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Original Article

Detection of clinical and subclinical Foot and Mouth Disease Virus in Cattle in Al-Najaf Province

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Abstract

Foot-and-mouth disease (FMD) is a highly transmissible disease caused by *Aphthovirus*s of the family *Picornaviridae*. This study aimed to investigate the serological approach (non-structural protein [NSP] analysis) of 3ABC enzyme-linked immunosorbent assay (ELISA) to diagnose FMD cattle in vaccinated and unvaccinated animals. A total of 84 serum specimens, including non-vaccinated, single-vaccinated, and multi-vaccinated samples, were collected from four districts in Baghdad Province, Iraq, to evaluate the antibodies to NSP of the FMD virus. The ELISA was used to detect antibodies (NSP) of FMDV in the serum of cattle. The result showed that the seroprevalence was estimated at 34% (29/84) in farm animals. The seroprevalence rates of FMD in relation to the age of infected animals were obtained at 21%, 7%, and 6% in 9-23-, 24-36-, and ≥ 36-month-old groups, respectively. The consequences of the examination of the sera from naive, immunized, and non-immunized infected farm animals applying 3ABC-ELISA were presented; accordingly, the incidence rates of FMD infection in non-vaccinated and vaccinated animals were 18 (75%) and 11 (18%) respectively. Negative results were recorded in the immunized group 49 (82%) higher than in the non-immunized group 6 (25%). Evaluation of NSP antibodies to isolate vaccinated animals from infected ones showed that the application of these assays was significantly useful for FMD prevention and control management programs in infected areas.

Keywords: Foot and mouth disease virus, Non-structural proteins, Vaccine

1. Introduction

Foot-and-mouth disease (FMD) is a contagious viral infection among numerous wild and domestic cloven-footed mammals and is an incredibly transmissible virus caused by *Aphthovirus*, a member of the family *Picornaviridae*. *Aphthovirus* spp. consists of 7 serotypes, including A, O, C, Asia 1, Southern African Territories (SAT) 1, SAT 2, and SAT 3 (1, 2). The structure of the viral genome encodes four structural proteins, including VP1, VP2, VP3, and VP4, and 10 non-structural proteins (NSPs). As there is no cross-immunity between serotypes, immunity to at least one kind does not confer protection against the others (3).

The virus often infects ruminants, and hosts include cattle, pigs, sheep, and goats. Serotype O FMD virus (FMDV) is responsible for about 70% of outbreaks globally. It has been reported that 6 of 7 serotypes are prevalent in Africa (O, A, C, SAT-1, SAT-2, SAT-3), 4 in Asia (O, A, C, Asia-1), and 3 in South America (O, A, C) (4). At some point of viral replication, antibodies against both the virion and the NSPs are produced. This led to the improvement of assessments for the detection

of the antibodies against NSP having the capability of distinguishing vaccinated from infected animals with the delivered benefit of detecting antibodies impartial of the need to be applied as the antigen of the infecting virus serotype. Vaccines ideally require the induction of antibodies, especially against structural antigens, because the used virus is inactivated and the compounds must contain small amounts or no NSPs. (5). At some stage in the producing technique of FMD vaccines, distinctive techniques are used for the identification and purification of the antigens (e.g., ultrafiltration, precipitation with polyethylene glycol, and chromatography), which usually reduce NSP (5). Those techniques permit the components of vaccines with excessive antigenic payloads; however, they can additionally pay attention to any NSP that can result in antibodies that intervene with the tests presently used for viral interest assessment (6).

Foot-and-mouth disease is characterized by fever and vesicles in the mouth and at the muzzle, teats, and toes of animals. Transmission can be by direct touch with infected animals, fomites, and devices, and possibly via inhalation, ingestion, and much more likely airborne approach. The incubation duration of FMD is variable and depends on the host, environment, course of exposure, and virus strain. The most common incubation duration is 2-14 days in farm animals. Inactivated virus vaccines protect against particular serotypes in the vaccine most effectively for 4-6 months. Billions of doses are used every year and protect animals against clinical infection; however, there is currently no viral persistence in the pharyngeal area; therefore, vaccinated animals may be carriers of infectious viruses (7).

Prevention and the management of the preliminary measures described in the FMD manage approach are the presence of early detection, caution systems, and the implementation of effective surveillance. The implementation of the FMD manipulate strategy varies from state to state and relies upon the epidemiological state of the affairs of the sickness as is well known; it is highly important for the livestock industry to adopt healthy biosecurity measures to prevent the emergence and spread of disease (8, 9).

In this study, the antibodies against the NSPs of FMDV in the serum of vaccinated and non-vaccinated cattle in Najaf Province in Iraq were detected using the enzyme-linked immunosorbent assay (ELISA) method.

2. Materials and Methods

2.1. Animals and Study Area

Serum samples gathered from 84 cattle from several animal organisms, including non-vaccinated, single-vaccinated, and multi-vaccinated, from 4 districts in Baghdad Province, Iraq, were studied to test antibodies to non-structural FMDV proteins. The cattle sera had been collected from 1st September 2020 to 1st April 2021. The samples belonged to three categories 9-23-, 24-36-, and ≤ 37-month-old.

Blood samples were collected using vein puncture into 10 mL vacutainer blood series tubes. The sera were decanted into sterile 2 mL Eppendorf tubes, and were then placed inside the aluminum racks with drawers and dividers and maintained at 20°C. Vaccination information of the collected samples was based on the health history recorded on a farm in Iraq. A trivalent vaccine FMD type of O, A, and Asia1 was used for vaccinating animals twice in 12 months. Najaf Province was sampled and examined, which revealed that this province has excessive animal density, and every 12 months suspected cases of FMD have been reported.

2.2. ELISA Assay

The enzyme-linked immunosorbent assay was used to detect antibodies to the NSP of FMDV in the serum from cattle. A commercial NSP kit (Bommeli, Switzerland) was employed to detect FMDV 3ABC antibodies by indirect ELISA, according to the manufacturer's instructions. The sera were examined for FMD antibody by NSP ELISAs in 2021.

2.3. Data Analysis

The collected data were entered into Microsoft Excel software and analyzed in Statistical Package for Social Services (SPSS; Inc, Chicago, USA) version 18.0. A p-value of less than or equal to 0.05 was considered

significant. The seroprevalence for FMD NSP antibodies became standardized with the aid of the following method:

Direct adjusted seroprevalence = ∑ Seroprevalence % × Reference population distribution at all age group strata (Fleiss, 1973)

The NSP seroprevalence in each age group changed as anticipated, in which the total number of animals counted in the specific age group was taken into consideration.

3. Results

The seroprevalence of FMD in cattle was calculated at 34% (29/84) using the NSP ELISA test. The results of field animal serum tested by 3ABC-ELISA on vaccinated and unvaccinated animals are presented in table 1. Accordingly, infection was also reported in the vaccinated group (18%).

The seroprevalence ratios of FMD were estimated at 21%, 7%, and 6% in the 9-23-, 24-36-, and ≥ 36-month-old groups, respectively (Table 2). The highest and lowest rates of infection were reported in the groups of 9-23-month-old and ≥ 36-month-old groups, respectively.

Table 1. FMD seroprevalence in vaccinated and non-vaccinated cattle in Najaf Province

No. of samples		No. of positive samples (%)	No. of negative samples (%)
Vaccinated	60	11 (18%)	49 (82%)
Non-vaccinated	24	18 (75%)	6 (25%)
Total	84	29	55

Table 2. Seroprevalence of FMD in Najaf Province based on NSP ELISA test

Age group (months)	No. Of samples	Result	
		No. of positive Samples (%)	No. of negative Samples (%)
9-23	45	18 (21%)	27 (32%)
24-36	18	6 (7%)	12 (14%)
≥36	21	5 (6%)	16 (19%)
Total	84	29 (34%)	55 (65%)

4. Discussion

Foot-and-mouth disease is a highly contagious viral disease among ruminants with global distribution. Monitoring for FMD control is a critical requirement before making reasonable efforts to manipulate the disease. In such large countries as Iraq, with an area of 438,317 km² and surrounded by neighboring countries where four FMDV serotypes (i.e., O, A, SAT-1, and SAT-2) have been reported, the inhibition of the illegal movement of animals along the borders and the vaccination of livestock are recommended to control and prevent FMD. The results of the current study indicated that FMD was endemic in Iraq and the virus was maintained particularly in the cattle population. This speculation became supported through the profile of NSP antibodies in cattle that confirmed a significant increase (21%) in the seropositivity of the examined animals in the 9-35-month-old group. This finding was consistent with those reported in other pieces of research (10, 11); moreover, the cattle movement and transhumance practices are common in Iraq. The low occurrence in the oldest age group (i.e., ≥36 months) was probably due to steady re-exposure to FMD (12); nevertheless, the results of studies have shown higher FMD seroprevalence in calves than in adult farm animals (12, 13).

After 9 months of birth (9-24 months), animals lose maternal immunity and become vulnerable mainly to FMD. As this age group is often traded, it is required to adopt control measures in this regard, the results were consistent with the findings reported in a study by Sarker, Talukder (14).

Modern inactivated FMD vaccines offer the best serological protection against an FMDV serotype and no longer provide inter seroprotection. All vaccinated farm animals triggered an excellent degree of antibodies in opposition to every vaccine strain. Vaccines will provide higher safety in opposition to defiance with a virulent disease that is homologous to that within the vaccine than to an antigenically unique virus (11, 15).

There are different reasons for the lack of vaccine response, such as vaccine factors, variable batch efficiency, bad management, non-attention to shelf lifestyles, and cold chain necessities Shabana and Krimly (16). Furthermore, population density and the nature and frequency of contact will affect the degree of challenge.

Veterinary vaccines are then formulated efficiently, and it is anticipated that they are protective even when animal susceptibility and pathogen exposure are excessive (17-19). Regardless of this, vaccine failure inside the area can still arise. The existing observations indicate that FMD has been circulating in large regions of Iraq. It is crucial to perform studies to investigate the effectiveness of vaccines and measure their protection through vaccination programs. Vaccine effectiveness needs to be monitored, especially when there are outbreaks inside a vaccinated population. Young farm animals play a vital function in FMD transmission, and it is important to reduce susceptibility in youth as much as possible.

Authors' Contribution

Study concept and design: A. A. H., N. A. A. W. A. A. and K. Z. K. A.
Acquisition of data: A. A. H.
Analysis and interpretation of data: N. A. A. W. A. A.
Drafting of the manuscript: K. Z. K. A.
Critical revision of the manuscript for important intellectual content: A. A. H., N. A. A. W. A. A., K. Z. K. A. and A. A. H.
Statistical analysis: G. S. B.
Administrative, technical, and material support: A. A. H., N. A. A. W. A. A., K. Z. K. A. and A. A. H.

Ethics

The experimental protocol applied in this research was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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