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HUMORAL IMMUNE RESPONSE OF GUINEA PIGS TO AN EXPERIMENTAL VACCINE AGAINST EQUINE HERPES-VIRUS INFECTION

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The aim of this study was to develop an experimental vaccine against herpesvirus (EHV-1) infection. For vaccine development weused a field strain of the virus isolated from the tissue of an aborted foetus from a horse herd in wich the disedse appeated in the form of mass abortion. Virus was adapted and multiplied in piglet kidney primary tissue culture, utilizing Eagle-s MEM with 2% calf fetal serum. Virus was collected 24-48 hours after inoculation, when the complete cell monolayer was affected by the cytopathogenic process (change). After elimination of cell detritus, virus inactivation was performed with 0,2% formalin solution. A 30% $Al(OH)_3$ v/v in gel-form was used for virus stabilization.

Antigenic properties of the vaccine were examined on 30 guinea pigs weighting 300 to 400 g. Vaccine was administered twice at a twenty-one-day-interval, as a 0,4 ml subcutaneous dose in a knee ridge. The humoral immune response (seroconversion) was chected 14 days after vaccination and re-vacination. using a serum neutralization test with 100 $TCID_{50}$ viruses on the PK-15 cell line. Values forthe antibody titer ranged from 1:4 to 1:64, 14 days after vaccination, while they were significanty higher 14 days after re-vaccination, ranging from 1:32 to 1:128.

Key words: equine herpesviirus infection EHV-1. inactivated vaccine, laboratory animals, serum-neutralization test.

INTRODUCTION

Equine herpesvirus infection (Equine viral rinopneumonitis-Equine abortion virus, with its two sub-types: subtype 1 EHV-1 and subtype 2 EHV-4), is widely spread in the horse population in the whole world. Having in mind considerable economical losses caused by this disease, an abligatory program of mass vaccination has been run in many countries.

EHV-1 subtype 1 causes abortions, perinatal losses, respiratory diseases, myeloencephalitis, paresis and vulvovaginitis. In free herds abortions appear in the form of mass disease or sporadically, when the virus exists endemically (Bagust 1971, Studdert et al. 1970). It is not known so far if abortions result from the primary infection, re-infection or infection reactivation during the period of pregnancy. They occur without primary signs of the disease, mostly in the period from 8 to 11 months of gravidity, usually only once which is the results of acquired immunity. Sometimes weakly vital foals are born,dying usually after a few days. Pathological signs of viraemia, parenchymatous degeneration of the myocardim, liver and kidneys, spleen and lymph-node oedema; congestion and swelling of the mucous membranes, catarrhal changes followed by petechia on gastric and intestinal mucous tissues, and lung congestion, oedema and interstinal pneumonia are evident in adult animals. Necrotic changes in the liver, a weak icterus, interlobular oedema, accumulation of serous liquid in pericardial, pleural and peritoneal cavities are obvious in aborted foetuses. An infection in the early stage of gravidity can even cause autolysis of the foetus.

EHV-4 subtype 2 induces infection of the upper tespiratory tract, mostly in foals in the first year of age, and sporadically abortions (Robert et al. 1988). Infection is manifested as a cold followed by fever, cough, rhinitis, pharyingitis, and sometimes serofibrinous arthritis. Animals improve quickly in good hygienic and nutritive conditions, otherwise complications in the form of bronchopueumonia or even lethality, are possible. The disease breaks out mainly in young animals once in a year, in the form of epizootics. In older categories it occurs as re infection with sporadic abortions. Congestion, erosions and necroses of the respiratory tract tissue as well as hyperplasia of the regional lymph tissue have been detected pathologically.

A principal source of infection are animals showing clinical signs of the disease or latently infected ones, disseminating the virus especially in stress situations (ablactation, transport, castration, traiming). Virus is disseminated by nasal, oral and genital secretions (most by the sperm of the infected stallion). The spread of the disease includes the mother - foetus infection i. e. infection through the placenta and aborted foetus.

Infection often remains unnoticed until abortion occurs, due to the unclearly pronounced clinical syptoms, absence of a good immune response as well as insufficient sensitivity of available diagnostic methods. Diagnosis is made by isolation of the virus from mucuous tissue of the naso-pharynx, leukocytes of the infected animal or from the tissue of the aborted foetus. Identification is performed mostly utilizing the method of direct immunofluorescence. The polymerase chain reaction method also presents a highly sensitive method (Ballagi-Paradany et al., 1990). The serum-neutralization test, ELISA, and complement fixation test are widely used to determine the serological status of the herd. DNA analysis is an important diagnostic method for examination of the epizootiological status. DNA-analysis and monoclonal antibodies against glycoproteins of the virus envelope are utilized for determination of the virus sub-types.

EHV-1 virus belongs to the family of Herpesviridae, subsp. Alphaherpesvirinae, 150-170 nm in size. The genome consists of ds DNA, containing 170 kpb. The capsid is formed of 162 capsomeres and around 20 proteins. The virus is susceptible to environmental factors, ether, chloroform, formalin, trypsin. It is relatively heat labile. It can be cultivated on cell culture media originating from the horse (kidneys, lungs, skin), sheep or pig, where it creates syncutal formations 1 to 5 days after inoculation. In lower titers virus can be multiplied on tissue cultures originating from cattle, rabbits and monkeys (Studdert 1970).

The virus is antigenically uniform. Both subtypes belong to the same serotype, which has been demonstrated by classic serological tests. Differences in some biological and genetic propertis exists among sub-types. EHV-1 subtype 1 (strain Armi 183, Kentucky, T 431) is very virulent, causing mainly abortions but rarely respiratory changes (O'Keefe et al. 1991; Robert et al. 1988; fifzpatricl 1984). On the other hand, the other subtype EHV-4 (T 473, H-45) is weakly varulent. The clinical picture of the infection is dominated by signs of respiratory disorders, seldom abortions. Comparison of the genome sequences of both strain shows 20% homology, pointing at their common origin (Ballagi-Paradany et al. 1990). DNA regions coding antigenic glycoprotein determinants, which are included in the virus penetration process and fusion of infected cells, show intertypical differences in tests with monoclonal antibodies (O'Keefe et al. 1991; Robert et al. 1988).

The am of this study was to prepare an experimental inactivated vaccine against herpesvirus infection of horses.

MATERIAL AND METHODS

Virus: A field strain isolated from the tissue of an aborted foetus from a herd with clinically pronounced infection in the form of abortions was used to prepare the experimental vaccine.

Cell culture: Virus was adapted and multiplied on a primary pig kidney cell culture.Eagle-s MEM culture medium with 2% of fetal calf serum was used in this experiment. Virus was collected 48 hours after inoculation, when CPE affected the entire monolayer. The virus suspension was frozen three times, and afterwards centrifugated at 2000 rpm to remove the cell detritus. The titer was $10^{8,5}$ $TCID_{50}$/0,1 ml.

Virus inactivation: A 0,2% formalin solution was used for virus inactivation

Adjuvant: 30% Al $(OH)_3$ v/v in gel-form

Standard methods were utilized to control virus inactivation, sterility, stability and safety of the vaccine.

EXAMINATION OF ANTIGENIC PROPERTIES OF THE VACCINE

Laboratory animals. For this investigation 30 guinea pigs weighing 300-400 g were used. A dose of 0,4 ml was administered s. c. in the knee ridge two times at intervals of 21 days. A control group included five animals. To check for seroconversion, blood was taken 14 days after vacctination, and re-vacctination.

Sarum neutralization test: The humoral immune response (seroconversion) was determined by micro serum neutralization test (SN). Double serum dilutions were prepared, starting from 1:2 and 100 $TCID_{50}$ virus on PK-15 cellular line. Results were interpreted after 4 days.

Statistics: Geometrical mean values were determined in this study. Student's t-test was utilized to determine statistically significant differences between mean values of the specific antibody titer. The frequency of the titer value of 1:64 was determined after the first and second vaccine administratous. utilizing Pearson's X^2 test.

RESULTS

Table 1. Distribution of values for the titer of neutralizing antibodies against EHV-1 in guinea pig blood sera obtained after two-fold administration of experimental vaccine.

Antibody titer	Number of investigated animals	
	14 days after 1.admin.	14 days after 2.admin.
< 1 : 2	5*	5*
1 : 2	–	–
1 : 4	4	–
1 : 8	8	–
1 : 16	13	–
1 : 32	3	6
1 : 64	2	20
1 : 128	–	4

* Control animals

The values for the specific antibody titer obtained after vaccination, ranged from 1:4 to 1:64 (Table 1) In 21 out of.30 animals titer values ranged from 1:8 to 1:16. After revaccination, the titer of specific antibodies increased significantly, extending from 1:32 to 1:128. The titer of 1:64 was observed in 20 out of 30 animals. Non-vaccinated animals from the control-group were seronegative. The difference between the geometric mean titer after vaccination and revaccination was statistically significant. (Table 2).

Table 2. Statistical significance between values of titers of neutralizing antibodies against EHV-1 in guinea pig blood sera obtained after single and two-fold administration of experimental vaccine.

Period of investigat.	GSV*	p**	X^{2+}
14 days after 1. administ.	13.00	–	–
14 days after 2. administ.	61.11	< 0.001	< 0.005

*Geometric mean value
**Statistical significance determined by t-test
+ Statistical significance determined by X^2 -test

Immunogenic properties of the vaccine were examined on 6 seronegative horses. Vaccine was administered twice at a 21 day interval, i. m., in the neck region. The titer of specific antibodies ranged from 1:64 to 1:256, 14 days after re-vaccination.

DISCUSSION

Basic protection against herpes virus infection of horses includes specific immunoprophylactic activities. Their efficacy is to a great extent determined by proper zooprophylactic and hygienic measures, keeping under quarantme, etc. Control of the disease is conducted by observing seroconditions in the herd, including early discovery and exclusion of latently infected animals.

Currently two types of vaccines are in use - "live" vaccines, made-up of attenuated virus strains, and "dead" vaccines containing inactivated virus (Bagust 1971; Studdert et al. 1970). Vaccinal strains were adapted and attenuated mostly by many passages, e. g. Rac H strain with 256 passages on pig kidney tissue culture (Mayer et al. 1992) on guinea-pig kidney etc. The lack of particular nucleotide-sequences has been confirmed by analyzing genomes of attenuated strains. a luck of nucleotice sequences (0,8 kpb) has ben determined in the terminal Ir genome-part of the RacH strain. These strains are not transferred horizontally, but sometimes they can reactivate latent infection (Mayer et al. 1992). The immunogenic value of vaccines containing inactivated virus is relative. Vaccines containing classic adjuvants mostly induce a weak immune stimulus, low antibody level and a short humoral immune response (Mumford et al. 1984), so they cannot prevent abortions (Sabine et al. 1988). If made up of horse-foetus tissue, they can awake haeemolytic anemia of foals.

Humoral and cellular immunity coponents are of great importance for the protection of horses against herpes-virus infection. The main effectors of the immunological defense are neutralizing antibodies and cellular immunity, determined by the surface of infected cells and glycoproteins of the virus envelope, such as glycoproteins gB and gH (Stokes et al 1988;1991).

Neutralizing antibodies are detectable one week post infection, reaching their maximal values in weeks three to five of the infection, and remaining unchanged until week 19. Afterwards, titer values can decrease to unmeasurable levels. Re-infection of stress can induce a repeated increase of antibody level

(Burrows et al. 1984). Antibodies belong mostly to the IgG class, appearing already on day 5, and reaching a maximal level at day 9, but their titer decreases rapidly (Stokes et al., 1991). Even though humoral and cellular immunity components develop during the infectionm, or after vaccination, they cannot affect viruses in latently infected cells (neurones, dorsal ganglia, endothelial cells of blood vessels, lymphocytes). Antibodies are present also in the period of abortion, bemg unable to prevent it.

Postvaccinal titers of serum-neutralizing antibodies of 1:100 or higher, are considered to be a solid protection against infection. This antibody titer provides resistance to infection, that is virus replication in the nasopharynx and viraemia. In this case the nasopharynx presents a barrier to infection and re-infection, providing also protection against abortions (Studdert 1974).

According to the OIE standards (Manual of Standards for Diagnostic Test and Vaccines 1992) the safety and power of the vaccine is checked by experiments on gravid mares or laboratory animals (golden hamser, guinea pig), seroconversion control or challenge infection (Cook et al. 1980).

Fovaurable results were obtained with our experimental vaccine. The vaccine with inactivated virus, was administered to guinea pigs twice at a twenty-one-day-interval, and resulted in specific antibody titers of 1:32 to 1:128. Considerably lower values were observed 14 days after primary application, ranging from 1:4 to 1:32. Horeover, high neutralization antibody titers were determined 14 days after re vaccination of seronegative horses (6 animals). We must emphasize that our experiment was not aimed at an examination of the resistance to challenge-infection.

A vaccination program is determined by the vaccine type, epizootic situation and serological condition in the herd. If infection is enzootically present in the herd, all age categories should be included in the vaccination program. Gravid mares are vaccinated in the third, fifth and seventh month of pregnancy, which provides the foetus with protection and a high level of colostral antibodies. Passive immunity protects foals in the first two to three months, being replaced with active protection without visible clinical symptoms. Foals should be subjected to vaccination after three months of age, which should be repeated at 6 month intervals. Stallions and other categories should be vaccinated twice yearly.

Deficiency of cross protection by both virus sub types presents a considerable problem in the vaccine production. Namely, serum neutralizing antibodies are type-specific, while cellular immunity provides protection against both subtypes. Glycoproteins of the virus envelope are considered as a promising factor in the production of new vaccines. Importance of the "main" gB glycoprotein (appearing at the surface of infected cell) in the induction of the immune response has been confirmed. Monoclonal antibodies against minor glycoprotein gH neutralize the virus (Cook et al., 1990). Vaccines resulting from genetic engineering will hoperfully provide protection against both subtypes of the virus (Ballagi-Pordany et. al. 1990. Bagust 1971), i. e. both forms of the disease (Sabine et al. 1989).

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HUMORALNI IMUNI ODGOVOR ZAMORACA NA EKSPERIMENTALNU VAKCINU HERPESVIRUSNE INFEKCIJE KONJA

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SADRŽAJ

Cilj našeg rada je bio priprema eksperimentalne vakcine protiv herpesvirusa (ehvi) infekcije konja. Vakcina sadrži terenski soj herpesvirusa konja,izolovanog iz tkiva abortiranog fetusa iz zapata gde se bolest pojavila sa kliničkom manifestacijom masovnog abortusa kobila. Virus je adaptiran i umnožen na primarnoj kulturi tkiva bubrega praseta, koristeći Eagle-s MEM obogaćen sa 2% telećeg seruma. Virus je sakupljan 24-48 časova posle inokulacije, kada je kompletna ćelijska kultura bila zahvaćena citopatogenim promenama. Posle uklanjanja ćelijskog detritusa, virus je inaktivisan sa 0,2%. rastvorom formalina. Kao stabilizator dodat je 30% A1(H)$_3$ v/v u gel obliku.

Antigene karakteristika vakcine ispitivane su na 30 zamoraca težine 300-400 g. Vakcina je aplikovana dvokratnou razmaku od 21 dan,po 0,4 ml subkutano u koleni nabor. Kontrola serokonverzije prateći humoralnu komponentu imuniteta, vršena je 14 dana posle vakcinacije odnosno revakcinacije, serum neutralizacionim testom sa 100 $TCID_{50}$ virusa na PK-15 ćelijskoj liniji.

Vrednosti titra antitela bile su od 1:4 do 1:64, 14 dana posle vakcinacije i značajno viši 14 dana posle revakcinacije kada su iznosile od 1:32 do 1:128.