Isolation and identification of fowl *Poxvirus* using PCR applied as a vaccine by nebulisation to newly-hatched chickens

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Newly-hatched chickens vaccinated with commercial fowl Poxvirus vaccine (BODIKAL® SPF, Vetrina d.o.o., Zagreb, Croatia) delivered by nebulisation did not show any clinical reaction to the given vaccine compared to negative (nonvaccinated) and positive (vaccinated by wing web) controls. To detect location and spread of vaccine virus, chickens were sacrificed and tissues were taken. Using commercially available kits, whole DNA was isolated from the tissue samples and viral DNA was detected using two different specific primer pairs in PCR reaction. Viral DNA was detected in trachea of chickens 20 hours and in lungs 7 and 14 days after vaccination by nebulisation. In positive controls, vaccinated by wing web method, viral DNA was detected at the site of vaccination in wing web tissue, and bone marrow on day 7 after vaccination, but none was detected in any other tissue of chickens in vaccinated groups during the trial. To define protection level after vaccination chickens were challenged into the wing web using velogenic field strain and skin reaction was measured and analyzed statistically. The size of the skin reaction in nebulised and wing web vaccinated chickens was significantly smaller compared to the negative control five days after challenge (day 26).

**Keywords**: chickens; Poxvirus; vaccination; nebulisation; PCR

**Introduction**

Poxviruses are group of the largest viruses with complex structure and with specificity to various groups of animals. Avipoxviruses are causing diseases in birds. In chickens fowl Poxvirus strain is causing cutaneous form of disease with large necrotic and crustous changes on the nonfeathered regions of the body and/or diphtheric form on the mucous membranes in the pharynx and upper respiratory system. In regions where pox is endemic specific vaccination using attenuated fowl or pigeon strain applied by wing-web vaccination around 8 weeks of age is indicated. Such method is labor consuming and hardly applied during production period.

Previous research by Gottstein et all. (2004) and Mazija et all. (unpublished) showed that pigeon or fowl strains, respectively, delivered to newly hatched chickens by means of nebulisation are safe and immunogenic. Poxvirus poses lots of nonessential regions in its genome that are used for insertion of heterologus genes and is suitable for development of recombinant vaccines. Such vaccines proved to be immunogenic and protective against inserted gene as well as carrier virus, for example in the case of avian influenza (Swayne and Mickle, 1997), but also showed that it could be a problem in repetitive application (Swayne et all., 2000). Some of those vaccines are already commercially available (Trovac AI H5, Merial), and proved to be effective in the case of single application.

This research was performed to confirm the previous results and show that fowl Poxvirus vaccine applied to newly hatched chickens is safe and immunogenic as well as to judge the PCR method is the
method of choice is simple, specific and sensitive enough to detect viral DNA that can be used for research of recombinant vaccines application.

Materials and methods

Chickens: 150 newly hatched male chickens, light line, were divided into 3 groups, with 2 repetitions per group and 25 chickens per repetition. Group A received vaccine by means of nebulisation during 60 seconds, while group B received vaccine by wing web method. Group C was not vaccinated and served as negative control. Chickens were fed and water was offered ad libitum.

Vaccine and methods of vaccination: Fowl pox vaccine BODIKAL® Pliva, Croatia, was used for vaccination of groups A and B. Chickens at group A were vaccinated by means of nebulisation using SONOVAC® 096 (Mazija and Štimac, 1999) and one dose per chicken while group B received the same vaccine by wing web method and in concentration according to the manufacturer instructions. Chickens were monitored clinically after vaccination till the end of the trial.

Tissue samples: Chickens were sacrificed 3 and 24 hours after vaccination and in weekly intervals starting from day 7 till day 21 and tissue samples of trachea, lungs, liver, spleen, thymus, bursa Fabricius, skin and bone marrow were taken. Each sample was frozen and subsequently used for DNA isolation using commercially available DNA isolation kit Macherey-Nagel Nucleospin Blood Kit (Germany) with some modifications.

PCR reaction: Detection of avian poxvirus was performed using set of primers previously described (Lee i Lee, 1997) that amplify 578 bp product of nuclear protein 4b. PCR was performed on 2 µl of DNA sample in 25 µl of reaction using GoTaq® Flexi DNA Polymerase (Promega, SAD) on GenAmp PCR System 2400 (Applied Biosystems, USA) during 35 cycles in following conditions: 94°C during 60 sec, 60°C during 60 sec, 72°C during 60 sec. PCR product was analyzed on 1% agaroze gel stained with ethidium bromide.

Challenge virus: Challenge was performed on day 21 of experiment using virulent field isolate. Virulent virus was applied to wing web and the skin reaction was measured using caliper in 5 days interval.

Statistics: Skin reactions were analyzed using ANOVA-LSD test in Statistica 7.1 (StatSoft, USA).

Results and discussion

Group A of chickens vaccinated by means of nebulisation didn’t show any sign from respiratory or other system, while group B vaccinated by wing web route gained skin reaction 5 days later. During the trial two chickens died because of yolk retention and technological error.

Using PCR reaction with specific primers viral DNA was detected in several samples. Specific 578 bp product was detected in vaccine (positive control) (Figure 1, line 1) as well as in tracheal tissue of group A 24 hours after vaccination (Figure 1, line 7). The same product was detected in the same group on day seven in lung tissue (Figure 1, line 10) as well as in group B in skin and in bone marrow tissue on the same day (Figure 1, line 15 and 14 respectively). Specific product was also detected in lung tissue of group A on day 14 (Figure 1, line 16). Other tissues of mentioned groups and group C didn't show any specific product till day 21 of the trial.
Skin reactions measured in 5 days interval after challenge on day 21 showed chickens of group A and B to have weaker skin reactions and are specifically protected compared to control group C on day 26 what is statistically significant (Figure 2).
Fowl Poxvirus is still causing serious problems in poultry production around the world especially in hot climatic regions. There it is rigorously immunoprotected starting in first week by wing web vaccination, following the second application after few weeks. Nevertheless the disease is still present in lower level.

European countries like Croatia have avian pox cases, usually in mild form. Epizootics of avian pox were more intensive five years ago and appeared in many countries including Croatia (Prukner-Radovič et al., 2006). The vaccination in Croatia is usually performed once, or in the case of enzootic type twice, depending on the type of production. Such approach is rather stressful for animals sometimes causing serious losses.

Croatia offered different way of immunoprotection proposed by Mazija and Štimac (1999) using nebulizer SONOVAC 096 where the most viral vaccines could be applied to newly hatched chickens already in the hatchery. Such approach is successfully used in vaccination against Marek’s disease (HVT-FC126), Newcastle disease, infectious bronchitis and Gumboro disease (Mazija et al., 1994; Mazija et al., 2000a; Mazija et al., 2000b; Rukavina, 1994).

Such success was the basis for further research in the field of nebulisation and pigeon Poxvirus was successfully used for vaccination of newly hatched chickens (Gottstein et al., 2004) and turkeys (Mazija et al., 2004, unpublished). Mentioned research as well as vaccination using fowl strain of avipoxvirus done by the same research group later, showed that vaccine didn’t cause any reaction from side of respiratory or any other system and by challenge proved the method to be protective. This was the basis for this trial performed in which the PCR method proved to be method of choice for simple, specific and sensitive way of viral genome detection and the dynamics of Poxvirus spread in different tissues.

This trial confirmed that nebulisation of vaccine is safe as chickens didn’t show any clinical reactions and immunogenic that was confirmed by the challenge on day 21. The PCR method showed to be sensitive and specific, and after all simple enough in detection of viral genome in different tissues. On the other hand dynamic of viral spread seems to be different depending on vaccine application methods. Vaccine delivered by nebulisation was detected in trachea 24 hours after...
vaccination, as well as on day 7 and 14 in the lungs, while after wing web method it was detected in tissues of skin reactions and in bone marrow on day 7. Virus was not detected in any other tissues till the day 21.

These results showed that nebulisation could be successfully used as a method of vaccination already in the hatchery. This is especially important in the investigation of the recombinant vaccines for detection of viral genome in tissue samples by PCR method.

References


