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Therapeutic and Prophylactic Potential of FMD Virus-specific Polyvalent Immunoglobulins during an FMD Outbreak in Cattle

Soliman R[1*], Saad MA[2], Darwish MD[3], Sayed RH[3], Abousenna MS[3], Shasha FA[3] and Hassan H[4]

[1]Department of Microbiology and Immunology, Faculty of Veterinary medicine, Cairo Univ., Egypt
[2]Veterinary Serum and Vaccine Research Institute, Agricultural Research Center, Cairo, Egypt
[3]Central Laboratory for Evaluation of Veterinary Biologics, Agricultural Research Center, Cairo, Egypt
[4]Institute of Animal Reproduction, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt
***Corresponding author:** rafikhamed1010@hotmail.com; mohamedsamy2020@hotmail.com

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ABSTRACT

Foot and mouth disease (FMD) is a highly contagious and economically devastating disease of cloven-hoofed animals worldwide. In the present study the therapeutic and prophylactic potential of polyclonal, polyvalent FMD virus-specific bovine antibodies has been investigated as a possible approach for rapid control, lessening of the severity of clinical signs and prophylactic protection of susceptible animals during FMD outbreaks. The prepared FMD virus-specific polyvalent immunoglobulins were concentrated, purified, filter sterilized (0.22nm) and its titer against the FMD virus serotypes, A, O and SAT2, was adjusted to 2.15, 2.25 and 2.10 $\log_{10}$ $TCID_{50}$/ml, respectively. In an experimental FMDV induced infection, the immunoglobulin therapy was given 4 days post experimental infection. The effect of different doses of immunoglobulins (4, 6 and 8ml) the severity of the clinical signs, healing of lesions and virus shedding was determined. A dose of 8 ml (2.0 $\log_{10}$ $TCID_{50}$/ml) of the prepared FMD virus-specific antibodies proved highly effective in reducing the severity of the clinical signs and inducing recovery within 48 - 96hrs post therapy, as compared with the control non-treated infected calves. A dose related recovery rate was recorded. Field trial was conducted and evaluated during the FMD outbreak in Egypt during 2016/2017. Significant reduction of the morbidity of the disease and 100% reduction of mortality were recorded. The prepared FMD virus-specific bovine polyvalent antibodies proved to be a drug of choice during FMD outbreaks protecting susceptible animals and inducing rapid recovery of diseased one associated with reducing clinical signs severity and reducing virus shedding.

Key words: Foot-and-Mouth Disease; Antibodies; Immunotherapy; Virus shedding.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious, devastating Disease and having economic impact with high morbidity rate in cloven-hoofed animals, including important livestock species such as cattle and buffalos (Alexandersen et al. 2003; Thompson et al. 2002; Yang et al. 2020). Following FMD virus infection around 25% loss of productivity of infected animals was recorded including loss or reduction in animals' milk production (Bradhurst et al. 2019; Ferrari et al. 2014). The loss records in national and international economy and trade following an outbreak make FMD the most priority concern for livestock owners. The losses associated with outbreak control and precautionary measures in addition to economic impact cost several million US dollars for every outbreak (SENASA 2006).

Foot and mouth disease are caused by FMD virus, the prototype member of the genus Aphthovirus of the family Picornaviridae. FMD virus occurs as seven distinct serotypes (Euroasiatic serotypes A, O, C, and Asia1 and South African Territories [SAT] serotypes SAT1, SAT2 and SAT3 (SENASA 2011). FMD virus can be transmitted by direct contact, indirect contact facilitated by contaminated materials and airborne droplets (Doel et al. 1984; Hayer et al. 2018; Arzt et al. 2019). The rate of spread and the incubation period, as well as the severity of disease, depends on many variables including the dose of virus particles received, the route of transmission, the virus strain, the animal species, animal age and the animal

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health condition (Arzt et al. 2011). The virus first replicates in the pharynx and within 24–48 hrs, it induces viremia and shortly thereafter generalized infection occurred with significant lesions in the mouth and feet of susceptible animals. The incubation period of FMD virus has a range between 2- 12 days (Yoon 2011) and the disease is characterized by high fever that declines rapidly after two -3 days. Mouth and tongue ulcers developed with excessive secretion of saliva. Ulcers in the inter-digital space of the feet induce lameness. Adult animals may suffer of weight loss for long period (Brito et al. 2013). Also swelling of testicles of mature animals has been recorded (Brito et al. 2011). Significant reduction of milk production occurs in infected dairy cattle (Ferrari et al. 2014). The FMD disease is subside in the most animals, while it is life threatening for new born animals due to the infection may lead to myocarditis and death. Viremia usually disappears after 3–4 days but the virus propagates to very high titers (>8 log10 infectious units per ml) at lesions sites and is shed in the air and body fluids. Between 5 and 10 days after their appearance, lesions resolve and virus is no longer found at the lesion sites and can only be recovered from pharyngeal fluid and tissues (OIE 2018; Knight-Jones and Rushton 2013). According to the FAO 2012 report (OIE 2012), 6.3 buffaloes and 7.5 million sheep and goats are at risk of FMD disease in Egypt, also about 40222 cases of FMD infection are suspected during outbreaks. According to official estimates 4658 animals died in the last outbreak, mostly calves. The practice of use of pathogen specific immunoglobulin for therapeutic and prophylactic settings for FMD virus and other infection has been recorded by several authors (McCullough et al. 1986; Wang et al. 2015). The aim of the present work is to prepare FMD virus-specific polyclonal bovine antibodies against the prevalent FMD serotypes in Egypt, namely serovars A, O and SAT2 and to evaluate its therapeutic and prophylactic potentials.

MATERIALS AND METHODS

Animals

Thirty apparently healthy calves were involved in this study for preparation of FMD virus-specific polyvalent antibodies and for FMD virus experimental infection.

Calves used for preparation of FMD virus-specific polyvalent antibodies: Nine calves 2 years old were used for hyper-immunization with the polyvalent FMD vaccine (FMD virus serotypes A, O, and SAT2) and preparation of FMD virus-specific polyvalent bovine antibodies.

Calves used in the Experimental infection (ex-infection)

Twenty-one calves were used in the experimental infection with different FMD virus serotypes. This test was done for experimental evaluation of the therapeutic efficacy of the prepared FMD virus-specific antibodies. These calves were grouped in 3 groups (7calves/group). Group 1 was infected with FMD virus serovar O, Group 2 was infected with serovar A, and Group 3 was infected with serovar SAT2.

FMD vaccine

Oil adjuvant polyvalent inactivated FMD virus vaccine against serovars A. O, and SAT2 produced by Veterinary serum and vaccine research institute, Abbasia, Egypt was used for preparation of FMD virus-specific polyvalent antibodies.

Preparation of FMD virus specific polyvalent bovine antibodies

The calves were hyper-immunized with the polyvalent FMD virus vaccine against serotypes A, O, SAT2. Three vaccine doses were given intramuscular for each animal at 2 weeks interval. One week after the last booster dose blood was collected from the immunized animals, serum was separated and its FMD virus-specific antibody titers were determined using serum neutralization test (SNT).

Separation and purification of the FMD virus-specific antibodies

Briefly the preparation of the FMD virus-specific immunoglobulins includes dilution of the collected cattle plasma with 2 volumes of saline and the pH was adjusted to 7.2 (2N Na OH). Ammonium sulphate (NH42SO4) was added to a final concentration of 33% and the mixture was stirred overnight at 10°C. The precipitate was collected, gauze filtered and dissolved in physiological saline to the initial protein content. The pH was adjusted to 5.8 + 0.1 using 1.76 N glacial acetic acid followed by drop wise addition of caprylic acid (Sigma) to a final concentration of 0.5% and the mixture was maintained under vigorous stirring for 1 hr at 18°C. After centrifugation at 1550 x g for 30 min the mixture was sterilized by filtration through 0.22 mm depth filter sheets. The FMD-virus specific antibodies were adjusted to contain 2.25, 2.28 and 2.15 $\log_{10}$ $TCID_{50}$/ml, against FMD virus serotypes A, O, SAT2, respectively.

FMD virus Experimental infection (ex-infection)

It was done in the Viral Large Animal Vaccines Evaluation Department, Central Laboratory for Evaluation of Veterinary Biologics-Abbasia-Egypt according to Nermeen et al. (2019).

Inoculation of FMD virus serotypes A, O and SAT2: After screening of the FMD virus specific antibody titer at different times, the calves were infected with 0.3ml containing 10^4 BID_{50} of the following FMD virus serotypes (Cox et al. 2006): A (Group 1), O (Group 2) and SAT2 (Group3). Virus inoculation was done by the intra-dermolingual route at different sites in the tongue. Infected calves were observed daily for FMD symptoms (temperature, vesicles in the tongue and or the feet). Animals showing symptoms were subjected to virus re-isolation and results were recorded.

Experimental Evaluation of the immunotherapeutic potential of the prepared FMD virus specific polyvalent antibodies

Four days post infection the calves in the 3 Groups were treated by the prepared polyvalent bovine FMD virus-specific polyclonal antibodies. Three different doses namely 4, 6 and 8 ml of the prepared antibodies were tested. In each group (containing 7 calves), each therapeutic dose was tested in 2 calves and the last 7th calf was left as untreated control. The antibody therapy was injected intramuscular (i/m) 4 days post infection. The therapeutic effects of the

different injected doses on virus shedding and on the severity of the clinical signs and healing of mouth and feet lesions were recorded.

Field evaluation of the therapeutic and protective efficacy of the prepared FMD immunotherapy

The therapeutic potential of the prepared FMD virus-specific antibody therapy was evaluated during the last FMD disease outbreak that affected cattle in Egypt at 2016/17. The diseased calves (455 calves) and adjacent apparently healthy ones (300 calves) were treated with 8 ml dose of the FMD antibody therapy that was injected i/m or intravenously (i/v) and the treated animals were subjected to daily observation for up to 4 weeks.

RESULTS

Titer of FMD virus-specific antibodies in sera of immunized cattle

Data presented in Table 1, show the average titers of the FMD virus- specific antibodies that were measured in serum of the immunized cattle one week after the third booster dose of the FMD vaccine. It reached to 142.2 +84.1, 177.8+76.9 and 104+67.9 SNT units/ml against FMD serovars A, O, and SAT 2, respectively.

Table 1: Titer of FMD virus-specific polyclonal antibodies in serum samples from immunized cattle measured with serum neutralization test

Serial number of the immunized calves	Titers of FMD virus-specific antibodies against the following FMD virus serotypes		
	A	O	SAT 2
1	256	128	64
2	256	128	64
3	128	128	128
4	256	256	256
5	64	256	128
6	128	64	64
7	64	128	64
8	64	256	64
9	64	256	32
X±SDn	142.2±84.10	177.7±76.9	104.0±67.9

n=9 calves.

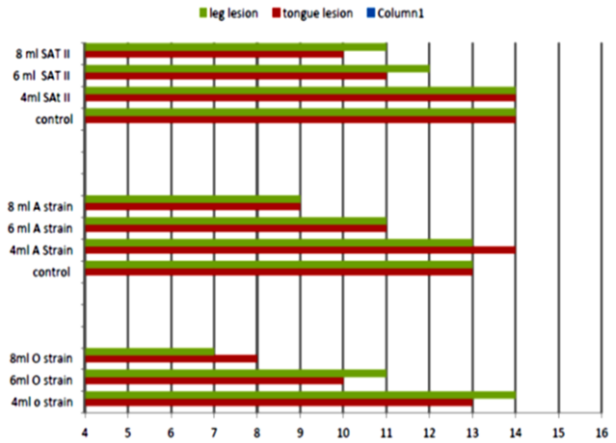

Fig. 1: The Therapeutic effect of FMD virus-specific polyvalent bovine antibodies on the recovery rates of FMD virus experimentally infected calves.

Therapeutic efficacy of FMD virus-specific polyvalent antibodies

Effect of the injected dose of FMD virus-specific polyclonal antibodies on the developed clinical signs in calves experimentally infection with the different FMD virus serotypes:

Ex-infected calves developed typical sings of FMD disease within 3-4 days post infection. Infected calves were treated just after the start of development of clinical signs (at the 4th day post infection) with the prepared polyvalent FMD virus-specific antibodies. Among the different the tested doses of the FMD virus specific polyclonal antibodies, the 8ml dose proved highly effective in lessening of the clinical signs and reducing the FMD associated lesions. A clear dose related rate of recovery was recorded where 8 ml dose containing ca.200 SNT units /ml of FMD virus specific antibodies could induce a rapid effect with a recovery rate from 48-96hrs as compared to 14 days required for spontaneous recovery in the control FMD virus infected non treated calves (Fig. 1).

Effect of FMD virus-specific polyclonal antibodies on FMD naturally diseased calves

From January to March 2017, a total of 455 buffalo and cattle heads with different ages, reared in different localities of Egypt manifesting typical clinical signs of FMD disease, including stop feeding and mouth and foot lesions, were included in a field trial for evaluation of the therapeutic potential of FMD virus-specific polyclonal polyvalent bovine antibodies. Also 300 adjacent apparently healthy cattle were included in the study. All animals were injected with 8ml of the prepared antibodies being given by the i/m or i/v routes.

All animals were treated with levamisole (600mg/100kg bwt) to avoid endo-parasite infestations along the trial. Treated animals were observed daily for clinical signs and lesion progress of FMD.

All treated diseased animals manifested significant improvement of their general health, appetite and rapid healing of FMD lesions within 24-72 hours post therapy without any side effects. The course of the FMD virus infection in the control non-treated diseased cattle was aggressive and continued up to 2 weeks before starting to recover. None of the FMD-specific immunoglobulin-treated adjacent apparently healthy calves developed FMD disease. In concern with recovery time the intravenous route proved more effective than the i/m route.

DISCUSSION

FMD as a renewed public and political high - profile disease has aroused the global concerns (Sobrino et al. 2001). It is a highly contagious disease that not only reduces animal commercial value by decreasing animal weight and milk production, but also it is the most important animal disease restricting animals and animal products trade (Lubroth 2012). For those reasons, it is important to prevent, control and even eradicate FMD.

In Egypt Foot-and-mouth disease (FMD) frequently causes outbreaks as a result of fault vaccination programs or introduction of new a FMD virus serotype with the

imported cattle through defective quarantine measures. These FMD outbreaks are usually associated with significant economic losses and social consequences associated with the severe clinical signs and mortality among affected cattle, particularly among newly borne diseases calves (Knight-Jones and Rushton 2013; OIE 2012). Also, the disease lesions are so painful preventing animal from feeding and reducing its productivity. Nevertheless, the efforts of the veterinarian to treat and reduce the severity of the clinical signs are almost based on expansive medical drugs that are more or less of limited value. Also, it should be realized that emergency vaccination to control FMD outbreaks takes at least 7 days until such vaccination protects animals (Barnett and Carabin 2002; Orsel et al. 2005). Within this time, the disease can spread further. Therefore, an intervention is required to face the FMD outbreaks that should not only provide rapid protection against FMD in outbreak situations but also lessen the severity of the clinical signs, reducing the mortality, enhancing healing of FMD lesions and prevent spreading of the disease through reduction of the virus shedding.

In the present work it was planned to provide and evaluate such rapid protection by passive immunization using polyvalent FMD virus-specific polyclonal antibodies of bovine origin. Although it is not a new intervention but, in this work, we intend to use a polyvalent antibody covering the FMD virus serotypes prevalent in Egypt and of bovine origin to avoid any possible side effects.

Immunoglobulins are an extremely gained the powerful of acceptance and convenience that can be widely used to prevent and treat emerging infectious diseases (Lu et al. 2020). Immunotherapy has been effective against both of infectious and non-infectious diseases (Chan 2009; Elsterova et al. 2016). The historical records of passive antibody therapies continuously prove the usefulness of implementation and impetus for developing.

The induction of neutralizing antibodies is the mechanism most frequently related to protection against FMD (Dunn et al. 1998) and a number of reports have provided in-depth information about such rapid protection that can be achieved by passive transfer of hyperimmune serum (Mateu and Verdaguer 2004), or neutralizing monoclonal antibodies (mAbs) (Christopher et al. 2012) or by the FMD specific camel nanobodies (Wang et al. 2015). The latter type of nanobodies has a major disadvantage where these small antibody fragments are rapidly removed from the circulation of mammals with an elimination half-life of several hours (Batra et al. 2002).

In the present work the use of antibodies of the same species aimed at reducing or preventing any possibility of development of allergic side effects following injection of these antibodies, a reaction known to occurs in case of using foreign antibodies. This might comment about the absence of any side effects, allergy, and clinical signs of serum sickness among treated animals for an observation period of 4 weeks post therapy. On the other hand, the use polyvalent antibodies were planned to cover the FMD serovars known to be prevalent in Egypt. Nevertheless, the use of polyclonal antibodies was decided to cover several FMD virus epitopes as compared to the monoclonal antibody (mAbs) types. Several authors have recorded that polyclonal antibody preparations may be more valuable to mAbs as the former contains antibodies to multiple epitopes as compared to the monovalent character of mAbs that greatly limit the anti-infection effect of antibody drugs (Batra et al. 2002).

In the present work the experimentally infection calves manifest the FMD clinical signs starting with fever and the lesions starts to develop in the mouth and feet 72-96 hours post infection with the different FMD virus serotypes, namely A, O and SAT 2. The immunotherapy was given at the fourth day post infection. Different doses including 4, 6 and 8ml/animal, containing an average of 200 $\log_{10}$ SNT units/ml against each FMD serovars (A, O and SAT2), were injected i/m. A dose related recovery rate was recorded and the 8 ml dose proved highly effective in reducing the severity of the clinical signs, healing of feet and mouth lesions and recovery of treated animals within 48-96hrs post treatment. This was associated with significant reduction of virus shedding. Similar results were obtained in the field trials that were so impressive to a degree that severely diseased newly born calves recovered completely after 48hrs post treatment. In the field trials the intravenous injection of the immunotherapy was more effective than the intramuscular route in relation to rate of recovery. There are some reports recording the therapeutic and prophylactic efficacy of passive immunization of cattle against FMD (Harmsen et al. 2009). The effect of the immunotherapy with FMD virus specific antibodies can be attributed to the fact that the prepared FMD specific antibody therapy is composed mainly of IgG antibody isotype, which is known by its good tissue penetration capability and a half-life of about 15-20 days (Arturo 1996). Also the FMD virus specific IgG is known to enhance phagocytosis of virus/antibody complexes by the reticuloendothelial system (McCullough et al. 1988). Furthermore, it blocks viral cell entry, which is the predominant mechanism of FMD virus neutralization (Mateu and Verdaguer 2004).

It should be considered that therapeutic antibody not only limited to extracellular pathogens, But also several records have suggested that some antibodies are active against some intracellular microorganisms. Some IgA mAbs can neutralize intracellular viruses by binding to viral proteins and interfering with viral assembly, and intracellular replication of Toxoplasma gondii has been reported to be interfered by a mAb. Additional evidence for intracellular antibody activity comes from the observation that IgG anti-DNA autoantibodies can enter the cytoplasm and nucleus of living cells (Christopher et al. 2012). Antibodies mediate antimicrobial function through a variety of mechanisms, including inhibition of microbial attachment, agglutination, viral neutralization, toxin neutralization, antibody directed cellular cytotoxicity, complement activation, and opsonization (Cox et al. 2006).

Although emergency vaccination to control FMD outbreaks is essential, the protection takes at least 7 days to protect susceptible animals (Salt et al. 1998). Within this time, the disease can spread further where the vaccinated animals might transmit the FMD virus to contact susceptible animals during the negative phase of

the vaccination process (4-7 days), wherever the airborne transmission route between farms is at highest risk approximately 5-20 days after the disease becomes detectable at the farm (Björnham et al. 2020). Therefore, there is a need for therapies that provide not only rapid protection against FMD but reduce shedding and prevent the spread of the virus. such emergency control could be conducted by hyper immune serum inoculation (Blancou 2002). Finally, it should be recognized that passive immunization of calves with bovine polyvalent FMD virus-specific polyclonal antibodies does not interfere with the laboratory diagnosis of this disease, which depends mainly upon the detection of non-structural protein of the FMD virus in animal serum using different serological tests, and it is recommended for effective prophylactic treatment during outbreaks.

Author's Contribution

SR, SMA and AMS conceived and designed the study in addition to writing the manuscript and providing the critical revisions. SRH, SFA, HH and DDM provided support to conduct the research study and collection the data. SR and AMS analyzed and interpreted the results. All authors approved the final version of the manuscript.

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