Cairo University
Faculty of Veterinary Medicine
Department of Internal Medicine
and Infectious Diseases

Some Epidemiological Studies on Foot and Mouth Disease (FMD) in Cattle and Buffalo in Egypt

Thesis
Presented by

EMAD DIAB MOHAMED IBRAHIM
(B.V.Sc., 2008, *Faculty of Veterinary Medicine*, Cairo University)

For the degree of M.V.Sc.
(Infectious Diseases, Cairo University; 2015)

Under supervision of

Prof. Dr. Magdy Mahmoud El-Sayed
Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Adel A. Fayed
Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo University

(2015)

Cairo University
Faculty of Veterinary Medicine
Department of Internal Medicine
and Infectious Diseases

Supervision sheet

Prof. Dr. Magdy Mahmoud El-Sayed
Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Adel A. Fayed
Professor of Infectious diseases
Faculty of Veterinary Medicine
Cairo University

(2015)

Cairo University
Faculty of Veterinary Medicine
Department of Internal Medicine
and Infectious Diseases

Name: Emad Diab Mohamed Ibrahim
Date of birth: 22/4/1986
Nationality: Egyptian
Degree: Master Veterinary Science
Specialization: Infectious Diseases
Title of the thesis: Some Epidemiological Studies on Foot and Mouth Disease (FMD) in Cattle and Buffalo in Egypt
Supervision:
Prof. Dr. Magdy Mahmoud El-Sayed Professor of Infectious diseases, Faculty of Veterinary Medicine, Cairo University
Prof. Dr. Adel A Fayed Professor of Infectious diseases, Faculty of Veterinary Medicine, Cairo University

Abstract

To investigate the current status of FMDv infection and to assure the vaccine efficacy used in some Egyptian governorates, a cross-sectional survey was conducted between October-2013 till July-2014 in ten Egyptian Governorates (Cairo, Al-Qaliubia, Giza, Alexandia, Al-Behaira, Al-Gharbia, Kafer El-Sheikh, Al-Fayoum, Al-Sharquia and Assiut). In this study >10,000 cattle and Buffaloes were studied, 529 serum samples out of them 321serum samples from non-vaccinated animals in FMD suspected foci and 208 serum samples from suspected free animals used for vaccinal Trials as well as 51 Saliva and tissue samples collected. The virus samples were serotyped by RT-PCR and the complete VP1 coding regions in the PCR products of positive samples were sequenced .The results confirmed the presence of three serotypes [A (13.63%), O (56.81%), SAT-2 (11.36%), A+ SAT-2 (2.27%)and A+O (2.27%)] of FMDv co-circulating in Egypt. Sequencing and phylogenetic analysis of VP1 further confirmed emergence of the East Africa-3 topotype (EA-3) of serotype O. Serotype O sequence was closely related to O/SUD/8/2008 with identity 93%, but differ from vaccinal strain (O/PanAsia-2) of ME-SA topotype by 14.6%. Meanwhile Serotype A and SAT-2 were closely related to recent Egyptian isolates and vaccinal strains type A/ EGY/1/2012 (Asia topotype, lineage Iran 2005) with identity 96.4% and vaccinal strain of SAT-2/EGY/9/2012 (topotype VII, lineage SAT-2/VII/Ghb-12) with identity 92% respectively. Serum samples were screened against the three FMDV serotypes circulating in Egypt (A/EGY/1/2012, O/EGY/4/2012 and SAT-2/EGY/9/2012) by using serum Neutralization Test (SNT). Results revealed that all three serotypes were circulating in all examined Governorates and the more prevalent serotype was SAT-2 (64.1%) followed by serotype O (61.9%) and serotype A (55.8%). The period of the study indicated that serotype A was more prevalent from October to December, SAT-2 more prevalent from January to May while serotype O started to increase from March till July. In relation to age, both cattle and buffalos less than 2 years old are more susceptible to FMD. Buffaloes showed high sero-positivite than cattle to serotype A; however no significant differences between cattle and buffalos was observed in serotype O and SAT-2. Vaccinal trial reviled that local vaccine has significant protection than imported for serotype O, however no significance in serotype A. on the other hand SAT-2 results were hard to explain as there was doubt about suspected FMD SAT-2 infection as the mean SNT titers for imported groups 1.77. The study concluded that there is species difference and age susceptibility to different FMDv serotypes in studied groups. Emerging of new topotypes of FMDv may require a change of vaccine production strategy. The present study recommended further studies for serotype O to confirm the immunogenic relationship between the vaccinal strain and the emerging new strains to provide maximum protection against circulating viruses.

Keywords: FMD- serosurveillance- FMD vaccination- FMD Epidemiology

ACKNOWLODGMENT

I am grateful to Allah Almighty the most beneficent for his graciousness bestowed upon me and the opportunity given to study and complete this thesis.

Frist, I would like to thank my family for their love and support, and without them I could not achieve this work

No words can adequately express my sincere gratitude and great appreciation to my supervisor ***Prof. Dr. Magdy El-Sayed*** *who offered me a lot of his time and who devoted his experience to provide me with the best possible pieces of advice and suggestion to this work.*

My deepest thanks to my supervisor ***Prof. Dr. Adel Fayed*** *for his precious supervision, helpful suggestion, kind advices, criticism, continuous encouragement*

I would like to thank heartily Prof. Dr. Sayed Rashwan Prof. in Veterinary Serum and Vaccine Research Institute for his help, helpful suggestion, and kind advices.

I would like to thank heartily Dr. Abdel-Hamid I. Bazid Faculty of Veterinary Medicine-University of Sadat city for his help in selection of PCR primers, helpful suggestion, and kind advices.

I would like to thank heartily Dr. Mohamed Fawzy Faculty of Veterinary Medicine- Suez Canal University for his help, helpful suggestion, and kind advices.

I would like to thank heartily Dr. Wagdy El-Ashmawy Faculty of Veterinary Medicine- Cairo University for his help in collection of some samples.

I would like to thank heartily Both Dr. Ahmed Ali Faculty of Veterinary Medicine- Beni-suef University for his great help and Dr. Omar Abdellatif Faculty of Veterinary Medicine- Cairo University for their helpful suggestion, kind advices and help in statistical analysis of my results.

I would like to thank heartily the all members of ME VAC Company and all members of Internal Medicine and Infectious Diseases Dept. Cairo University.

Table of contents

List of Tables

List of figures

1. Introduction

Foot and mouth disease (FMD) is an economically important disease of livestock. The disease is highly contagious with morbidity rates up to 100% causing severe losses in production. Therefore, FMD is a major economic concern for livestock-health in many developing countries and a continued threat to disease free countries ***(Knowles and Samuel, 2003; Ko et al., 2009)***. Although eradication efforts including vaccination started since 1900s, the disease still infects millions of animals around the world and remains major barrier to commerce of animals and animal's products ***(Depa et al., 2012)***.

The Foot-and-Mouth Disease virus (FMDv) is a small positive sense single stranded RNA (ssRNA) virus (approx. 9.3kb) belongs to family Picornaviridae, genus Aphthovirus ***(Belsham, 1993)***. There are seven antigenically distinct serotypes of FMDv (A, O, C, South African territories (SAT) types 1-3 and Asia 1) and many subtype variants (i.e. topotypes). This antigenic variation limits the control of FMD as infection or vaccination with one serotype of FMD does not protect against other serotypes and sometimes other subtypes within the same serotype ***(Paton et al., 2005).***

In Egypt, the disease is enzootic and outbreaks have been reported since 1950. FMD serotypes SAT2, A and O were described in Egypt ***(Aidaros, 2002; Shawky et al., 2013).*** Vaccination in Egypt is the only approach to control FMD. The immunogenicity of FMD vaccine depends to a large extent on the production of FMD virus on tissue culture and the stability after virus inactivation procedures and

formulation into vaccines ***(Crowther et al., 1995).***

The FMD vaccine used in Egypt before 2012 was cell culture inactivated bivalent vaccine prepared from the local strain O1/3/1993 and type A/1/EGY/2006. After isolation and molecular characterization of the recent FMD serotype SAT-2 ***(Shawky et al., 2013)***, a modified local trivalent inactivated vaccine containing O Pan-Asia 2 , A Iran/05 and SAT2/2012 was developed.

Since Egypt became endemic with FMD, the current study was designed to investigate the current status of FMDv infection in some Egyptian governorates as well as the currently used vaccine efficacy. The aims of this study were:

- Conducting epidemiological study on current FMD status in some Egyptian governorates:

- The Seroprevalence of different serotypes of FMDv in Egyptian livestock cattle and buffaloes

- Isolation and characterization of FMDv serotypes by VP1 sequence and sequence analysis.

- The efficacy of both locally produced and imported vaccines against detected FMDv serotypes in examined governorates.

- The effect of different factors (species, age and sex) on immune response induced by differently used FMDv vaccines.

2. Literature

2.1. Historical background of foot-and-mouth disease:

The first description of the foot-and-mouth disease (FMD) was probably occurred in 1514 ***(Fracastorius, 1546)*** who described a similar disease in cattle in Italy. In 1897, the causative agent of FMD was described as a filterable agent ***(Loeffler and Frosch., 1897)***. The disease was successfully reproduced by ***(Waldmann and Pape, 1920)*** in guinea pigs which showed a similar disease as in cattle, with lesions on the feet and tongue and the general loss of condition. Early estimates of FMDv size done by ***(Galloway and Elford, 1931),*** and the electron microscopy studies of ***(Bachrach and Breese, 1958; Bradish and Brooksby, 1960)*** which showed that its morphology was similar to that of poliovirus.

2.1.1. Discovery of FMDv Serotypes and sub-serotypes

FMDv was proved to be caused by more than one serotype by ***(Vallee and Carre, 1922)***, who recognized two serotypes and named them on their place of origin, O for the department of Oise in France and A for Allemagne. Their work was confirmed by ***(Waldmann and Trautwein, 1926)*** in Germany, who called them A and B. Then serotype C was discovered. Ultimately decided to call them Vallee O, Vallee A and Waldmann C, that has been reduced to serotypes O, A and C.

In the 1940s, three additional serotypes were described by ***(Galloway et al., 1948),*** found in Southern Africa and were named

accordingly as Southern African Territories types SAT-1, SAT-2, and SAT-3 ***(Brooksby, 1958)***. Finally Asia1 was found in sample from Pakistan in 1954 ***(Valarcher et al., 2005)***.

As early as 1927, two isolates of a serotype A virus could be differentiated by cross-neutralization tests ***(Bedson et al., 1927)***. During the major epidemic in Mexico between 1946 and 1954 field strains belonging to serotype A broke the immunity induced by a vaccine from another strain of A serotype ***(Galloway et al., 1948)***. These variations within serotypes were demonstrated by the extensive outbreaks of Western Europe during 1965-1966 by serotype O which broke the immunity of cattle by the control programs.

Work in the early 1930s by ***(Hecke, 1931)*** has shown that the virus could be grown in vitro, but ***(Frenkel and Ribelin, 1956)*** who showed that large-scale production of the virus could be obtained by using surviving tongue epithelium from cattle. After that it was replacement of tongue epithelium by pig kidney or Baby Hamster Kidney (BHK) cell in both monolayers and suspensions ***(Capstick et al., 1962; Mowat et al., 1962)***.

2.2. Economic importance of FMD

Foot and Mouth disease is a devastating disease and causing significant economic losses to affected countries' livestock industries ***(Garner et al., 2002; James and Rushton, 2002)***. Most industrialized and developed countries are free from FMD while most of developing nations remain infected. The main impacts of FMD were: reduced milk yields, weight loss, abortions and delayed conceptions, perinatal

mortality and lameness in draught animals in additional to indirect and invisible losses as showed in Table 1. Consequently, this disease becomes a significant barrier for the international trade ***(Rweyemamu and Astudillo, 2002)*** separating the international market into two separate international markets; FMD-free market and FMD-endemic market ***(Ekboir et al., 2002)*.**

In 1976 systematic FMD control programs were launched in India, where FMD affection average 15% of the nation's livestock each year and the estimated loss exceeded 200 million dollars annually ***(Ellis, 1993)*.** In the United Kingdom, the losses of recent epidemic in 2001 exceed 12 billion dollars and culling of 3.9 million animals ***(Aggarwal et al., 2002)*.** In an economic study of the potential impact of FMD in Australia, the estimated cost for outbreak control by vaccination in two studied regions varied from 1 to 4 billion dollars with additional costs from 11 to 21 billion dollars ***(Garner et al., 1997)*.** In the event of an outbreak of FMD in Australia, ***(Garner et al., 2002)*** estimated that implementing a zoning approach in Australia would be less costly than if control without zoning was implemented.

Several studies on the economic impact of FMD in Southeast Asian countries have been undertaken ***(Perry et al., 2002; Perry et al., 1999; Randolph et al., 2002)*** as during the 1997 FMD outbreak in Taiwan, 6,000 farms were infected and 20 million doses of vaccine were used to control the outbreak ***(Kitching, 1999)*.** In Table 1 conclusion of FMD economic losses.