Efficacy of Country Specific Vaccine for the Control of Foot and Mouth Disease in Cattle Population of Cholistan Desert, Pakistan

Ehtisham ul Haq Khan¹, ², Manzoor Hussain¹, Aatka Jamil¹, Amman Ullah³, Hafiz Fiaz Ahmad⁴, Faraz Munir Khan⁵, Shumaila Manzoor¹, Muhammad Abubakar⁴* and Muhammad Afzal¹

¹FAO Project ‘Progressive control of foot and mouth disease in Pakistan’ (GCP/PAK/123/USA), Islamabad, Pakistan
²Livestock and Dairy Development Department, Punjab, Pakistan
³Animal Health Program, National Agriculture Research Center, Islamabad, Pakistan
⁴National Veterinary Laboratory, Park Road, Islamabad, Pakistan

ARTICLE INFO

Received: Apr 13, 2016
Accepted: Aug 19, 2016
Online: Aug 20, 2016

Keywords
Foot and mouth disease
Vaccine
ELISA
Protection
Cholistan Desert
Pakistan

ABSTRACT

Present study was designed with the objective to find out the sero-prevalence of foot and mouth disease (FMD) in animals and to evaluate the role of FMD vaccination as a control strategy for this disease. Serological analysis of randomly sampled animals (n=373; 110 from Greater Cholistan and 263 from Lesser Cholistan) using non-structural protein ELISA indicated that 62.2% animals in the Desert had previous exposure to FMD virus. To determine the efficacy of vaccine for prevention of the disease, 7,500 cattle heads located at 131 ‘tobas’ both in Lesser and Greater Cholistan were vaccinated using a trivalent FMD vaccine containing A, Asia-1 and O serotypes. Booster dose was administered after 30 days of primary vaccination followed by immunization after every 6 months. Blood samples were collected at day 0 and at the time of booster dose. Sera samples were analyzed for titres against all 3 serotypes using Solid Phase Competitive Blocking ELISA. At the time of vaccination (day 0), 31.8-64.5% animals showed protective titres indicating previous exposure of animals against all 3 serotypes of the virus. This protective titre further increased from 64% to 87% animals against different serotypes after primary vaccination (day 30). Field observations indicated that after booster dose, none of the vaccinated animals showed any clinical sign of the disease; whereas, FMD outbreaks were recorded in un-vaccinated animals located in the study area. In conclusion, the high quality FMD vaccine, administered following the standard operating procedures, can protect animals against this disease in Desert Production System.

INTRODUCTION

Foot and mouth disease (FMD) is an extremely infectious viral disease of animals. It affects domestic cattle, buffaloes, small ruminants as well as wild ruminants (Alexandersen et al., 2002). Foot-and-mouth disease virus (FMDV) is the etiologic agent of this devastating disease that usually causes low mortality, but its high morbidity and contagiousness can lead to serious economic consequences (Guzman et al., 2008). Therefore, FMD control and eradication in endemic countries like Pakistan directly correlates with the uplift of rural economy. This could eventually result in improved quality of life particularly for rural population in the country (Perry and Rich, 2007). There are seven serotypes of FMDV worldwide. In Pakistan, the disease is endemic and three serotypes including O, A and Asia 1 are currently prevalent in cattle and buffalo populations (Klein et al., 2008; Jamal et al., 2011; Abubakar et al., 2015 a & b). Many studies have been carried out on prevalence of FMDV infection in cattle and buffalo and there is yearly data available about these species. As reported by Abubakar et al. (2012) the overall prevalence of FMDV in cattle and buffaloes in Pakistan was 33.2%, while in cattle alone; it was 37.1%, higher than that in buffaloes (28.7%).
Since infectious diseases are an important factor that limits output and productivity of livestock, it is critical to understand their status in different production systems for precise impact estimation and devising appropriate mitigating strategies. Most of the previous studies carried out are focused on different farming systems but there are very few studies about the prevalence of FMD in livestock population of desert areas (Abubakar et al., 2014, 2015a). Livestock is even more important source of livelihood for desert inhabitants as crop agricultural activities are almost non-existing. Furthermore, FMD has serious economical consequences on economy and livelihood of livestock farmers in a desert ecosystem in terms of reduced milk production, weight loss, calf mortality, reduced reproductive performance and minimal veterinary services available to avoid secondary infections.

Cholistan desert is situated in the South–West of Punjab province and is spread over an area of 26,300 square kilometers with patches of highly saline soils and brackish water. The length of the desert is about 480 kilometers and width from 32 to 192 kilometers (Akbar et al., 1996). Geologically, Cholistan desert comprises of two natural regions: (i) Greater Cholistan located on an area of 13,630 square kilometers (district Rahim Yar Khan) (ii) Lesser Cholistan spread over an area of 12,370 square kilometers (districts Bahawalpur and Bahawalnagar). Greater Cholistan lies to the South-West of Hakra River and extends to the border with India (Akhter and Arshad, 2006). Lesser Cholistan extends North-East from the Hakra River to the end, along the bank of Sutluj River. The climate of the area is arid, hot subtropical and monsoonal. It is characterized by great annual and daily variations in temperature and rainfall. The bioclimatic system falls in the category of “tropical desert “and is famous for bush formation. The only source of fresh water is the surface water collected in natural or man-made depressions called ‘toba’ mainly during monsoon season. The water in these “tobas” does not last long due to seepage and high rate of evaporation (Akram et al., 1986).

Cholistan desert is a typical rangeland and contributes significantly towards country’s supply line for live animals and their products (milk and meat). The desert is rich in livestock (about 0.57 million cattle, 0.6 million sheep and goats and camel) and is the main source of livelihood of more than 50,000 families. The backbone of Cholistan economy is livestock breeding and it has the major importance for satisfying the area’s major needs for cottage industry as well as milk, meat, fat and other animal products. Because of the nomadic way of life, the main wealth of the people is their animals. Animals are reared on free grazing land and water available in existing tobas. Once these ‘tobas’ are dry, few farmers have the facility to move to the nearest available well.

Since there is little or no movement of animals into the desert, the prevalence of FMD and other infectious diseases in the cattle was assumed to be very low. Government of Punjab also indicated to establish a disease free zone in Cholistan based on this assumption. Though it is assumed that FMD is the most prevalent infectious disease of livestock in Cholistan (Khan, 2010), there is hardly any data available to support this statement. Therefore, the present study was designed with the objective to find out sero-prevalence of FMD in cattle population of Cholistan Desert and evaluate the role of vaccination as a control strategy for the disease.

MATERIALS AND METHODS

Study population
Two cross sectional surveys were carried out from November 2013 to May 2014 in the cattle population of Cholistan desert of Pakistan. In the first survey, seroprevalence of FMD in cattle population was determined. Afterwards, about 7500 animals were vaccinated against FMD in 131 randomly selected tobas (Toba is a pond, where rain water is collected and stored after rains and camels were gathered for drinking before stating their browsing of the day). In the 2nd cross sectional survey, around 4% of the vaccinated animals were sampled for evaluation of efficacy of FMD vaccines in study population.

Sample size
For 1st cross sectional survey, a two-stage cluster-sampling scheme was used for estimating the required sample size. A true FMD prevalence of 33% was assumed for the Cholistan area, same as reported for the rest of country (Abubakar et al., 2012). As herd composition, management and husbandry practices are similar across the desert; a random effect of one was assumed, same as for simple random sampling. Using a confidence limit of 5% and a confidence level of 95%, required sample size was calculated as 340 samples from two clusters. The calculations were made in Stat Calc application in Epi Info® software.

Using the same sample size, blood samples were collected from two clusters in Cholistan i.e. Greater Cholistan and Lesser Cholistan. Within each cluster, it was planned to collect 28 samples per toba from 12 tobas in each cluster (7 ‘tobas’ in Lesser and 5 in Greater Cholistan). A total of 373 blood samples (including 263 in Lesser and 110 in Greater Cholistan) were collected randomly from animals available at these ‘tobas’ at the time of sampling.

All sampled animals had no history of vaccination against FMD. Blood samples were collected before animals were vaccinated to determine the soro-
prevalence of FMD in cattle population of Cholistan (day 0).

**FMD Vaccination and 2nd cross sectional survey**
This survey was carried out in five different areas of Cholistan desert. These areas were further divided into 131 locations (tobas) selected randomly for vaccination/sampling with the assistance of the staff from Cholistan Development Authority (CDA) and the Livestock & Dairy Development Department Punjab, Rahim Yar Khan. Five teams of CDA identified ‘tobas’ with desired number of animal population for vaccination and later, blood sampling for sero-monitoring. A map showing selected tobas is given in Fig 1.

A total of 7,500 animals were ear tagged and given primary vaccination against FMD. The vaccine used was manufactured by ARRIAH Russia containing serotypes ‘A’ (Turk-06); ‘Asia-1 (Sindh-08) and ‘O’ (PanAsia-2) with >6 PDs. Number of ‘tobas’ and animals vaccinated by each team are given in Table 1.

Afterwards, around 4% of the vaccinated animals were sampled for the evaluation of vaccine efficacy against FMD in these desert cattle.

![Selected Tobas in Cholistan](image)

**Fig. 1: A map showing location of study area in Cholistan.**

**Sample collection**
Blood samples were collected from jugular vein of each animal in 10 ml sterile vacutainer tubes. After sampling, animals were given a code by ear tagging and this code was used to label the vacutainers containing the sample. Then the blood was allowed to clot by placing it overnight at room temperature. The serum was collected in cryo-vials from the clotted blood and transported using an icebox to the National Veterinary Laboratories, Islamabad, Pakistan. These samples were stored at -20°C till used for the detection of antibodies to FMD virus.

**Serological analysis**
In the first cross sectional survey, antibodies against non-structural protein (NSP) in serum samples were detected using, 3ABC-trapping indirect ELISA kit (IZSLER Brescia, Italy). Briefly, sera samples were diluted (1:10) and then added to pre-coated micro plates with the 3ABC antigen captured by the monoclonal antibodies (M-Ab). Plates were incubated for one hour at room temperature. After washing to remove unbound material, an anti ruminant IgG (peroxidase conjugated M-Ab) was dispensed in the whole plate. This conjugate binds to antibodies present in serum sample against NSP of FMDV. After incubation, unbound conjugate was removed by washing and TMB chromogen/substrate was added. As a result of colorimetric reaction, color developed in proportion to amount of antibodies present in the sample. This colorimetric reaction was stopped by adding stop solution. The OD values were read at 450 nm by using ELISA reader (Dekker et al., 2008).

For 2nd cross sectional study, all the samples were tested for the presence of antibodies against structural protein (SP) of all three prevalent serotypes (A, O, Asia-1) of FMD. For this purpose, solid phase competitive blocking ELISA (SPCE) was used (IZSLER Brescia, Italy). In this assay, anti-FMDV monoclonal antibodies and FMDV antigen were pre-coated on the plate. These MAb were sero-specific and acted as catching antibodies. Diluted test sera (1/10) were incubated (1 hour) with trapped antigen so that specific antibodies present in test sera could bind to the antigen. Then anti FMDV MAb, conjugated with peroxidase was dispensed. After incubation, unbound conjugate was removed by washing. Then TMB substrate/chromogen was delivered to all wells of plate. A colorimetric reaction was developed in the wells having negative samples and vice versa. After the addition of stop solution, the OD value was read by using ELISA reader. Percent inhibition produced by test and reference sera was calculated using following formulae.

\[
\text{Inhibition (\%)} = 100 - (\text{serum OD}/\text{reference OD}) \times 100
\]

**RESULTS**

**Sero-prevalence of FMD**
Serological analysis of the sampled animals (1st cross-sectional survey) indicated that on an average, 62.2% animals both in Lesser and Greater Cholistan had previous exposure to FMD virus (Table 2). Although the number of animals suffered from the disease was higher in Greater Cholistan than those in Lesser Cholistan, however, the difference was non-significant (P >0.05).

Exposure of animals to FMD virus was also evident from the analysis of serum samples for antibodies to structural proteins of the virus (Table 3). Animals sampled at day 0 (at the time of primary vaccination) both in district Bahawalpur (Lesser Cholistan) and district Rahim Yar Khan (Greater Cholistan) showed
Livestock production system in Cholistan is considered as a closed system in general. Thus livestock is bred and reared in the desert with almost negligible movement of animals from out to inside of the desert. As FMD has never been reported from this region, it has been generally assumed that prevalence of FMD is minimum, if any, in this livestock production system. It has also been assumed that FMD viruses, if circulating in this desert, may belong to only one serotype. The absence of basic requirement for contact with infected animals and higher animal density required for the maintenance of FMD and other TADs are two of the many reasons behind these assumptions. However, the results of this study strongly reject this concept as a 62.2% sero-prevalence against NSP of FMD virus in cattle population of Cholistan was observed. The sero-prevalence documented in this study showed high value when compared to the previous reports of Hafez et al. (1994) which was 16% in Saudi Arabia; 12.8% by Gelaye et al. (2009), 14.05% by Mohamoud et al. (2011) in Ethiopia; 17.6% by Dukpa et al. (2011) in Bhutan and 19.33% by Nawaz et al. (2014) in Pakistan. On the other hand, the seropositivity findings of this survey were almost equal to the overall sero-prevalence of 61% reported by Mwiine et al. (2010) in Uganda.

Since animals in Cholistan are not vaccinated against FMD, such a high prevalence of antibodies against NSP clearly indicates a large-scale exposure of this population to FMD virus. These findings indicated that a large number of cattle population is moving inside the desert, possibly carrying all type of infections prevalent in the livestock population of Pakistan (Abubakar et al., 2015a). In desert, livestock are usually kept in mixed herds, consisting of all types of livestock. Thus a herd will usually consist of cattle, sheep, goats and camels (Farooq et al., 2009). As large number of cattle has been found to be exposed to FMD virus in this study, it is likely that all other livestock are also exposed.

FMD usually spreads through contact or aerosol to susceptible livestock. Water scarcity is the main constraint for livestock. Rainwater, harvested in the “Tobas”, mainly provides water. The location, availability of water points and amount of precipitation, dictate the mobility-pattern of livestock (Chaudhry et al., 2004). Various herds usually come in contact with each other when they share a toba for drinking water. Thus infected animals contaminate water in these tobas and that spreads to other livestock when they drink this contaminated water. This risk of disease transmission is heightened during draught when small tobas get dry and animals move to a fewer larger tobas for drinking water. Thus, these tobas play an important role in the transmission and maintenance of livestock diseases in this desert.

### Table 1: Vaccination of cattle against FMD around different ‘Tobas’ in Cholistan Desert

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Field team</th>
<th>Tobas Covered (No.)</th>
<th>Total Cattle Population</th>
<th>Primary vaccination</th>
<th>Booster vaccination</th>
<th>6 Monthly vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chanainpir</td>
<td>36</td>
<td>10655</td>
<td>1793</td>
<td>1764</td>
<td>1711</td>
</tr>
<tr>
<td>2</td>
<td>Jugaipir</td>
<td>30</td>
<td>7651</td>
<td>1221</td>
<td>1000</td>
<td>999</td>
</tr>
<tr>
<td>3</td>
<td>148 DB</td>
<td>34</td>
<td>10400</td>
<td>1486</td>
<td>1476</td>
<td>1390</td>
</tr>
<tr>
<td>4</td>
<td>Head Farid</td>
<td>16</td>
<td>8040</td>
<td>2000</td>
<td>1795</td>
<td>1540</td>
</tr>
<tr>
<td>5</td>
<td>Qila Derawar</td>
<td>15</td>
<td>7530</td>
<td>1000</td>
<td>982</td>
<td>880</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>131</td>
<td>44276</td>
<td>7500</td>
<td>7171</td>
<td>6620</td>
</tr>
</tbody>
</table>

### Table 2: Prevalence of Non Structural Protein (NSP) antibodies against FMD virus in cattle sera of Cholistan Desert

<table>
<thead>
<tr>
<th>Area</th>
<th>Tobas (#)</th>
<th>Number of samples</th>
<th>NSP antibody positive (#)</th>
<th>Prevalence %age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater Cholistan</td>
<td>5</td>
<td>110</td>
<td>65</td>
<td>59.1</td>
</tr>
<tr>
<td>Lesser Cholistan</td>
<td>7</td>
<td>263</td>
<td>167</td>
<td>63.5</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>373</td>
<td>232</td>
<td>62.2</td>
</tr>
</tbody>
</table>
The milk dealers and other people who frequently visit these herds may be another source of such wide spread of FMD in desert. In Lesser Cholistan, milk is usually used to meet daily needs and rest is sold to the middlemen who travel on motorbike to collect and sell milk in the nearby towns. This frequent movement may also be a factor in carrying the virus and transmitting into the Desert. Since animals are freely moving in the desert in search of feed, once infected, owners come to know after several days and sometimes after a month. Such animals could be a potential source of spreading the disease to other healthy animals.

One of the important factors in transmitting FMD virus to the susceptible population of the desert is drought. In search of water and fodder, these animals are moved to the nearby towns. During this period, there are chances that FMD virus if present in local animals; it is contracted by the animals coming from the desert. Subsequently, this animal is also a source of infection to other animals in the herd. These factors, movement outside the desert during dry periods and return and communal watering may have resulted in such a widespread prevalence of FMD and other diseases in the livestock population of this desert.

Results indicated that FMD virus is circulating in large ruminants of Cholistan desert and around 62.2% animals both in Lesser and Greater Cholistan have previous exposure to the viral infection. This provided a justification to undertake preventive vaccination to determine if FMD vaccine can also protect animals in the desert production system.

In the 2nd cross sectional study, vaccinated animals showed a very favorable sero-conversion that ranged from 77-87% for all three serotypes of FMD virus. Although FMD outbreaks were recorded in non-vaccinated cattle in the desert during the period of study, yet FMD vaccines did not show any clinical signs of the disease. Thus vaccination can help to protect a very large proportion of the susceptible cattle against FMD. Moreover, the herd immunity developed after vaccination was enough to stop further spread of FMD infection in this population. This shows that the vaccine used has a very high protective efficacy against all three prevalent serotypes of FMD. Furthermore, vaccinated animals showed high level of antibodies against all 3 serotypes of FMD virus.

Table 3: Antibodies against structural proteins of FMD virus serotypes in vaccinated cattle in Cholistan

<table>
<thead>
<tr>
<th>District</th>
<th>Total samples tested</th>
<th>Day 0</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>Bahawalpur</td>
<td>257</td>
<td>125 (49.0)</td>
<td>131 (51.0)</td>
</tr>
<tr>
<td>Rahim Yar Khan</td>
<td>110</td>
<td>71 (64.5)</td>
<td>46 (41.8)</td>
</tr>
</tbody>
</table>

*Values in parentheses indicate % animals protected.

Efficacy of country specific FMD vaccine in Cholistan

The study indicated that drought is a crucial period when a large number of animal populations have to move to nearby towns in search of water and fodder. Since most probably, this practice results in transmission of FMD virus to animals coming from the Desert, it would be important that CDA and Livestock Department Punjab should foresee such situation and vaccinate animals at those ‘tobas’ with expected movement to the near-by towns.

**Author’s contribution**
EHK and MH designed and executed the study. AJ, SM and MA were associated with laboratory analysis of samples and interpretation of results. HFA and FMK assisted in all field activities. AU, AJ, MA and MF mainly were involved in write-up of the manuscript as well as technical assistance during the period of the study.

**Acknowledgments**
The study was carried out under the Project, “Progressive Control of Foot and Mouth Disease in Pakistan” GCP/PAK/123/USA, implemented by FAO with the
financial assistance of USDA. Support provided by the staff of Cholistan Development Authority as well as Livestock & Dairy Development Department, Govt. of Punjab, (Bahawalpur and Rahim Yar Khan) to undertake field activities are highly acknowledged.

REFERENCES


Dekker A, D Sammin, M Greiner, I Bergmann, DPaton, S Grazzioli, K de Clercq and E Brocchi, 2008. Use of continuous results to compare ELISAs for the detection of antibodies to non-structural proteins of Foot and Mouth disease virus. Vaccine, 26, 2723-2732.

Dukpa K, ID Robertson and TM Ellis, 2011. The seroprevalence of foot-and-mouth disease in the sedentary livestock herds in four districts of Bhutan. Preventive Veterinary Medicine, 100: 231-236.


