

## SAFETY EVALUATION OF GUMBOKAL® IM FORTE SPF LIVE INTERMEDIATE VACCINE AGAINST INFECTIOUS BURSAL DISEASE

Original scientific paper

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**ABSTRACT.** - Safety of GUMBOKAL® IM FORTE SPF, live intermediate vaccine against infectious bursal disease vaccine, was evaluated in compliance with European Pharmacopoeia. A ten-fold dose ( $10^{5.0}$  TCID<sub>50</sub> per chicken) of the vaccine was given to one-day-old as well as 15-day-old specific pathogen free chickens by ocular route. Chickens were observed daily for the next 21 days when they were sacrificed and their bursae were submitted for histological examination. No clinical signs or mortality were recorded during the observation period in both groups. In the bursa of most birds vaccinated at one day of life, intermediate atrophy and mild inflammation and fibrosis were found. These alterations were manifested by 50% lymphocyte depletion with a moderate vacuolisation in approximately 50% of lymphoid follicles, whereas in other lymphoid follicles only a mild lymphoid depletion and minor vacuolisation were noted. In the bursae of chickens vaccinated at the age of 15 days no atrophy of the mucosa epithelium was found, but only mild to moderate lymphoid depletion and/or vacuolisation of lymphoid follicles. However, the lymphoid follicles in these birds were of normal oval shape and of the same structure as the interstitial connective tissue. The results recorded in both groups indicate not only mild to medium severe alterations of the bursa, but also the tendency of their recovery and repopulating with B cells. Although the vaccine GUMBOKAL® IM FORTE SPF does not fully comply with the European Pharmacopoeia requirements, it can be considered as a safe live infectious bursal disease vaccine of intermediary type.

**Key words:** infectious bursal disease, live vaccine, safety

### INTRODUCTION

Infectious bursal disease (IBD), an immunosuppressive disease of young chickens, has been re-

sponsible for major economic losses in the poultry industry worldwide, particularly for the past decade. The disease affects lymphoid organs, i.e. lymphoid cells in bursa of Fabricius, resulting in

lymphoid depletion and the final destruction of the bursa as the predominant feature of the pathogenesis of IBD (van den Berg, 2000). Chickens are highly susceptible to the virus between 3 and 6 weeks after hatching, when the bursa of Fabricius reaches maximum development (Müller *et al.*, 2003). In the late 1980's, the emergence of very virulent IBD virus (vvIBDV) in Western Europe, changed IBD situation from mainly subclinical infection causing less than 1% mortality and satisfactorily controlled by vaccination to severe infection causing mortality up to 25% in broilers and 60% in layers (van den Berg *et al.*, 1991). It was no longer possible to protect broilers with vaccines produced from mild vaccinal strains because it was obvious that vvIBDV could break through immunity provided by highly attenuated vaccine strains (van den Berg *et al.*, 1991; van den Berg, 2000). Therefore, it was necessary to introduce more invasive strains in vaccine production. On the other side, it is well known that less attenuated strains may cause lesions in the bursa follicles followed by immunosuppression, particularly if 'hot' vaccines are used (Guittet *et al.*, 1992). Bolis *et al.* (2003) reported that stronger vaccines give better protection to the bursa irrespective of the damage in the bursa tissue. Although their immunogenicity is a must, it is important for live IBD vaccine to be considerably safe.

The aim of this trial was to evaluate safety of GUMBOKAL® IM FORTE SPF (Veterina Ltd., Croatia), a live intermediate vaccine against IBD, in compliance with Eu. Ph. (1998) for testing live, freeze-dried vaccine against avian infectious bursal disease (Gumboro disease).

## MATERIALS AND METHODS

### Vaccine and vaccination

GUMBOKAL® IM FORTE SPF vaccine (Veterina Ltd., Croatia) was used in this study. GUMBOKAL® IM FORTE SPF is a live, freeze-dried, IBD vaccine of intermediary type that contains VMG 91 strain of IBD virus in titre of  $10^{4.0}$  TCID<sub>50</sub> per dose.

The vaccine was dissolved in distilled water in a way that 0.1 ml (2 droplets) of the solution con-

sisted 10 regular vaccine doses i.e.  $10^{5.0}$  TCID<sub>50</sub>. Each trial chicken was vaccinated with a droplet (0.05 ml) of the solution in each eye. As the result, each chicken was vaccinated with ten-fold vaccinal dose ( $10^{5.0}$  TCID<sub>50</sub>) in 0.1 ml volume of the solution.

### Birds

Two groups, A and B, each consisting of 15 specific pathogen free (SPF) leghorn type chickens (SPAFAS, USA), were used in this trial. Each group was placed in separate isolator until the end of experiment. Feeding and watering of the birds were ad libitum.

### Trial procedure

Chickens of group A were vaccinated at age of one day, whereas chickens of group B were vaccinated at age of 15 days. Birds of both groups were observed daily for following 21 days and then killed for histological examination of bursas of Fabricius. When collected, each bursa was labelled and individually treated. The bursas were fixed in 10 % buffered formalin.

### Histology

Bursas were paraffin embedded and thin sections were made from each bursa, stained with haematoxylin and eosin and examined in a light microscope.

## RESULTS

During the observation period of 21 days after vaccination neither mortality nor clinical signs were recorded in both trial groups. No symptoms that could be attributed to the vaccination with ten-fold dose were noted, as well.

### Bursa histology of group A

In birds vaccinated with ten-fold dose of GUMBOKAL® IM FORTE SPF at age of one day, predominantly mild and intermediate alterations were found on day 21 after vaccination.

In bursas of most of the birds (9), intermediate at-

rophy and mild inflammation and fibrosis were found. These alterations were manifesting in 50% lymphocyte depletion with intermediate vacuolisation in approximately 50% of lymphoid follicles, whereas in other lymphoid follicles only mild lymphoid depletion and mild vacuolisation were noted (Figure 1).

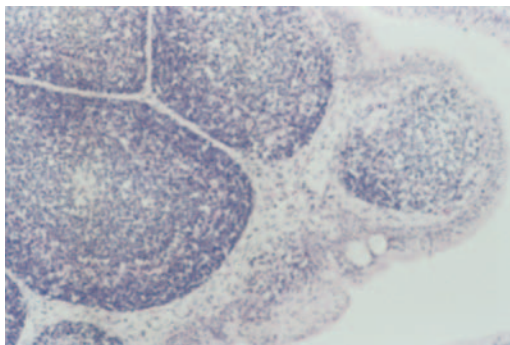


Figure 1  
Folding of the mucosa of the bursa of Fabricius 21 days after vaccination with the vaccine GUMBOKAL® IM FORTE SPF in a chick vaccinated at one day of life. Moderate atrophy, mild inflammation and fibrosis, H&E, magnification x50.

Slika 1  
Nabor sluznice burze 21 dan nakon vakcinacije vakcinom GUMBOKAL® IM FORTE SPF u pileta vakciniranog u dobi od jedan dan. Srednje jaka atrofija, blaga upala i fibroza. HE, povećanje 50x.

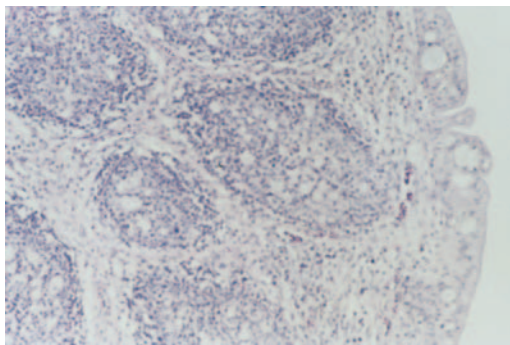


Figure 2  
Folding of the mucosa of the bursa of Fabricius 21 days after vaccination with the vaccine GUMBOKAL® IM FORTE SPF in a chick vaccinated at one day of life. Severe atrophy and pronounced inflammation and fibrosis, H&E, magnification x50.

Slika 2  
Nabor sluznice burze 21 dan nakon vakcinacije vakcinom GUMBOKAL® IM FORTE SPF u pileta vakciniranog u dobi od jedan dan. Jaka atrofija i izražena upala i fibroza. HE, povećanje 50x.

Severe atrophy as well as pronounced inflammation and fibrosis of bursas were recorded in two chickens. This was manifested in more than 75% lymphoid depletion in at least 90% of the lymphoid follicles (Figure 2).

Fibrosis and inflammation of bursas were found in four birds. Incomplete fibrosis was found in two chickens whereas complete fibrosis with infiltration of inflammatory cells, i.e. granulocytes, was found in other two chickens (Figure 3).

### Bursa histology of group B

Mild to moderate alterations, mainly in lymphoid follicles, were found in bursas of most of the chickens vaccinated with GUMBOKAL® IM FORTE SPF at age of 15 days and examined 21 days after vaccination.

In bursas of 11 chickens, no atrophy of epithelium was found, but mild to moderate lymphoid depletion and/or vacuolisation of lymphoid follicles were noted. However, lymphoid follicles were regularly shaped as well as interstitial conjunctive tissue (Figure 4).

In bursas of four chickens, mild atrophy of epithelium as well as mild depletion and/or vacuolisation in one third of the lymphoid follicles was found. Most of the follicles were regularly shaped

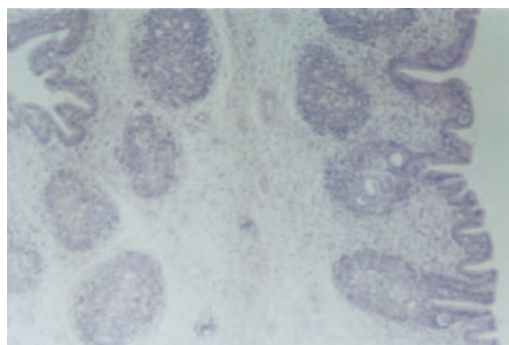


Figure 3  
Folding of the mucosa of the bursa of Fabricius 21 days after vaccination with the vaccine GUMBOKAL® IM FORTE SPF in a chick vaccinated at one day of life. A partial fibrosis and inflammation, H&E, magnification x50.

Slika 3  
Nabor sluznice burze 21 dan nakon vakcinacije vakcinom GUMBOKAL® IM FORTE SPF u pileta vakciniranog u dobi od 1 dan. Djelomična fibroza i upala. HE, povećanje 50x.

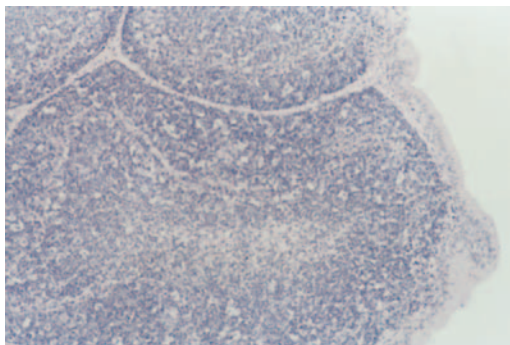


Figure 4

Folding of the mucosa of the bursa of Fabricius 21 days after vaccination with the vaccine GUMBOKAL® IM FORTE SPF in a bird vaccinated on day 15 o age. A mild to moderate lymphoid depletion and vacuolisation of the lymphoid follicles, H&E, magnification x50.

Slika 4

Nabor sluznice burze 21 dan nakon vakcinacije vakcinom GUMBOKAL® IM FORTE SPF u pileta vakciniranog u dobi od 15 dana. Blaga do srednje jaka limfoidna deplecija i vakuolizacija limfoidnih folikula. HE, povećanje 50x.

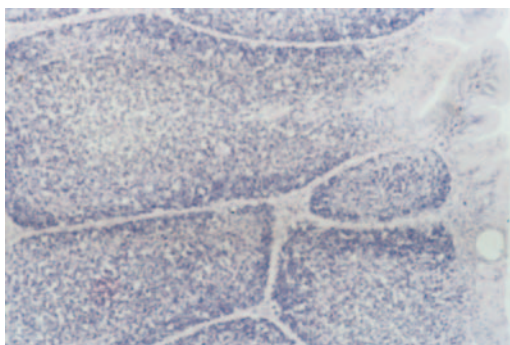


Figure 5

Folding of the mucosa of the bursa of Fabricius 21 days after vaccination with the vaccine GUMBOKAL® IM FORTE SPF in a bird vaccinated on day 15 of age. A mild atrophy of the epithelium and a mild depletion and vacuolisation of the lymphoid follicles, H&E, magnification x50.

Slika 5

Nabor sluznice burze 21 dan nakon vakcinacije vakcinom GUMBOKAL® IM FORTE SPF u pileta vakciniranog u dobi od 15 dana. Blaga atrofija epitela i blaga deplecija i vakuolizacija limfoidnih folikula. HE, povećanje 50x.

with clearly distinguished cortex and medulla. Interstitial conjunctive tissue was also regularly shaped (Figure 5).

## DISCUSSION

The aim of this study was to evaluate safety of GUMBOKAL® IM FORTE SPF, live vaccine against IBD, in compliance with the European Pharmacopoeia and therefore ten-fold dose of the vaccine was given to SPF chickens. Increased dose did not cause any clinical symptom or mortality in birds vaccinated at age of one day or in those vaccinated at age of 15 days. According to van der Berg (2000), the reason for mortality in chickens infected with IBDV would be related to the excessive release of cytokines and fever induction, independent of the damage of the bursa of Fabricius. Further, light hybrids are known to be more susceptible to the mortality induced by IBDV than heavy hybrids. This is due to the differences between the two types of birds in the major histocompatibility complex from allele B, and it does not interfere on the induction of bursal lesions in birds infected with IBDV (Nielsen *et al.*, 1998). SPF birds used in this study were of Leghorn type i.e. light hybrids, and therefore it can be concluded that the vaccine is safe regarding clinical symptom and mortality of vaccinated birds, even if given in ten-fold dose.

Histological changes of bursa of Fabricius found 21 days after vaccination were of different intensity in birds vaccinated at the age of one day and in birds vaccinated at age of 15 days. Generally, more pronounced and more extensive alterations in bursa of Fabricius in this study were found in the birds vaccinated as day-old. This finding seems to be unusual since it is known that the highest age susceptibility is during maximum development of bursa of Fabricius, which is between 3 and 6 weeks of age (van den Berg, 2000; Müller *et al.*, 2003). This age susceptibility can be broader, but only in case of vvIBDV strains (van den Berg *et al.*, 1991; Nunoya *et al.*, 1992). Therefore, it could be expected that more intensive alteration would be found in the birds vaccinated at age of 15 days, since number of susceptible cells present in bursa of Fabricius is significantly higher if compared to those in day-old chickens. However, it must be considered just a single sampling of bursas on 21<sup>st</sup> day after vaccination has been done, as proposed by European Pharmacopoeia (1998) for testing live, freeze-dried vaccine against avian infectious bursal dis-



ease (Gumboro disease). The acute phase of the disease lasts for about seven to ten days, during which bursal follicles are depleted of B cells and the bursa becomes atrophic. Chickens that survive the acute disease clear the virus and recover from its pathologic effect and bursal follicles are repopulated with B cells (Kim *et al.*, 1999; Sharma *et al.*, 2000). However, IBDV strains vary in their pathogenicity and they range from strains that cause intense loss of the bursal stroma and of the follicular microenvironment that sustains cell B differentiation and those that cause little or no bursa damage. IBDV isolates and strains that induce partial follicular lymphocyte loss in the acute phase of infection will show follicles with extensive lymphocytic regeneration areas during the recovery phase (Pope, 1991). It is possible that, although intense bursa damages could occur in the chickens vaccinated at age of 15 days, recovery and B cell repopulation of the bursas were significantly efficient, since histological examination of the bursas has fallen in age of their maximum development i.e. between 3 and 6 weeks of age. In other words, bursas of the chickens vaccinated as day-old did not fully recover until the age when the bursas were taken for histological examination i.e. at age of three weeks. This means that age of chickens for bursa examination may considerably affect its result. Further, the European Pharmacopoeia regulates safety testing of live IBD vaccine in SPF chickens of the minimum age stated on the label. Most of IBD vaccines are intended for vaccination of birds as young as day-old since young commercial chickens possess maternal antibodies that interfere with vaccinal strains and prevent post-vaccinal damage of the bursas (van den Berg and

Meulemans, 1991; Saif, 1998; Müller *et al.*, 2003). Since SPF birds do not possess specific maternal antibodies, they seem not to be appropriate indicator for safety test. Moreover, examining bursa damage caused by vaccination within first three weeks of live, i.e. before its maximum development, may miss present maximum recovery and repopulation of the bursas. According to the European Pharmacopoeia, IBD vaccine complies with the safety test if, 21 days after inoculation of the vaccine, no chicken shows lesions of the bursa of Fabricius. According to Abdel-Alim and Saif (2001) and Mass *et al.* (2001) the intensity of microscopic alterations in the bursa of Fabricius may also be quantified to evaluate the level of immune protection where less microscopic alterations means less immune protection. In the same way Bolis *et al.* (2003) concluded that stronger vaccine strains give better protection to the bursa, irrespective of the damage in the bursa tissue. Thus, complying the safety test proposed in the European Pharmacopoeia significantly reduces immune protection afforded by the vaccine if not completely diminishes it. Criteria that determine safety of live IBD vaccines should be adjusted to various types of the vaccines allowing certain microscopic lesion of the bursas.

In this trial it was found that GUMBOKAL® IM FORTE SPF, intermediate live IBD vaccine, when given in ten-fold dose to SPF chickens, causes no mortality or clinical symptoms. Histological examination has shown mainly mild to intermediate lesions of the bursas, but with tendency of their recovery and B cell repopulation. It can be concluded GUMBOKAL® IM FORTE SPF is a safe vaccine.

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## EVALUACIJA NEŠKODLJIVOSTI INTERMEDIJARNE VAKCINE PROTIV ZARAZNE BOLESTI BURZE GUMBOKAL® IM FORTE SPF

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**IZVADAK.** - Istražena je neškodljivost intermedijarne vakcine protiv zarazne bolesti burze GUMBOKAL® IM FORTE SPF prema zahtjevima Europske farmakopeje. Za istraživanje su upotrijebljene dvije skupine pilića slobodnih od specifičnih patogena (SPF). Prva skupina pilića vakcinirana je okularno deseterostrukom dozom vakcine ( $10^{5.0}$  TCID<sub>50</sub> za pile) u dobi od jedan dan. Druga skupina vakcinirana je također okularno deseterostrukom dozom vakcine u dobi od 15 dana. Pilići su promatrani 21 dan nakon čega su žrtvovani, a Fabricijeve burze histološki pretražene. Tijekom razdoblja promatranja, ni u jednoj skupini nisu zamijećeni klinički znakovi bolesti niti je bilo uginuća. Histološki nalaz u burzama u većine pilića vakciniranih u dobi od jedan dan upućuje na srednje jaku atrofiju te blage upalne i fibrotične promjene. U približno 50% limfoidnih folikula je ustanovljena 50%-tna deplecija limfocita s osrednje izraženom vakuolizacijom, dok se u ostalim folikulima uočava samo blaža limfoidna deplecija i slabija vakuolizacija. U burzama pilića vakciniranih u dobi od 15 dana nisu nađene atrofične promjene u epitelu nabora sluznice. Vidljiva je samo blaga do srednje jaka limfoidna deplecija i/ili vakuolizacija u limfoidnim folikulima, ali su oni normalnog ovalnog oblika i iste su građe kao i vezivnotkivna stroma. Rezultati u obje skupine pilića pokazuju da nije riječ samo o blagim do srednje jakim promjenama u burzama nego i o tendenciji oporavka burza i njihove repopulacije limfocitima. Iako vakcina GUMBOKAL® IM FORTE SPF u cjelosti ne slijedi zahtjeve Europske farmakopeje, može se smatrati neškodljivom živom vakcinom intermedijarnog tipa protiv zarazne bolesti burze.

**Ključne riječi:** zarazna bolest burze, GUMBOKAL® IM FORTE SPF, živa vakcina, neškodljivost