DIAGNOSIS OF CARRIER STATE OF FOOT AND MOUTH DISEASE VIRUS IN VACCINATED AND UNVACCINATED CATTLE BY RT-PCR

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ABSTRACT

The aim of present work were to investigate the carrier state to foot and mouth disease virus in vaccinated cattle (vaccinated carriers) and unvaccinated cattle (unvaccinated carriers). Reverse transcriptase polymerase chain reaction (RT-PCR) technique was employed to detect FMDV from esophageal and pharyngeal fluid samples collected from both vaccinated and unvaccinated cattle. Results have been shown that the persistence of FMDV is significantly higher in unvaccinated cattle in comparison to vaccinated animals. It have been concluded that high vaccination trials were play a role in the elimination of carrier status from cattle.

INTRODUCTION

Foot and mouth disease (FMD) is an contagious, acute viral disease of cloven-hoofed animals such as cattle, buffaloes, sheep and goats,characterized by fever, lameness, depression, loss of appetite, moreover, the appearance of vesicles on the feet and in, or around, the mouthis considered the typical lesions of the disease[1]. The disease is caused by foot and mouth disease virus (FMDV), a member of*Aphthovirus* genus which is belonging to picornaviridae family,the viral particle is icosahedral in shape containing smooth surface with a diameter of about 30nm. Its particle composed of a single-stranded RNA of positive polarity, approximately 8500 nucleotides long[2].In general, Infected cattle clear the systemic infection within 8–15 days[1]. Following the resolution of acute infection, FMD virus can persist in the

oropharynx for years[3]. Animals from which live-virus can be recovered 28 days post infection are defined as persistently-infected[4] and up to 50% of FMD-recovered ruminants become persistently infected, irrespective of their vaccination status[5]. After virus clearance from blood and other affected sites, the virus, however, can be found in the nasopharynx of persistently infected ruminants and is associated with the basal layers of the epithelium[6,7]. However, virus isolation from carriers is intermittent.The mechanisms of persistence of FMDV in host species have not been described. There is experimental evidence that increasing the vaccine dose may reduce virus excretion and may also reduce the frequency of carriers in vaccinated cattle. This is confirmed by the observation that the probability of an animal becoming a carrier is higher in groups receiving a lower vaccine dose[8,9]. Although vaccination by itself does not produce carriers, vaccinated ruminants when exposed to FMDV some can become carriers (vaccinated carriers). When discussing vaccination policies as a mean of controlling FMD outbreaks, the perceived carrier risk appears to be the main argument against such policy. There is epidemiological evidence to support the hypothesis that carrier animals may be the origin of outbreaks of acute disease when brought into contact with susceptible animals[10].Reverse transcriptase - polymerase chain reaction (RT-PCR) technique has been used to detect FMD viral RNA in oropharyngeal fluid samples to identify carrier animals. These RT-PCR methods seem to work very well[11,12].

For many years, FMD has occurred periodically as outbreaks and sporadic cases in Basrah where the disease is considered as enzootic.Since 2002, vaccination campaign has become a routine practice in cattle. This has been applied once a year, however this is not sufficientto completely prevent or control the disease[13]. Foot and mouth disease in Basrah are registered before and might become an endemic contagious diseases therefore the aim of the present work were to investigate the carrier state in vaccinated and unvaccinated cattle.

MATERIALS AND METHODS

Animals and study design

One hundered and seventy clinically healthy local cattle breeds of different ages (eighty cattle were vaccinated and ninety were unvaccinated) reared in Basrah

governorate at the period started from September 2014 to September 2015 were used in present work. Esophagyeal and pharyngeal fluid samples were collected in sterile cups according to Siutmoller and Gaggero (1965)[14]. Briefly, the cup was passed through the mouth into the pharynx and by movements directed dorsally and laterally attempts were made to scrape quickly the surface epithelium of the pharynx and soft palate. The cup was then passed down into the upper portion of the esophagus and withdrawn, preferably after the animal had swallowed, depositing the pharyngeal scrapings together with mucus and saliva into the cup. Particular care was taken to ensure that each specimen contained some visible cellular material.Immediately after collection, each sample was mixed with an equal quantity of sterile phosphate buffered saline (PBS)and the mixture was then shaken vigorously.Samples were centrifuged at 4000 rpm for 10-15 min. Supernatants were kept at -20°C until use.

Viral RNA extraction

Viral RNA was extracted from supernatants usingQIAamp Viral RNA Mini Kit (Qiagen)following the manufacture'sinstructions. The RNA was kept at -20°C until use.

Oligonucleotide primers

The universal primers VN-VP1F (5′-AGYGCYGGYAARGAYTTTGA-3′) and VN-VP1R (5′-CATGTCYTCYTGCATCTGGTT-3′)were used for the one-step RT-PCR method[15]. These primers amplified an 821-bp dsDNA amplicon(Figure 1) covering the entire capsid protein VP1-coding sequence of FMDV serotypes O, A, and Asia 1. The primers were synthesized commercially by the Bioneer Company, Republic of Korea.

One-step RT-PCR for detection of FMDVs

DNA amplification was performed using AccuPower RocketScript RT-PCR PreMix Kit following the manufacture's instractions. Reverse transcription and PCR amplification were performed simultaneously in one tube using optimized components provided with the kit. The tube was firstincubated at 42°C for 30 min to synthesize cDNA, followed by initial denaturation step at 95°C for 5 min; then 35 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min. The reaction was then incubated at 72°C for 10 min as a final elongation step[15].

Gel electrophoresis

The amplified DNA was detected by gel electrophoresis. Ten microliters of the PCR product was loaded onto a 1.5% (w/v) agarose gel prepared in TBE buffer and stained with ethidium bromide. Ten microliters of 100bp ladder (BioLabs UK) were also loaded on the gel as a molecular weight marker to determine the size of the PCR product.The gel was visualized usingan ultraviolet transilluminator and photos were taken for documenting.

Statistical analysis

Statistical package for social science (SPSS) was used to analyze the data. Chi-sequare (X^2) test was used to assess the significance of differences between groups. P value ≤ 0.05 was considered to be statistically significant.
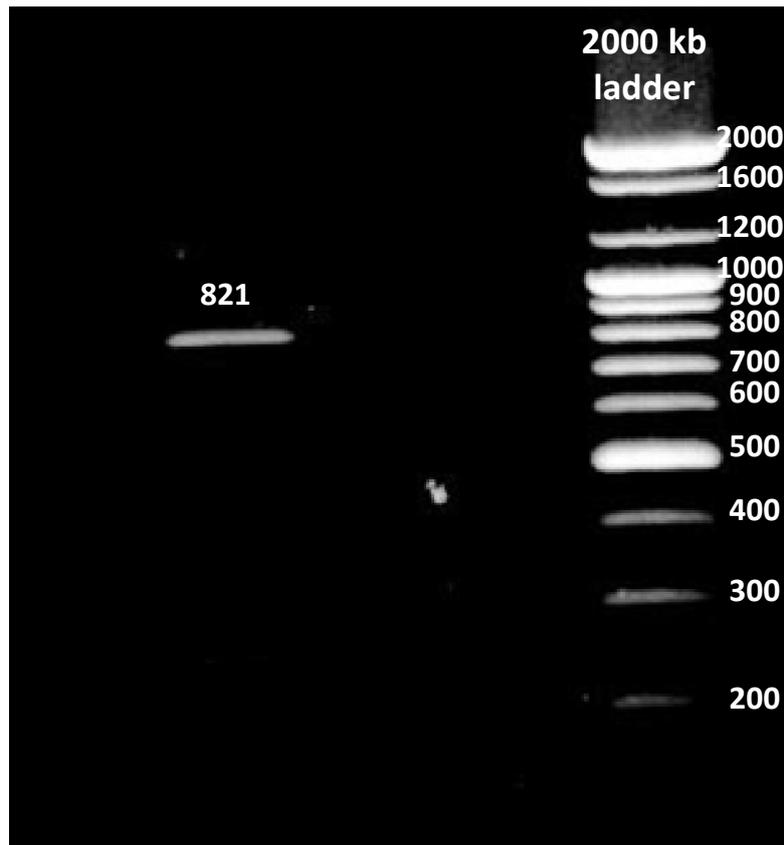
RESULTS

A total of170 esophageal/pharyngeal fluid sampleswas testedby one step RT-PCR using one pair ofa universal primer. FMDV was detected directly in38 out of 170 (22.6%) samples. FMDV was detected in 7 out of 80 (8.75%) of vaccinated cattle, and in 31 out of 90 (34.4%) ofunvaccinated cattle with FMD vaccine (Table 1).

Table1: Result of RT-PCR. The table shows the presence of FMDV in vaccinated and unvaccinated cattle. Significant difference was detected between the two groups (P<0.05).

Status	No.	Positive	%
Vaccinated	80	7	8.75
Unvaccinated	90	31	34.4
Total	170	38	22.6

(P<0.05)

Figure 1: RT-PCR product of FMDV visualized by ethidium bromide stained agarose gel electrophoresis. Positive result (821bp band) appears clearly on the gel.

DISCUSSION

Reverse transcriptase PCR (RT-PCR) is a more sensitive technique than virus isolation to detect carriers of FMDV and moreover easier to perform and less time consuming[16-20]. The polymerase chain reaction (PCR) can identify small quantities of viral genome present in the sample. The PCR identifies only part of the viral genome and would be positive even if the genome was itself fragmented and not associated with any live virus.

Foot-and-mouth disease (FMD) is one of the most important diseases of both a veterinary as well as an economic point of view. Outbreaks with devastating economic consequences still occur and remain a terrible threat to our country.The possibility of transmission of FMDV by persistently infected carriers is an important issue for the control of FMD[21]. Results showed that 22.6% of cattle are carriersfor the virus.Once animal become carrier can play a role in transmitting the disease to

susceptible contact animals. In Botswana, the prevalence of carrier African buffalo has been described to be very high (50–70%) under free-living conditions [22,23]. A survey in Asiatic Turkey found that 15–20% of cattle and sheep are carriers to FMD[24] and in Brazil, a survey for carriers detected around 50%[14]. The prevalence rate of carriers in a population depends on the species, the incidence of disease, vaccination coverage and the immune status of the population.

The current study has shown that the percentage of carriers of vaccinated cattle is significantly lower than those of unvaccinated. McVicar and Sutmoller[25] found that 14 out of 30 of vaccinated cattle and 19 out of 32 of unvaccinated cattle are persistently infected at day 28 after challenge.The variation in the number of carriers seen in the various studies may be due to the use of different assays.Effective vaccination can protect the development of the carrier state in cattle by reducing the prevalence of carrier animals under field conditions. Therefore, the strategy for eliminating carriers from a population should be to maintain high vaccination coverage for a sufficient length of time to ensure that the probability of any carriers remains low.

Further investigations is greatly required to fully understand the mechanisms involved and the role of carriers in the epidemiology of FMD.role in the elimination of carrier status from cattle.

تشخيص الحالات الحاملة لفيروس الحمى القلاعية في الابقار الملقحة والغير ملقحة بواساطة تفاعل سلسلة البلمرة العكسي(RT-PCR)

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الخلاصة

كان الهدف من الدراسة الحالية هو التعرف على الحالات السريرية للابقار الحاملة لفيروسالحمة القلاعية في الابقارالمحصنة (حاملات ملقحة) والابقارغير المحصنة (حاملات غير ملقحة). اذ تم استخدام تقنية تفاعل سلسلة البلمرة العكسي (RT-PCR) للكشف عن فيروس الحمى القلاعية(FMDV) من عينات سوائل المريء والبلعوم التي تم جمعها من كلا الابقارالملقحة وغير الملقحة. وقد أظهرت النتائج أن تواجد فيروس

الحمى القلاعية(FMDV) هو أعلى بكثير في الابقار غيرالملقحة بالمقارنة مع مجموعة الابقارالملقحة. استنتج من هذة الدراسة أن ممارسة تغطية تلقيح عالية يمكن أن تلعب دورا في القضاء على الحالات الحاملة بين الابقار.

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