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RESEARCH ARTICLE

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

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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 sero-

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 PD_{50}. 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.

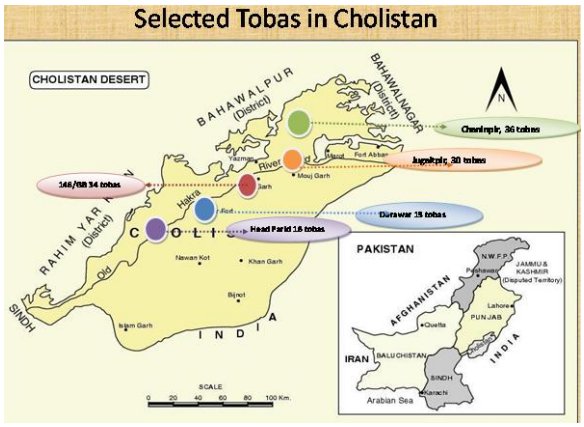

Fig. 1: A map showing location of study area in Cholistan.

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 -20ºC 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.

Inhibition (%) = 100 - (serum OD/reference OD) × 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

the presence of antibodies to all 3 serotypes of FMD virus present in the country. The level of antibodies increased significantly when samples collected were tested at day 30 following primary vaccination. This level was higher in those samples collected from animals in district Rahim Yar Khan that those in district Bahawalpur. Further, no clinical case of FMD was reported by the farmers or the local veterinary staff in vaccinated animals after booster dose. Only two animals showed mild clinical symptoms after primary vaccination but ELISA and virus isolation attempts proved negative. However, clinical cases of FMD were reported from unvaccinated animals in the surrounding tobas. Blood samples were also collected periodically to determine the immune response in animals after vaccination. Results are shown in Table 3. Since the animals in Cholistan were not vaccinated against FMD by the CDA staff for the last about one year, high titre at the time of vaccination (day 0) indicated previous exposure of animals against all 3 serotypes of the virus. This titre was further increased after primary vaccination (day 30).

DISCUSSION

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 form 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

S. No.	Field team	Tobas Covered (No.)	Total Cattle Population	Primary vaccination	Booster vaccination	6 Monthly vaccination
				No. of Animals		
1	Chaninpir	36	10655	1793	1764	1711
2	Jugaitpir	30	7651	1221	1000	999
3	148 DB	34	10400	1486	1476	1390
4	Head Farid	16	8040	2000	1795	1540
5	Qila Derawar	15	7530	1000	982	880
	Total	131	44276	7500	7117	6620

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

Area	Tobas (#)	Number of samples	NSP antibody positive (#)	Prevalence %age
Greater Cholistan	5	110	65	59.1
Lesser Cholistan	7	263	167	63.5
Total	12	373	232	62.2

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

District	Day 0				Day 30			
	Total samples tested	Samples with protective titres against serotype*			Total samples tested	Samples with protective titres against serotype		
		O	*A*	*Asia-1*		*O*	*A*	*Asia-1*
Bahawalpur	257	125 (49.0)	131 (51.0)	109 (42.4)	216	161 (74.5)	170 (78.7)	123 (60.0)
Rahim Yar Khan	110	71 (64.5)	46 (41.8)	35 (31.8)	70	60 (85.7)	61 (87.0)	54 (77.0)

*Values in parentheses indicate % animals protected.

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 vaccinates 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. Cholistan Development Authority (livestock Wing) has the main responsibility for prevention and control of animal diseases in the area. However, it was apparent during the study that availability of resources including travel expenses, human resources and budget for medicines/vaccines are limiting factors for controlling livestock diseases in the Desert. Also, there is no dispensary or hospital inside the Desert and it takes a while to inform the veterinary staff and adopt measures to control the disease and/or treat the sick animals. Although Punjab Livestock Department tried to establish community based farms where all necessities to keep animals were provided, it seems that the farming community was not motivated and this effort failed to bring better livestock management practices in the desert. This study provides an in-sight of FMD control options in Cholistan desert, an area where farmers are entirely dependent on the productivity of animals and their sale mainly during Eid-ul-Azha festival and/or distress sale when their lives are at risk. However, when water and rain-fed bushes are available, this desert greatly improves livelihood of the farmers. Similarly, farmers have very little awareness how to prevent or control different diseases and how animal productivity can be enhanced. If the Livestock Department could create awareness amongst the farmers about the measures that can be adopted to increase animal productivity and provide veterinary coverage to protect animals in a short period of time, livelihood of the farmers can be largely improved in Cholistan Desert.

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.

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