ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/261171690

Locally Prepared Live and Inactivated Bovine Herpes Virus 1 gE-Negative Vaccines Induce Immune Response in Calves and...

Article · January 2009

CITATIONS
0

READS
53

4 authors, including:

Gabr F El-Bagoury
Benha University
32 PUBLICATIONS **12** CITATIONS
SEE PROFILE

Ehab El-Nahas
Benha University
17 PUBLICATIONS **31** CITATIONS
SEE PROFILE

Alaa El-Kholy
Veterinary Serum and Vaccine Research Instit...
15 PUBLICATIONS **145** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Virulence determinants in respiratory and enteric viruses View project

All content following this page was uploaded by Ehab El-Nahas on 28 March 2014.

The user has requested enhancement of the downloaded file.

Locally Prepared Live and Inactivated Bovine Herpes Virus 1 gE-Negative Vaccines Induce Immune Response in Calves and Allow Serological Differentiation

El-Bagoury, G.F.*; Amer, D. I. ; El-Nahas, E.M.* and El-Kholy, A.A. ****

*Departments of virology, Faculty of Veterinary Medicine, Moshtohor, Benha University, Egypt. ** Veterinary serum and vaccine research institute، Abbassia, Cairo.

Abstract

Based on a glycoprotein E (gE) deleted bovine herpesvirus - 1 (BoHV-1) Abu Hammad strain, a modified live virus as well as an inactivated virus marker vaccine have been developed that allow differentiation between immunized and BoHV-1 infected cattle. These vaccines were evaluated for safety and efficacy in calves and compared with the commercial locally prepared inactivated BoHV-l vaccine. No adverse effects were observed in any of the calves vaccinated with the gE-negative vaccines. Both gE negative and the commercial inactivated vaccines revealed a significant increase in humoral immune response at 3rd week post booster vaccination no significant change could be detected among them as detected with serum neutralization test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA). After vaccination, calves vaccinate with gE-negative vaccines did not produce antibodies against gE, but these antibodies were detectable within 2 weeks in vaccinated calves with a local prepared inactivated BoHV-l vaccine as demonstrated using gE-blocking ELISA to detect antibodies against gE. These results demonstrate the efficacy of a gE-negative BoHV-l vaccine and the detectability of antibodies against gE. The combined use of the marker vaccine and the gE-blocking ELISA makes it possible to differentiate between vaccinated animals and infected animals. This possibility may be very useful in BoHV-l control programmes.

Introduction

Bovine herpesvirus-1 (BoHV-1), a member of the family Herpesviridae,subfamilyAlphaherpesvirinae, genus Varicellovirus is a major pathogen of cattle. Primary infection induces various clinical manifestations such as infectious bovine rhinotracheitis

(IBR), infectious pustular vulvovaginitis, abortion and generalized systemic infection (**7 and 9**). The BoHV-1 genome consists of a linear dsDNA molecule of about 140 Kb (**13**). It encodes several glycoproteins that are expressed on the viral envelope and membranes of infected cells. While some of these are essential for virus replication (**11**), other glycoproteins are not essential for virus replication and represent potential targets for deletions aiming the development of marker vaccines. Glycoprotein E (gE) is one of such non-essential proteins (**4 and 14**). Although it is conserved among other members of the Herpesviridae family, the role of gE in the invitro growth characteristics may vary in function of the virus species and the host cell (**2**). Vaccination with conventional vaccines has been the most important control strategy world-wide. Although these vaccines can induce good clinical protection (**5**), cattle vaccinated with conventional vaccines cannot be distinguished from cattle infected with the field virus by serological tests. For that reason, we conducted a trial for comparison of the efficiency of inactivated and live gE-negative vaccines with the commercial inactivated vaccine associated with a companion diagnostic test for gE-negative BoHV1 vaccines.

Materials and methods

2.1. Experimental vaccines: both attenuated and inactivated BHV-1 gE negative vaccines were made from gE-negative BoHV-l strain genetically constructed in VSVRI (Dr. Alaa A. EL-Kholy) through a project sponsored by the Academy of Science and Technology, Cairo, Egypt. Prior to inactivation the titer of the virus $10^{6.5}$ TCID50/ml. Also locally prepared inactivated BoHV-l (Abu Hammad strain) vaccine was included in the experiment. The vaccines were produced and provided by VSVRI.

2.2. Animals: male mixed breed (Friesian and local) calves, 6-9 months old and free of BoHV-l (Abu Hammad Strain) using

serum neutilization test (SNT) according to **(6)** and Enzyme Linked Immunosrbent Assay (ELISA) using IBR test kit (Idexx lab B.V., the Netherlands) as described by the manufacture. All calves were healthy and adapted to their new surroundings. All calves remained BoHV-l seronegative until the start of the experiment.

2.3. Experimental design: The calves were randomly divided in four groups of three calves each and housed in identically conditioned isolation stables as follow:

Group I: Each of three calves was vaccinated intramuscularly with 2ml of live modified BoHV-1 gE negative vaccine and were boostered at 21 days post the first ones.

Group II: Each of three calves was intramuscularly immunized with 2ml of killed modified BoHV-1 gE negative vaccine and was boostered at 21 days post the first ones.

Group III: Each of three calves was intramuscularly immunized with 2ml of locally prepared inactivated IBR- vaccine and received booster doses of the vaccine 21 days post the first ones.

Group VI: Three calves were left as non vaccinated controls. Each of these calves was intramuscularly injected with physiological saline and was left as control.

2.4. Serum samples: All sera were collected from groups I,II,III,VI on the day of vaccination (zero day) ,then at the 1st and 2nd week post vaccination and 1st, 2nd ,3rd ,4th ,6th and 8th week post booster vaccination and were examined for antibody response to vaccination by the Serum Neutralization Test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA) in addition to detection of antibodies against gE with gE-blocking ELISA

2.5. Serum neutralization test (SNT): It was carried out as described by **(6).**

2.6. Enzyme linked Immuno Sorbent Assay "ELISA" Test Kit:-

2.6.1. The CHEKIT-Trachitest Serum Screening ELISA Test Kit "IBR Antibody Test Kit",Idexx Laboratories B.V.,The Netherlands Cat No 240-R421. This kit provides a rapid, simple, sensitive and specific method for detecting antibodies against (BoHV-1)

2.6.2. BoHV-1 gE Antibody test Kit:- Herdchek-Anti IBR gE is an enzyme immunoassay for the detection of antibodies in bovine serum samples to the gE antigen of BoHV-1.

2.7. Virus isolation and detection: nasal swabs were collected from all calves before and post vaccination. They were tested for virus shedding of BoHV-1 from vaccinated calves using MDBK cell culture inoculation and PCR.

2.8. Polymerase chain reaction: It was carried according to **(1)** using one set of primer gB1(Forward) 5' CACGGACCTGGTGGACAAGAAG 3' gB2 (Reverse) 5' CTACCGTCACGTGAGTGGTACG 3' for gB gene synthesized by Operon Biotechnologies group as described by (**3).**

RESULTS

3.1. Humoral immune response in calves vaccinated with Live modified BoHV-1 gE negative, inactivated modified BoHV-1 gE negative and inactivated BoHV-1(Abu Hammad) vaccines: Statistical analysis of the serological data between groups I and III revealed a significant increase in the SN-antibody titer against BHV-1 virus on the 3rd week post booster vaccination also there was a significant increase between groups II and III in the SN-antibody titer against BHV-1 virus on the 3rd week post booster vaccination but there was no significant change could be detected among the groups I, II, and III on the 3rd week post vaccination as revealed in (Table 1) and (Fig 1a). The same results were observed by ELISA as shown in (Table 2) and (Fig 1b).

Table (1): Mean BoHV-1 Antibody titers (expressed as in log10 TCID 50) in sera of vaccinated calves using serum neutralization test

Date of samplig	gP I	gP II	gP III	gP IV
Zero Day	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1st W.P.V	0.23 ± 0.08	0.28 ± 0.04	0.34 ±0.08	0.00 ± 0.00
2nd W.P.V	0.58 ± 0.10	0.49 ± 0.17	0.59 ± 0.04	0.00 ± 0.00
3rdW.P.V	0.71 ± 0.16	0.76 ± 0.15	0.85 ± 0.00	0.00 ± 0.00
1st W.P.B.V	1.00 ± 0.17	0.93 ± 0.12	1.11 ± 0.17	0.00 ± 0.00
2nd W.P.B.V	1.15 ± 0.22	1.08 ± 0.17	1.31 ± 0.08	0.00 ± 0.00
3rd W.P.B.V	1.25 ± 0.08	1.22 ± 0.86	1.41 ± 0.00	0.00 ± 0.00
4th W.P.B.V	1.23 ± 0.05	1.11 ± 0.17	1.27 ± 0.15	0.00 ± 0.00
6th W.P.B.V	1.13 ± 0.20	0.98 ± 0.00	1.18 ± 0.08	0.00 ± 0.00
8thW.P.B.V	1.10 ± 0.17	0.97 ± 0.12	1.02 ± 0.17	0.00 ± 0.00

gP I : Live Modified BoHV-1 gE negative vaccine gP II : inactivated Modified BoHV-1 gE negative vaccine gP III : inactivated BoHV-1 vaccine gP IV: control non vaccinated group W.P.V : week post vaccination W.P.B.V : week post booster dose vaccination

Table (2): Mean anti-BoHV-1 antibody titers in sera of vaccinated calves using CHEKIT trachitest serum screening ELISA test kit, Idexx"

Date of samplig	gP I	gP II	gP III	gP IV
Zero Day	5.93 ± 1.25	4.73 ±1.56	6.60 ± 1.25	5.40 ± 1.01
1st W.P.V	130.43 ± 9.70	91.00 ± 5.29	119.50 ± 4.27	6.17 ± 1.15
2nd W.P.V	152.33 ± 9.50	102.17 ± 5.96	168.00 ± 16.09	6.83 ± 0.85
3rdW.P.V	229.70 ± 10.50	114.90± 3.05	218.00 ± 12.50	7.60 ± 0.529
1st W.P.B.V	255.17 ± 18.50	169.60 ± 9.01	223.90 ± 5.35	6.40 ± 0.80
2nd W.P.B.V	298.67 ± 4.50	259.00 ± 4.35	292.20 ± 7.62	5.47 ± 0.95
3rd W.P.B.V	304.43 ± 3.60	272.70 ± 8.73	302.10 ± 2.10	7.77 ± 0.55
4th W.P.B.V	267.47 ± 3.41	231.17 ± 10.50	277.50 ±18.0	7.37 ± 0.907
6th W.P.B.V	254.60 ± 7.05	219.83 ± 1.73	265.80 ± 6.02	5.87 ± 1.19
8thW.P.B.V	254.10 ± 4.40	217.40 ± 2.30	259.80 ± 10.42	5.83 ± 1.00

Anti - BoHV-1 Ab titre represented as ELISA percentage value Value< 35% mean negative-Value≥ 35% to 45% mean suspect-Value≥ 45% mean positive

Table (3): Mean anti-BoHV-1 antibody titers sera of vaccinated calves using (BoHV-1) gE antibody test kit

Date of samplig	gP I	gP II	gP III	gP IV
Zero Day	0.93 ± 0.04	1.03 ± 0.03	1.02 ± 0.01	0.99 ± 0.01
1st W.P.V	0.94 ± 0.01	1.02 ± 0.03	0.43 ± 0.01	1.04 ± 0.02
2nd W.P.V	0.97 ± 0.01	1.04 ± 0.03	0.40 ± 0.02	1.03 ± 0.02
3rdW.P.V	0.99 ± 0.05	1.05 ± 0.03	0.38 ± 0.04	1.06 ± 0.00
1st W.P.B.V	1.01 ± 0.01	1.08 ± 0.04	0.41 ± 0.02	1.00 ± 5.01
2nd W.P.B.V	1.06 ± 0.01	1.15 ± 0.02	0.38 ± 0.06	1.07 ± 0.02
3rd W.P.B.V	1.02 ± 0.01	1.17 ± 0.01	0.45 ± 0.02	1.08 ± 0.01
4th W.P.B.V	1.00 ± 0.02	1.08 ± 0.04	0.35 ± 0.03	1.10 ± 0.01
6th W.P.B.V	1.04 ± 0.02	1.04 ± 0.02	0.34 ± 0.01	1.11 ± 0.01
8thW.P.B.V	1.00 ± 0.01	1.13 ± 0.03	0.30 ± 0 .01	1.12 ± 0.01

Anti-BHV-1 Ab represented as (S/N) sample/negative Ratio S/N < 0.60 the sample is positive S/N > 0.6 or equal to 0.7 sample should be retested S/N > 0.7 the sample is negative

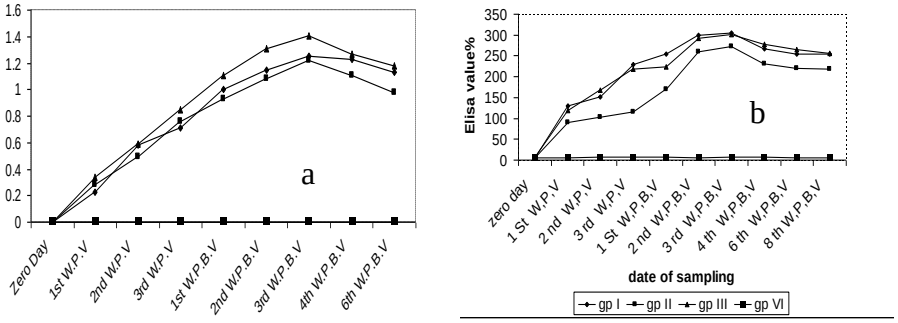

Fig (1) (a) mean BoHV-1 neutralizing antibody titers expressed as $\log_{10}$ of highest serum dilution that inhibited the BoHV-1 cytopathic effect.(b) Anti -BoHV-1 Ab titre represented as ELISA percentage value for gP I : Live Modified BoHV-1 gE negative vaccine gP II : inactivated Modified BoHV-1 gE negative vaccine gP III : inactivated BHV-1 vaccine gP IV: control non vaccinated group W.P.V : week post vaccination W.P.B.V : week post booster dose vaccination

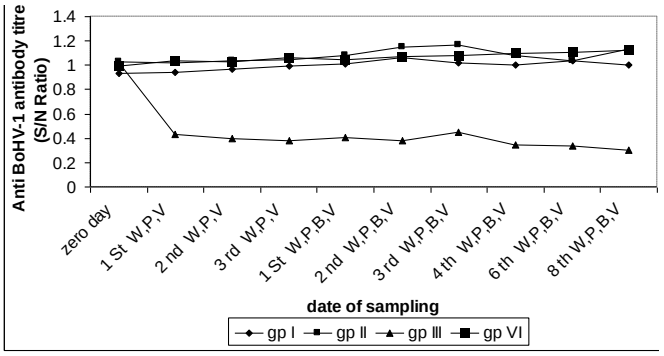

Fig (2) Anti-BHV-1 Ab represented as (S/N) sample/negative Ratio for gP I : Live Modified BoHV-1 gE negative vaccine gP II : inactivated Modified BoHV-1 gE negative vaccine gP III : inactivated BoHV-1 vaccine gP IV: control non vaccinated group W.P.V : week post vaccination W.P.B.V : week post booster dose vaccination

4.5. Detection of gE antibody titer in sera of vaccinated calves using BoHV-1 gE antibody ELISA test kit: In ELISA (BoHV-1 gE Test kit) there was a significant increase in ELISA ratio between groups I, II and groups II, III on the 3rd week post

vaccination. Also there was a significant increase in ELISA ratio between groups I, II also groups I, III and groups II, III on the 3rd week post booster vaccination as revealed in (Table 3) and (Fig2).

4.6. Virus shedding: no infectious virus was detected in the nasal swabs collected from vaccinated calves at groups I, II and III on the 3rd, 4th, 6th week post booster vaccination as observed by absence of CPE and negative results for PCR assay as shown in figure (3).

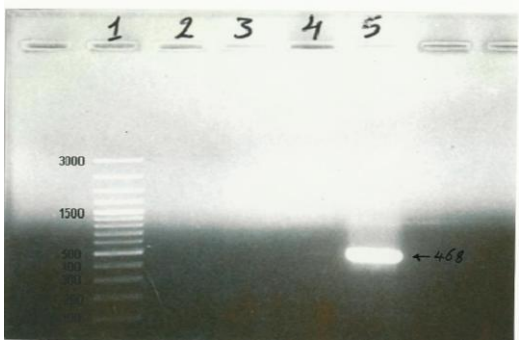

Fig (3): Agrose gel electrophersis of PCR amplicons from gB gene of BoHV-1 genome separated on 1.5% Agrose gel and stained with ethidium bromide. Lanes (1) Gene Ruler 100pb DNA ladder plus (Fermentas, USA),(2,3,4) DNA extracted from nasal swabs from vaccinated animals and (5) Genomic DNA from BoHV-1 gE-negative strain Notice the unique discrete bands of PCR amplicons at the size of 468 bp

Discussion

Control of BoHV-1 in Egypt depends mainly on vaccination of susceptible animals. Marker vaccines, which allow serological differentiation between vaccinated and field virus infected animals, may be of value in BHV-1 eradication programmes (**16)**. Glycoproteins gC, gE, gI, gG and gM are non-essential and thus may be deleted with little or no effect on virus production in vitro or in vivo. **(8)**. So in this study BHV-1 gE negative viruses were used as a marker vaccinal strain for production of live and

inactivated vaccine type and compared with the inactivated vaccine produced from a natural BoHV-1 Abu Hammad strain with respect for humoral immune response that assayed by serum neutralization test and ELISA techniques. Moreover using especial ELISA kits for serological differentiation between antibody produced against both natural and marker vaccine.

Results of humoral immune response indicated that there was no significant change could be detected among the groups I, II, and III on the 3rd week post vaccination as shown in table (1 and 2) and fig (1(a) and1(b)) . This comes in agreement with **(10 and 15**) and this could be discussed according to **(12**) who discovered that the majority of the wild-type strains have the gE gene and the antigenic variability of gE is low.

BoHV-1 gE antibody ELISA test kit were sensitive and detected gE antibodies only in group III vaccinated with inactivated Abu Hammad strain within the 1st week of vaccination and extend along the period of experiment table (3). Fig (2). This result indicated that the combined use of the marker vaccine and the gE ELISA makes it possible to differentiate between vaccinated animals and infected animals .this possibility may be very useful in BoHV-1 control programmes.as recorded by **(8).**

PCR assay indicated no execration of the marker virus vaccine in discharges as shown in figure (3) that reduce the population outbreak, virus transmission and also latency and this comes in agreement with by **(7 and 8**) who showed that significant protection was induced by the gC, gE and gG mutants, whereas the protection levels were lower for the gI and gE/gI viruses. Finally, after dexa-methasone reactivation, the gC and gG mutants could be re-isolated suggesting that these mutants caused latency, whereas no gE,gI or gE/gI virus was isolated.

References

1. **Alegre, M., Nonni, M., and Fondevila, N. (2001):** Development of a multiplex polymerase chain reaction for the differentiation of BHV-1 and BHV-5.Vet. Med. B.48:613-621.
2. **Balan P., Davis-Poynter N., Bell S., Atkinson H., Browne H. & Minson T. (1994):** An analysis of the in vitro and in vivo phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or the putative gJ. J. Gen. Virol. 75(6):1245-1258.
3. **El-kholly, A.A (2005):** Molecular and immunological detection of bovine herpes virus-1 in clinical specimens. The Egypt. Jour. of immunolog.12 (2)125-136.
4. **Franco A.C., Rijsewijk F.A.M., Flores E.F., Weiblen R. & Roehe P.M. (2002):** Construction and characterization of a glycoprotein E deletion mutant of bovine herpesvirus type 1.2 strain isolated in Brazil. Braz. J. Microbiol. 33:274-278.
5. **Frerichs, G.N., Woocls, S.B., Lucas, M.H. and Sands, J.J. (1982):** Safety and efficacy of live and inactivated infectious bovine rhinotracheitis vaccines. Vet. Rec. 111, 116-l 22.
6. **Fulton, R.W., Confer, Burge, L.J.,A.W.,Porino, I.J., Doffay, J.M., Payton, M.E., and Mock, R.E., (1995):** antibody viral vaccines containing BHV-1, PI-3and BRSV immunogens and subsequently revaccination at day 140. Vaccine,13:725-733
7. **Jones C. (2003):** Herpes simplex virus type 1 and bovine herpesvirus 1 latency, Clin. Microbiol. Rev. 16:79–95.
8. **Kaashoek, M.J., Rijsewijk, F.A., Ruuls, R.C., Keil, G.M., Thiry, E., Pastoret, P.P., Van Oirschot, J.T. (1998):** Virulence, immunogenicity and reactivation of bovine herpesvirus 1 mutants with a deletion in the gC, gG, gI, gE, or in both the gI and gE gene. Vaccine 16: 802-809.
9. **Kaashoek, M.J., Rijswijk, F.A.M., Van Oirschot, J.T. (1996):** Persistence of antibodies against bovine herpesvirus 1 and virus reactivation two to three years after infection.Vet. Microbiol. 53, 103–110.
10. **Mars M.H., De Jong M.C.M., Franken P. and Van Oirschot J.T. (2001)**: Efficacy of a live glycoprotein E-negative bovine herpesvirus 1 vaccine in cattle in the field. Vaccine, 19, 1924–1930.
11. **Rebordosa, X., Pinol, J., Perez-Pons, J.A., Lloberas, J., Naval, J.,Serra-Hartmann, X., Espuna, E., Querol, E. (1996):** Glycoprotein E of bovine herpesvirus type 1 is involved in virus transmission by direct cell-to-cell spread. Virus Res. 45, 59–68.
12. **Rijsewijk, F.A., Kaashoek, M.J., Langeveld, J.P.M, Maris-Veldhuis, M.A., Magdalena, J. and Van Oirschot, J.T. (2000):** Epitopes in

glycoprotein E and on the glycoprotein E/ glycoprotein I complex of bovine herpes virus-1are expressed by all of 222 isolates and 11 vaccine strains. Arch. Virol. 145, 921–935.

13. **Schwyzer, M., Ackermann, M., (1996):** Molecular virology of ruminant herpesviruses. Vet. Microbiol. 53, 17–29.
14. **Spilki F.R., Silva A.D., Hubner S., Esteves P.A., Franco A.C., Driemeier D., Roehea P.M. (2004) :** Partial protection induced by a BHV-1 recombinant vaccine against challenge with BHV-5, Ann. NY Acad. Sci. 1026 247–250.
15. **Trapp, S., Konig, P., and Beer, M., (2003):**convenentional and markedBHV-1 vaccines in Germany: abrief review . Berl. Munch.TierarztlWochenschr 116(5-6):208-215.
16. **Van Oirschot, J.T., Kaashoek, M.J., Rijsewijk, F.A.M., (1996):** Advances in the development and evaluation of bovine herpesvirus 1 vaccines. Vet. Microbiol. 53, 43–54.

View publication stats