In Vitro Preparation And Testing Of Anti-Salmonella Vaccine Against Abortion In Sheep

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Abstract

In March 2016, the microbiology laboratory of the Faculty of Veterinary Medicine Cluj-Napoca, 3 type of antisalmonella vaccine for sheep were prepared. For type 1, 24 hours salmonella on brain heart infusion (BHI) broth culture, heat inactivated for 1 hour at 60°C, then with formaldehyde in a concentration of 3 ‰. Variant 2 - the culture supernatant obtained on solid BHI medium, washed with PBS, frozen-thawed 6 times, centrifuged at 4000 rpm for 15 minutes, filtered through 0.2 µm Millipore filter Orange Scientific. Variant 3 - suspension of cell walls remaining after centrifugation suspended in PBS and inactivated with formaldehyde.

After the bacterial and fungal sterility control, the three types of vaccine were administered in rabbits by the subcutaneous route at a dose of 1 ml/individual along with 1 ml of plant extract adjuvant (decoction of *Ewernia prunastri*) sterilized by filtration.

There have been two booster inoculations of the initial administration, 7 and 14 days after. Before each dose of vaccine, blood was sampled from the marginal auricular vein in order to control the immunogenicity by anti-somatic "O" serum antibody (Ab) titration using slow microplate agglutination test (Widal reaction). After three inoculations with the vaccine variant 1, Ab serum titer reached 1/128, and in types 2 and 3, 1/512 after 2 inoculations, decreasing to 1/256 after the second booster administered with no immunomodulator.

Keywords: *anti-Salmonella vaccine against abortion in sheep, adjuvant, Ewernia prunastri.*

INTRODUCTION

Salmonella abortion of sheep and goats is a contagious infectious disease that occurs enzootic evolving since the third gestation month until the fifth. Depending on the time of abortion onset, can occur in early pregnancy when it manifest by simply expelling the fetus without clinical signs in the mother, but if it occurs in late pregnancy, after removal of the nonviable or dead lamb, the ewe is presented with serious clinical signs like loss of appetite, fever and other complications that can

cause mortality in 10% of the cases (Leterrier, 2009).

In the worldwide epidemiology of salmonella abortion in sheep, numerous serovars of *Salmonella enterica* species are involved, which is capable of producing infections in other species of animals including humans. These infections are found worldwide, but are more common in Western Europe and Asia. *Salmonella* infections that cause abortions have been reported in France, Spain, Germany, Cyprus, Italy, Switzerland, Russia,

and Bulgaria. This disease was once very common in southwest England and Wales, but is now rare in the UK (Martín-Atance *et al.*, 2012).

In sheep, especially *Salmonella typhimurium* and *S.dublin* are isolated, but most specific during an abortion is *Salmonella abortus ovis* (Brougere-Picoux, 2004). *Salmonella abortus ovis* serovar in Romania was recorded for the first time in 1937 by Suhaci and Isopescu quoted by Volintir in 1975 and since then they have an endemic evolution in sheep flocks.

Consecutive to outbreaks evolution strong immunity is observed and no cases are usually registered for 2-3 years, but after this period new cases are observed. These observations and high costs as a result of antibiotic therapy in case of disease outbreaks led to the conclusion that the most effective and cost reducing method of prophylaxis remains vaccination.

Immunoprophylaxis of sheep salmonella abortion is currently based on the use of inactivated and live vaccines. Inactivated vaccines are relatively easy to produce but are generally poorly immunogenic, so the requirement for parenteral inoculation of repeated quantities of antigens and the use of adjuvants (Sviridenko and Ozerov, 1982). A few of the inactivation techniques or solutions used over time to obtain vaccines from inactivated germs are presented: phenol (Sviridenko, 1982), heat, formalin, (Nicolas *et al.*, 1981b), heat and formalin (Nicolas *et al.*, 1979a) or by freeze-thawing (Girginov *et al.*, 1971). The use and preparation of live vaccines to reduce the virulence of *Salmonella abortus ovis* strains were also tasted (Uzzau *et al.*, 2005).

Immunoprophylaxis with killed or live vaccines is currently the most common in enzootic areas. They are represented by *Salmonella* strains in liquid medium inactivated with phenol, formaldehyde or heat and adjuvated with aluminum hydroxide gel. Extracts of various species of lichens are used as immunostimulatory products in humans, but no information on their use as adjuvants for vaccines is currently available.

MATERIALS AND METHODS

The strain used in vaccine preparation was isolated in February 10, 2016 from an sheep abortion (provenience Jucu de Sus). Primary isolation was performed by direct inoculation on MacConkey agar (selective medium). On culture

media containing bile salts *Salmonella abortus ovis* is behaving atypically: slow growth, lactose-negative colonies (colorless) pin point in size after 24 hours of incubation, reaching 2 mm in diameter after 48 hours. Genus identification was made based on the selective medium colonies using API 20E gallery. Serological group confirmation was achieved by the rapid slide agglutination test in the presence of anti-Salmonella polyvalent serum followed by a second agglutination using group sera type OA, OB, OC, OD. The isolated strain agglutinated with polyvalent serum and OB group serum.

The vaccine strain was subjected to purity control through morphological examination of smears and examination of cultural characters on different selective media for Enterobacteriaceae family. Then the 3 possible vaccines were prepared. The culture medium used was the liquid and solid brain-heart infusion (BHI).

Type 1 was prepared from a 24 hours culture of BHI medium incubated at 37°C, inactivated for 1 hour at 60°C and then using formaldehyde in a concentration of 3 ‰.

For type 2 and 3, vaccine culture was prepared on solid BHI medium inoculated on its surface, incubated 24 h at 37°C, washed with PBS and inactivated with formaldehyde 3 ‰ and heat for 48 hours. Variant 2 was represented by the clear liquid resulting from the aforementioned vaccine culture subjected to 6 freeze-thawing cycles, centrifugation at 4000 rpm for 15 minutes and filtration through a filter with porosity of 0.2 µm Millipore Orange Scientific.

Variant 3 was represented by the suspension of the remaining cell walls and membranes after centrifugation for the preparation of the 2nd version, adjusted to the initial volume of the culture with PBS. In order to increase the immunogenicity of the three types of vaccine was prepared and used a plant adjuvant represented by a decoction of *Evernia prunastri* buffered with sodium bicarbonate and sterilized in a filter of a porosity of 0.2 µm Millipore Orange Scientific.

After determining the bacterial and fungal sterility of the three types of vaccine, they were administered to rabbits by the subcutaneous route at a dose of 1 ml vaccine + 1 ml adjuvant. For the immunogenic control of the of the vaccine, 1 ml blood was taken prior to the first vaccine inoculation, 7 days after the first administration,

Table 1. The results of the humoral immune response determined by slow agglutination test after 3 inocuations of the vaccine

Vaccin type	Initial titers before the inoculation	Titers after the first inoculation	Titers after the second inoculation	Titers after the third inoculation
Broth culture inactivated in 60°C	0	32	64	128
Culture supernatant freezed-thawed	0	512	512	256
Suspension of the cell wall after freezing-thawing	0	512	512	256

14 days after the 2nd administration and 14 days after the 3rd administration. From the resulted serum, titration of specific anti *Salmonella* antibodies by slow agglutination reaction (Widal reaction) was carried out.

RESULTS AND DISCUSSIONS

Antisalmonella antibody titer in the serum after the first blood sampling was absent from all three rabbits sampled. Following titration of sera obtained after four blood samples were obtained the following results (Table 1).

Harmlessness of the three variants of the vaccine has been very good, so the vaccine and immunomodulators used was well tolerated by the rabbits. At the site of inoculation were not observed undesirable local reaction such as inflammation, abscess or nodules.

After the first inoculation, the titer of serum antibody is increasing up to 1/32 for broth culture inactivated at 60°C and 1/512 of the other two variants (Table 1). Following the first boost with the vaccine associated with immunomodulatory serum antibodies titer increase to 1/64 in the vaccine inactivated by heat in broth culture solution, while the other two variants titers of 1/512 is maintained. After the second booster vaccine administered without adjuvant, serum antibody titer increases to 1/128 for the first variant while in the other two the titer decreased to 1/256.

Compared to the first version of the vaccine is observed that the other two stimulated much efficiently the immune system since the first inoculation. Reduced activity of the first vaccine could be due to the lower concentration of the antigenic mass (decreased bacterial cell number)

while type 2 and 3 were very rich in antigenic mass.

Since we did not find bibliographic information about the humoral immune response after vaccination with this vaccine we tried to compare the results with other antisalmonella vaccines used in poultry by Cambir (1999). This vaccine was also very dense in antigen mass, obtained by culturing the yeast and in the form of a fine atomized powder with a grain size below 0.2 mm. The product was used in large daily doses from 0.1 grams to 0.15 grams per chicks and adult chicken. Anti-salmonella antibody titers against somatic "O" antigen dose by slow agglutination method by Widal had a value of 1/320.

Titers obtained by us in rabbits have varied depending on the vaccine type between 1/128 and 1/512. We consider that higher titers of serum antibodies to the vaccine obtained by us in rabbits could be due to the immunomodulator used, which contains in addition using acid (natural antibiotic) and selenium, with a beneficial role in mammals.

Gram negative bacteria generally stimulate the production of antibodies in serum at lower levels than Gram positives because they have some wall structure fractions that are immunosuppressants. The presence of serum antibodies can be revealed by serological techniques (SAT) and Elisa in serum of females after abortion, the titer is usually greater than 1/20 (Martin-Atance *et al.*, 2011). According to other authors threshold of suspicion is 1/320 and threshold of positivity of 1/640, but the method of determination is not mentioned (Leterrier, 2009). If in doubt, do not hesitate to recheck the animals after fifteen days to see if the antibody titer is increased.

It has been found that the vaccine consisting of inactivated cultures of *S. abortusovis* induce both types of immune response, humoral and cell-mediated, which confers protection against natural or experimental infection with virulent *S. abortusovis* strains (Cagiola *et al.*, 2007).

CONCLUSIONS

All three variants of the vaccine were well tolerated after subcutaneous administration in rabbits. At the site of inoculation were not detected inflammatory or other negative reactions.

Type 2 consists of supernatant from frozen culture containing the ribosomal structure and induced the synthesis of high antibody titers from the first inoculation.

Type 3 also determines high antibody titers that are amplified after the first booster.

We found that the best immune response is observed following administration of concentrated vaccine type associated with immunomodulators.

The use of immunomodulators results in protective serum titers in rabbits with no need for vaccine booster in concentrated types.

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