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PCR for Detection of Ovine Herpesvirus-2 in Cow and Sheep of Iran

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Abstract: Malignant Catarrhal Fever (MCF) is one the most important viral diseases of livestock. Aetiologically, there are 2 forms of the disease, one is associated with Alcelaphine Herpes Virus-1 (AlHV-1) of the wildebeests and the other (SA-MCF) is associated with Ovine Herpes Virus-2 (OvHV-2). In order to detect the epidemiological condition of the disease (Ovine herpes virus-2 in cattle and sheep) in Iran, we collected 100 whole blood samples from clinically ill cattle with MCF, healthy cattle and sheep (more than one year-old). The specimens undergone PCR method in several stages. The 1st stage was performed by pair primers 556 and 775. The reviced a result a band of 422 DNA base pair in 100% of infected cattle and sheep and 75% in healthy cattle. In the 2nd stage, using pair primers 556 and 555, in the Semi-nested PCR, 3 bands of base pairs 238, 340 and 420 of DNA were traced in the specimens. It is noteworthy, that the specimens colleted from sheep and clinically infected cattle with MCF, had all three bands and the healthy cattle specimens had only the 2 DNA band of 420 and 340, which were belonged to OvHV-2 genome.

Key words: Ovine herpes virus-2, sheep, cattle, PCR, semi-nested PCR, Iran

INTRODUCTION

Malignant Catarrhal Fever (MCF) is a sporadic but almost invariably fatal viral disease affecting many species of Artiodactyla and is characterized by a generalized lymphoadenopathy and mucopurulent nasal and ocular discharges. This disease has worldwide distribution. MCF is also defined by the appearance of characteristic lymphoid cell aggregates in non-lymphoid organs, vasculitis and lymphoid hyperplasia in lymphoid organs and can be caused by several viruses of the gammaherpesvirus family (Murphy *et al*., 1999). There are at least 2 herpesviruses implicated in the etiology of MCF. Wildebeest-associated or African MCF is caused by infection with Alcelaphine Herpesvirus-1 (AlHV-1). AlHV-1 is spread to susceptible ruminants from wildebeests, which are asymptomatic hosts for the virus. Sheep Associated MCF (SA-MCF) predominates in Europe and North America, where it is often found in cattle or bison in contact with sheep (Muller-Doblies *et al*., 1998; Hussy *et al*., 2002).

Histopathological examination is the most widely used diagnostic procedure to confirm clinical suspicion of SA-MCF. Lymphocytic infiltration and vasculitis in the brain and other organs are the most significant lesions (Rovnak *et al*., 1998). In 1990, a DNA sequence with high homology to AlHV-1 was discovered in lymphoblastoid cells from SA-MCF-diseased ruminants and strongly implicated the corresponding virus, the putative OvHV-2, with the etiology of SA-MCF (Murphy *et al*., 1999). Subsequently, Baxter *et al*. (1993, 1997) developed a PCR protocol to demonstrate OvHV-2 DNA.

Nowadays, PCR method is primarily used for detecting DNA of ovine herpes virus 2 in cattle and sheep. In some countries the presence of this virus has been confirmed by this method (Kleiboeker *et al*., 2002). But so far, there has not been conducted any studies about it in Mid. East region and Iran, therefore in this study we detected the OvHV-2 genome through report Seminested PCR.

MATERIALS AND METHODS

Samples: Total 100 specimens of whole blood containing EDTA anticoaggulative agent were colected. The samples were obtained from: cattle with clinical symptoms of MCF

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(n = 14), healthy cattle (n = 36) and healthy sheep in a age range of more than one year (n = 50). The samples were taken from the stocks of different parts of the country, namely Chaharmahal-o-Bakhtiyari, Isfahan, Tehran and East Azarbaijan provinces of Iran.

Sample preparation: EDTA-blood samples (10 mL) were centrifuged at 18°C for 35 min at 1,400×g. Buffy coat cells were resuspended in 4 volumes of sterile 0.2% NaCl to lyse erythrocytes. After 1 min, 7.2% NaCl was added to reconstitute isotonicity. The cells were further washed in phosphate-buffered saline and stored at -20°C (Muller-Doblies *et al*., 1998).

DNA was purified from PBLs by proteinase K digestion and phenol-chloroform extraction, dissolved in water, and quantified by measuring the optical density at 260 nm (Laird *et al*., 1991).

PCR: A 2-step PCR amplification was used with previously described (Li *et al*., 1995). Primer set 556 (5-AGTCTGGGGTATATGAATCCAGATGGCTCTC-3) and primer set 775 (5-AAGATAAGCACCAGTTATGCAT CTGATAAA-3) in the first step and primer sets 556 and 555 (5-TTCTGGGGTAGTGGCGAGCGAAGGCTTC-3) in the 2nd step. All amplification reactions were performed in a 50 μL volume containing 5 μL 10X PCR buffer); 2 mM MgCl2; 200 mM (each) dATP, dCTP, dGTP and dTTP (Roche Applied Science, Germany Co.); 1 μM (each) primer and 1 U of Taq DNA polymerase (Roche Applied Science, Germany Co.). Thermal cycling conditions for both steps were 5 min at 95°C and then 34 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min; this was followed by a final 10 min at 72°C for extension. Five microliters of the PCR mixture from the first step was used as target for the amplification in the 2nd step. For product 336+visualization, 10 μL of the amplified PCR products was analyzed by 1.5% agarose gel electrophoresis and ethidium bromide staining (Li *et al*., 1995; Hussy *et al*., 2001).

RESULTS

Figure 1 shows a PCR product of samples using a pair primer 775 and 556. As shown in the Fig. 1, the band 422 is from samples contained Ovine herpes virus-2 genome. Of from 14 cattle with clinical symptoms of MCF, all 14 samples entirely (100%) and also all of the 50 samples from healthy sheep (100%) had the band 422 bp pertaining to the DNA of Ovine herpes virus-2 (Columns 2, 3, 4, 5, 6 and 7 in Fig. 1). But of 36 healthy cattle, only 27 cases (75%) were positive for OvHV-2 genome (Column 6 and 7 in Fig. 1).

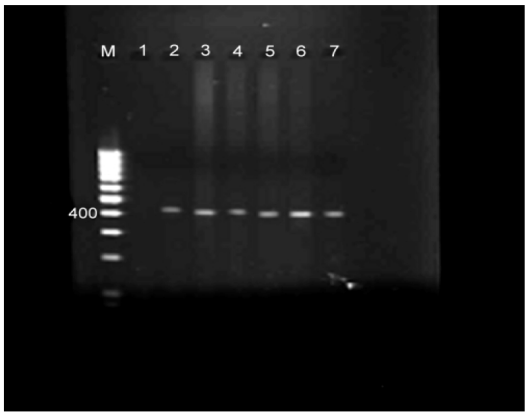

Fig. 1: PCR products samples using pair primers 556 and 775 (Column m: DNA 100 bp Marker; Columns 1: Negative control, Columns 2 and 3: Samples from cattle with clinical symptoms; Columns 4 and 5: Samples from sheep older than one year; Columns 6 and 7: Samples from healthy cattle)

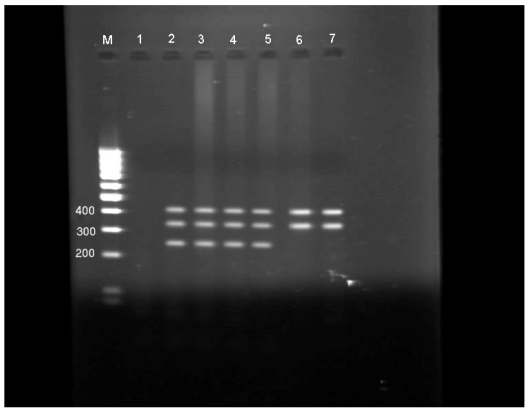

Fig. 2: Seminested PCR products samples using pair primers 555 and 556 (Column m: DNA 100 bp Marker; Columns 1: Negative control; Columns 2 and 3: Samples from cattle with clinical symptoms; Columns 4 and 5: Samples from sheep older than one year; Columns 6 and 7: Samples from healthy cattle)

In Seminested PCR method using 556 and 555 pair primers, there were three specific bands pertaining to Ovine herpes virus-2 genome in the weights of 238, 340 and 420 bp in the tested samples (Fig. 2). All 14 tested samples from the cattle with clinical symptoms of MCF and from sheep had DNA bands of 238, 340 and 420bp. But in 27 samples from healthy cattle having DNA of Ovine herpes virus-2, there were only 2 bands of 340 and 420 bp (Columns 6, 7, in Fig. 2).

DISCUSSION

Today, the key roles of ovine carrier have been definitely determined as the main vector of gama-herpesvirus, which causes Malignant Catarrhal Fever in its form of SA-MCF. For this reason, application of techniques detecting the specific sequenses of Ovine herpes virus type II, has been increasingly welcomed by virologists as a reliable method for diagnosis of the infection. The recent investigation reveal a ubiquitous distribution of Ovine herpes virus type II and epidemiologically there is a further closely historical adaptation with sheep. The transmission patterns of two forms of the MCF, namely WA-MCF and SA-MCF, differ completely (Li *et al*., 1994, 2004). Therefore, programming for eradication of the disease in the different countries, the transmission pattern should be determined (Li *et al*., 1995). For this purpose, the present study has been conducted for the first time in Iran, in order to detect the transmission pattern and causative agent of MCF.As the result it is propose that transmission pattern of infected sheep and cows are in accordance with SA-MCF form of the disease in Iran. In some Australian and American strains, there has been impossible to detect MCF by OvHV-2 primers, so there has arisen suspicion that, in some non-African countries, MCF may has an etiology rather than OvHV-2 and AlHV-1 may be involved in the disease (Li *et al*., 1995, 2004).

According to the study of Li *et al.* (1998), the most of the lambs up to three-month old had not any infection of OvHV-2, but almost all them will catch the virus from the infected sheep in the age of one year and more (Li *et al*., 1998). Li *et al.* (1994, 1998) reported that, it is possible to have a OvHV-2 free sheep, on condition that before 3-month of age it has been isolate the sheep from the infected sherd.

Hua *et al.* (1999) demonstrated that the amount of the viruses in the nasal discharge is more than in the blood, so the direct transmission by nasal discharges is considered the most route way of infection in the susceptible animals.

Evaluation of nucleotide sequence of this virus has showed that, there are at least four region, having the attachment potential to the primer 555 from of them, the first two are more specific, so as the target DNA concentration decreases, the non-specific responses will be far less than before (Li *et al*., 1995).

The results of this study indicate the entire infected sheep with OvHV-2 were more than one-year old and it is in aggrement to the other findings of SA-MCF pattern in the other countries. The genetic diversity of the protein structure of OvHV-2 tegument 140 KDa has not yet precisely investigated, but it seems that this protein like the tegument of other herpes viruses may have high genetic diversity, so this can explain the absence of band 238 bp, in spite of presence of bands 420 and 340 bp. In this study, in 20% of apparently healthy cattle the band 238 bp was not detected. This may be either due to the genetically diversity of the tegument gene, or due to the absence of the sequences in the apparently healthy cases. Li *et al.* (1995) informed a protein that in spite of antiviral antibodies in serum, had been expressed, so some of the infected cases had not the chance to exhibit disease. As a result, on according to the time and efficiency of immune response the progression of the clinical symptoms may be faild.

CONCLUSION

Since, the main purpose of this study, however, was characterizations and determination of MCF herpes virus circulating in Iran, by this findings, it is now possible to confirm the presence of the SA- MCF form of disease. The results of this study may pave the way for the future researches, especially the more works on the genetic diversity, determination of different strains of the virus and providing more precisely plans for diagnosis in country.

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