The immunogenicity and efficacy of a commercially available Infectious Bovine Rhinotracheitis (IBR) virus vaccine against a Pakistani field IBR strain

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Summary
Infectious bovine rhinotracheitis (IBR) is a highly communicable disease of cattle and wild ruminants that is caused by Bovine alphaherpesvirus 1 (BoHV-1). For IBR control, several developed countries have adopted the immunization and eradication programs focusing on IBR-positive animals. In Pakistan, livestock producers are importing commercially available vaccine of BoHV-1, but no studies on the efficacy of these commercial vaccines against local isolates are available. Therefore, the present study was aimed to evaluate the efficacy of a commercially available vaccine of BoHV-1 against local field isolates of virus. The rabbit model was used and the vaccine was evaluated for immunogenicity and protection after challenge with a highly virulent strain of a field virus. The immune response was measured by virus neutralization titers (VNT). This vaccine induced a humoral response in rabbits but that was not sufficient to completely protect the vaccinated animals against the wild-type BoHV-1 strain challenge. While a low virus titer compared to control rabbits was observed in the vaccinated rabbits ($p<0.05$), there was no sterilizing immunity or freedom from infection. However, complete freedom from disease, for example, the absence of pyrexia was noticed in the vaccinated group. In conclusion, the present study demonstrated that imported vaccine stock provoked only a partial protection against indigenous isolate of BoHV-1. However, tests performed on rabbits are preliminary, as only those performed on the source species can determine more reliable results.

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Introduction

Infectious bovine rhinotracheitis is a highly contagious disease of cattle and wild ruminants that is caused by Bovine alphaherpesvirus 1 (BoHV-1).

The virus is a double stranded DNA virus belonging to family *Herpesviridae* (Mahajan *et al*. 2013). Isolates of BoHV-1 have been classified into three subtypes BoHV-1.1, BoHV-1.2a and BoHV-1.2b based on genomic analysis. Subtype-1 is the representative strain of IBR and prevalent in Europe, North and South America. Subtype 2a is associated with respiratory and genital disease and is widespread in Brazil (Edwards *et al*. 1990). Subtype 2b is involved in genital disease such as infectious pustular vulvovaginitis (IPV) and balanoposthitis but is not associated with abortion and predominates in Northern Ireland and Australia. In Columbia, BoHV-1 has been reported (Ruiz-Saenz *et al*. 2012). IBR have been eradicated from Finland, Austria, Sweden, Denmark, Switzerland and Norway.

After infection of conjunctiva, oral and nasal cavity, BoHV-1 substantiates to its latent form in sensory neurons of trigeminal ganglion and pharyngeal tonsils. After genital infection, it establishes latency in sacral ganglion. With the passage of time, the virus reactivates from latency. Animals with latent infection keep shedding BoHV-1 and serve as a reservoir of the virus for naïve animals. Various factors such as parturition, inclement weather conditions, malnutrition, transportation stress, chronic diseases and administration of corticosteroid can lead to the reactivation of the virus. Vaccination is known to reduce the amount of virus shedding following reactivation (Raaperi *et al*. 2014).

In North America, the control of BoHV-1 was done by using both modified live vaccine (MLV) and inactivated vaccine. The uses of MLV of BoHV-1 are associated with abortions when administered to pregnant animals. Two pre-breeding doses of MLV with an annual booster of multivalent combination viral (Marsden *et al*.) vaccine decrease the incidence of abortion and fetal loss (Fulton *et al*. 2015; Walz *et al*. 2017).

In Pakistan, prevalence of BoHV-1 is increasing posing a serious threat to country's economy. A recent study has indicated a prevalence of 69% of BoHV-1 in cattle of Pakistan.

In order to minimize economic and stock losses, Pakistani farmers are importing commercially available BoHV-1 vaccines. However, no study is available about the efficacy of these vaccines for protection from indigenous isolates of the virus. Keeping in view this scenario, the present study was designed to evaluate the efficacy of a commercially available imported vaccine of BoHV-1 against field isolates.

Materials and methods

Vaccine

To evaluate the efficacy of a commercially available vaccine, an imported inactivated commercial vaccine against BoHV-1 was procured from the market and stored at 4˚C till use. The vaccine contains inactivated whole BoHV-1.

Field virus

A local wild-type BoHV-1 strain was obtained from Institute of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore. The virus was isolated from immunosuppressed cattle in a recent study (Rehman *et al*. 2020).

Titration of virus

Mardin-Darby Bovine Kidney (MDBK) cells, derived from a kidney of normal adult steer, were used for virus titration and neutralization assays. The virus was titrated by tissue culture infectious dose 50 ($TCID_{50}$)/mL. For this purpose, 15,000 MDBK cells were added in each well of a 96-well cell culture plate. These cells were inoculated with tenfold dilutions of virus which were prepared in maintenance medium. After 72 hours of incubation, each well was observed for the percentage of cell death under inverted microscope and an infectious dose was determined by Spearman Kerber method (Hierholzer and Killington 1996).

Experimental animals

The seronegative male white rabbits 7-8 weeks of age and 1.5-2.5 kg of weight were procured. Rabbits were divided into two groups of 8 animal each: the experimental group and the control group. Before the start of experiment, rabbits were allowed to adapt the environment for 1 week (Machado *et al*. 2013). All the experimental procedures were performed as per approval of Ethical Review Committee for the Use of Laboratory Animals of UVAS, Lahore, Pakistan.

Experimental design

The rabbits of the vaccine group were vaccinated with a commercially available imported vaccine via sub cutaneous and the control group was mock vaccinated with phosphate buffered saline. The rabbits were inoculated on day 0 and a booster dose was administered on day 15. Temperature and clinical signs were recorded daily from day 1 to day 28 post vaccination (dpv). Blood samples were collected on weekly basis from pre-vaccination until

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the end of experiment (42 dpv). On day 28, post-vaccination, rabbits were challenged by 1 mL of $4x10^7$ $TCID_{50}$/mL of the wild-type BoHV-1 strain by intranasal route (0.5 mL per nostril).

Temperature was recorded and nasal swabs were collected daily for 14 days of post-challenge. Rabbits were examined daily for nasal discharge, respiratory distress, eye discharge and conjunctivitis (Ruiz-Sáenz *et al*. 2013).

Virus Neutralization Test

Serum complement was inactivated by incubating in a shaking water bath at 56˚C for 30 minutes (Liu *et al*. 2015).

Two-fold dilutions of the serum were made from 1:2 to 1: 256 in wells of a 96-well cell culture plate. A 100 µl of 100 $TCID_{50}$/mL of the wild-type BoHV-1 strain was added in each well containing the diluted serum. The plate was incubated in CO_2 incubator for 2 hours at 37˚C. The MDBK cells (10,000) were added in each well and the plate was again incubated at 37˚C for 72 to 96 hours in 5% CO_2. Virus neutralization titer was the reciprocal of highest serum dilution that completely prevented appearance of cytopathic effects (Raza *et al*. 2016).

Post-challenge isolation and titration of virus

After challenging with the field virus, nasal swab and ocular discharge samples were collected and transported to laboratory in microfuge tube with transport medium.

The swab samples were processed and the supernatant was filtered through 0.22-micron syringe filter. Tenfold dilutions of filtrate were prepared for titration of the virus (Vargas *et al*. 2016).

Statistical analysis

The data were analyzed using descriptive statistics. Comparison of means was done by Analysis of variance using Bonferroni procedure for least significant difference of neutralizing antibody titer, virus excretion and body temperature in control group as well as in vaccinated group. The statistical significance was set at 0.05 in all the cases.

Results

Antibody Response in the Rabbits

At day 0, before the start of experiment, the antibody titer was zero in all the experimental and control rabbits.

Two weeks post-vaccination, average 1:4 titer was observed in the experimental group. After booster vaccine, the antibody titer increased significantly: average neutralization titer was increased to 1:32 and 1:128 at 21 and 28 dpv, respectively.

All individuals of control group remained seronegative up to 28 day, however, after challenge with the field isolate of BoHV-1, a sudden peak in antibody titer was noticed with the maximum titer of 1:64 on day 42 post-challenge.

Neutralization titer also increased in vaccinated individuals soon after challenge but this peak was less steep as compared to the control group.

Statistical analysis revealed a significant difference ($p<0.05$) in virus neutralization titer between the two groups throughout the experiment except in the beginning of experiment (Figure 1).

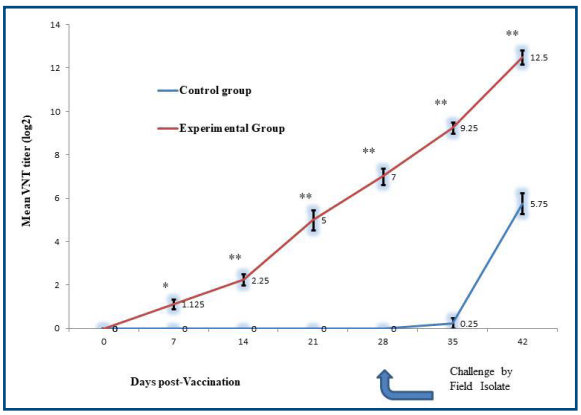

Figure 1. *Humoral immune response induced by subcutaneous administration of inactivated vaccine in rabbits. Experimental group was vaccinated with 1mL dose of subcutaneous injection at day 0 and day 14 and control group was mock vaccinated with 1ml of Phosphate Buffered Saline. On 28 day, both groups were challenged with the field isolate of BoHV-1 (arrow). Neutralizing antibody titer was determined in serum, these values represent the average titer of each group ± SEM at different time periods, **p<0.005, *p<0.05.*

The effect of vaccine on rabbits

The temperature of control group was between 101.2°F and 101.8°F from day 1 to day 28.
Temperature of vaccinated individuals was also normal throughout the length of experiment; however, a slight peak was noted two time points: first one day 2 and second on day 16 with temperature of 102.5°F and 102.7°F. None of the experimental animal showed any clinical signs: nasal secretion, eye secretion, conjunctivitis or dyspnea after vaccination. The statistical analysis did not reveal significant difference between vaccinated and control groups from day 0 to day 28 except a rise in the body temperature seen on day 2 and on day 16 ($p<0.05$) (Figure 2).

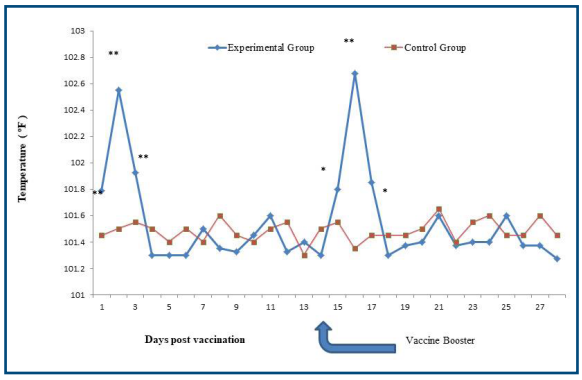

Figure 2. *Temperature pattern among rabbits of vaccinated and control group rabbits from day 1 to day 28 post vaccination. Values represent the mean of each group, **p<0.01, *p<0.05.*

Protection against challenge of field virus

Rabbits were challenged with 1mL of 1×107 TCID50/mL of field virus and were observed daily for the development of any clinical signs or rise in the body temperature.

In the control group, there was a slight increase in temperature (101.7ºF) 48 hours after challenge which peaked at 103.75 on day 3 post-infection.

After day 3, body temperature started to decrease on days 4 and 5 (102.4ºF and 102.0ºF, respectively) and reached to normal body temperature on day 6 (101.7ºF). In the vaccine group, no change in temperature was observed from days 1 to 14 post-infection.

The statistical comparison revealed a significant difference (p<0.05) between vaccinated and control groups from day 2 to day 5 post-infection (Figure 3). Clinical signs such as nasal secretions, ocular secretions, conjunctivitis or dyspnea were not seen in any of rabbits from both groups.

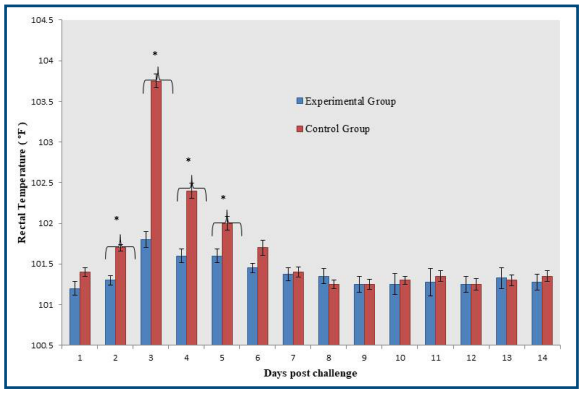

Figure 3. *Mean rectal temperatures of both vaccinated and control groups after challenge with field isolate of BoHV-1. Values indicate the mean ± SEM of each group with a significant difference of *p<0.05.*

Decrease in viral shedding

A decrease in virus shedding in the vaccinated group compared to control group was observed (Figure 4). In the control group, the highest virus titer was observed on day 3 post-challenge, however, the control group completely recovered from infection on day 10 as no virus shedding was noticed. In the vaccine group, the virus titer was 1.4 which decreased with time and rabbits were completely recovered from infection on day 8, without obtaining any peak of virus shedding. The statistical analysis revealed a significant difference (p<0.05) on days 2, 3 and 4 between the two groups.

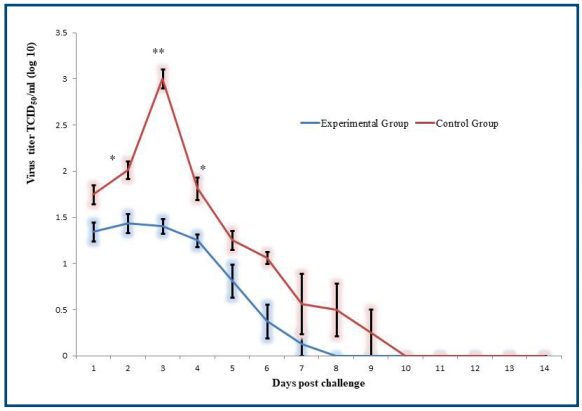

Figure 4. *Virus titration to measure the virus load in nasal swabs of immunized and control groups after challenge with a local field isolate of BoHV-1. Virus titers were calculated by $TCID_{50}$/mL of individuals nasal swabs soaked in 1mL of DMEM. Values represent the mean ± SEM of both groups at different time periods, *p<0.05, **p<0.01.*

Discussion

Infectious bovine rhinotracheitis (IBR) could pose a serious challenge to emerging dairy industry of Pakistan as well as threaten food security of poor farming communities of Pakistan by compromising livelihood. The present study was designed to evaluate the efficacy and immunogenicity of a commercially available imported Infectious bovine rhinotracheitis (IBR) vaccine after challenge with a local field isolate. Our results showed that the induced humoral response was not sufficient to completely protect vaccinated rabbits against the field virus. No sterilizing immunity or freedom from infection was observed in the vaccinated rabbits but complete freedom from disease was noticed. The present study demonstrated that imported vaccine induced only a partial protection against wild-type BoHV-1 strain.

The only possible way to control and eliminate the IBR disease is through vaccination but if attenuated vaccines are used in those areas which have high serological prevalence of BoHV-1, there is a risk of development of highly pathogenic strains through

recombination of vaccine strains and field strains. Upon using attenuated vaccines, there are risks of reversion vaccine strain to pathogenic form, establishment of virus in latent form and induction of abortion in pregnant animals (Thiry *et al*. 2006)

Inactivated vaccines are safe for administration in pregnant animals. Upon using inactivated vaccines, there are no risks of conversion of the virus to its latent form, shedding of virus and there is no hindrance with the induction of immunity by subsequent inactivated vaccines. However, in case of inactivated vaccines, cell-mediated immunity is not sufficiently stimulated, therefore, multiple doses of vaccines and adjuvants are needed to stimulate adequate immunity. Many studies suggest that the use of inactivated vaccine could generate sufficient protection to control BoHV-1 in cattle (Sambamurti *et al*.).

Owing to the non-availability of seronegative cattle, research studies suggest the use of rabbits or guinea pigs for the evaluation of BoHV-1 vaccines. Both, rabbits and guinea pigs can be used to evaluate the immune response of a BoHV-1 vaccines as these animals' immune system are similar to cattle (Kamaraj *et al*. 2009; Parreño *et al*. 2010). A rabbit model was used in the present to demonstrate that vaccines are competent to prevent IBR by increasing the virus neutralization titer and decreasing viral shedding after challenging with wild-type BoHV-1 strain corroborating.

Commercially available BoHV-1 vaccines differ from one another in protection from infection and prevention of disease after field virus challenge (DesCôteaux *et al*. 2003). In the present study, a commercial inactivated BoHV-1 vaccine induced good immune response as mean serological neutralizing titer reach up to 7 (log2) at 28-day post immunization. These titers increased to 9.25 (log2) and 12.5 (log2) on days 7 and 14 post challenge in the vaccinated group. In the control group, serological titer was zero up to 28 days post-vaccination but increased to 5.75 (log2) after 14 days post challenge. After challenge, pyrexia was observed in animals of control group which peaked to 103.75ºF, while in the vaccinated group, rabbits' body temperature was in the normal range throughout the length of experiment. Although virus titer was observed in both the vaccinated and control groups, less virus titer was observed in the vaccinated group in comparison to control group (figure 4) corroborating previous studies (Ruiz-Sáenz *et al*. 2013). The manufacturing company of the vaccine has not disclosed the viral strain that they have used to make this vaccine therefore from the results it depicts that they have used a strain that is different from the local circulating strain. The antibody titer was 1:128 on days 21 and 28. This high antibody titer is unable to provide complete protection against the homologous challenge. The probable reason for this may be that the killed/inactivated vaccines are unable to stimulate adequate cell mediated immune response. The cell-mediated immune response is a critical defense mechanism to provide complete protection against BoHV-1 infection (Levings and Roth 2013; van Drunen Littel-van den Hurk 2007).

There are a few limitations of the present study, only one vaccine was evaluated. The use of more vaccines may elucidate the spectrum of immune responses against local BoHV-1 strains Moreover, side-by-side comparison of immune response induced by imported vaccines and vaccines made from the local strains could shed further light on the immunogenicity of vaccines. Further, vaccine efficacy and challenge studies in the target species (cattle) could be extremely helpful in establishing the immune correlates of BoHV-1 vaccination for the control of IBR in Pakistan.

Author Contributions

Conceptualization, MR,SR, FNA and; methodology, HIA; formal analysis, HIA,SR and SF; writing original draft preparation, HIA,SR and SA; writing, review and editing, HIA, MR, FNA, SR, SF and SA.

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