similar to an earlier study by Hasan and Alsaad [39], who discovered two genotypes in the province of Nineveh with prevalence rates of 12.95% for BVDV1 and 1.01% for BVDV2. BVDV-1 has only been identified in the Iraqi provinces of Basrah and Nassirya, according to Jarullah et al. [30]. These two genotypes were also discovered in Turkey and Iran [28, 40]. According to the phylogenetic analysis of BVDV in various countries, the global distribution rate of BVDV-1 was 88.2%, which is significantly greater than that of BVDV-2, which was 11.8% [41].

IV. Conclusion

The conclusions of this study indicate that BVDV infection is present among cattle herds in the Thi-Qar regions within Iraq. Epidemiological studies must be performed at the national level to confirm the disease's spread and to identify animals with persistent infection. Gene sequencing for local isolate in future studies to examine the transmission patterns among ruminant species in Iraq, including cattle. It has been demonstrated that BVDV may infect a variety of ruminant animals, including sheep, buffalo, and camels.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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