

CLINICAL STUDY ON LIVE INFECTIOUS BURSAL DISEASE VACCINE GUMBOKAL® IM FORTE SPF IN BROILERS

Original scientific paper

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ABSTRACT. - Immunogenic properties and side effects of GUMBOKAL® IM FORTE SPF, a live infectious bursal disease vaccine, have been studied under field conditions. A commercial Ross 308 broiler flock of 10,350 chickens was vaccinated via drinking water on day 12 of life. Antibody titres of the infectious bursal disease virus were assessed using FlockCheck ELISA kit on day 5, 12, 17, 26 and 40 of life. At the age of 12 days, when the vaccine was administered, most of the birds had negative specific antibody titres whereas significant increase in antibody titres was recorded afterward, reaching relatively high and uniform mean antibody titre at the age of 40 days (mean titre = 2049, standard deviation = 824). Histological examination performed on day 5 as well as on day 28 after vaccination did not reveal either significant fibrosis or inflammation of the bursa of Fabricius. Furthermore, post-vaccinal Newcastle disease virus antibodies were at protective levels showing no adverse effect of GUMBOKAL® IM FORTE SPF to the vaccination against Newcastle disease. No other adverse or side effects of the vaccine were noticed. Mortality rate (overall for 42 days = 3.85%) and average body weight (final body weight on day 42 = 2,147 g) were within standards for the hybrid.

GUMBOKAL® IM FORTE SPF is an immunogenic vaccine against infectious bursal disease, which does not cause any adverse effects in broiler production under field conditions.

Key words: infectious bursal disease, live vaccine, broilers

INTRODUCTION

Numerous viral diseases are capable of seriously affecting poultry. Some of them, including infectious bursal disease (IBD), are currently of a

great concern in many countries. Economic importance of this disease is manifested in two ways, mortality and immune suppression of infected chickens. Concern regarding IBD is focused towards classical, variant and very virulent

strains of IBD virus (IBDV). Until 1987, the strains were of low virulence causing less than 2% specific mortality, and were satisfactorily controlled by vaccination (van den Berg, 2000). Apart from the so called classical strains, IBDV isolates that emerged in the US in late 80's had been affected by antigenic drift against which classical IBDV vaccines did not afford satisfactory protection (Jackwood and Saif, 1987). In contrast to classical and American variant strains, new very virulent strains arose in Western Europe (Chettle *et al.*, 1989). Although very virulent strains are closely related to classical IBDV strains and no antigenic drift was detected (van den Berg *et al.*, 1996; Pitcovski *et al.*, 1998; Etarradossi *et al.*, 1999), these strains were of increased virulence causing mortality of even 25% in broilers, 60% in layers and up to 100% in SPF chickens (van den Berg *et al.*, 1991). Very virulent IBDV (vvIBDV) strains spread apparently all around the world with exception of Australia, New Zealand, Canada and US (van den Berg, 2000) and seem to be the major concern regarding this disease worldwide. Croatian poultry industry was affected with vvIBDV in 1995 (Savić *et al.*, 1997) and the virus spread to free-range chicken and backyard poultry, too (Savić, 1999). Since IBDV is very resistant to vaccination and this disease occurs despite strict hygienic measures, vaccination is inevitable under high infection pressure and mandatory to protect chickens against infection during the first weeks after hatch (Müller *et al.*, 2003). Therefore, numerous vaccines and vaccination programs have been studied and proposed worldwide. First attempts of controlling IBD have been done using non-attenuated field strains of the virus with moderate pathogenicity. In the early 1970s, young birds were immunized with attenuated vaccinal virus propagated in embryonated eggs or cell cultures, or nonvirulent viral strains (Lasher and Shane, 1994). In the mid-1980 a passive immunization of very young birds by using inactivated oil-emulsion vaccines in breeders was introduced (Lucio and Hitchner, 1979). Therefore mild live vaccines could not be successfully used in such progeny since maternal antibodies readily neutralize highly attenuated vaccinal viruses. Intermediate vaccine strains, cloned or with less passages in chicken embryos or cell cultures, were intro-

duced with the objective of immunizing chicks with high maternal antibody titers. They substituted vaccines produced with very attenuated or nonvirulent strains, called mild vaccines (McFerran, 1993; Lukert and Saif, 2003). The aim of this trial was to evaluate immunogenicity and side effects of GUMBOKAL® IM FORTE SPF (Veterina Ltd., Croatia), an intermediate live IBD vaccine, in broilers under field conditions. The broilers were vaccinated via drinking water when 12-day-old and monitored until the end of fattening period i.e. when 42-day-old.

MATERIALS AND METHODS

Vaccines

GUMBOKAL® IM FORTE SPF and PESTIKAL® La Sota SPF vaccines (Veterina Ltd., Croatia) were used in this study. GUMBOKAL® IM FORTE SPF is a live IBD vaccine of intermediary type that contains VMG 91 strain of IBD virus in titre of $10^{4.0}$ TCD₅₀ per dose. PESTIKAL® La Sota SPF is a live lentogenic Newcastle disease (ND) vaccine that contains LaSota strain of ND virus in titre of $10^{6.0}$ EID₅₀ per dose.

Birds and vaccination

Flock of 10,350 12-day-old ROSS 308 broilers, originated from 41-week-old commercial breeder flock, was vaccinated with GUMBOKAL® IM FORTE SPF via drinking water lot number 2924012. Drinking water was withdrawn for 2 hours to all birds prior to vaccination. Contents of ten vaccine vials, each containing 1,000 individual doses, were suspended in 60 L of cold water free from chlorine and other disinfectants. Water containing the vaccine was given to the broilers through drinking facilities what allowed for all birds to drink-up the water within an hour. The same method was used to vaccinate the birds against ND with PESTIKAL® La Sota SPF at age of 17 days. No other vaccines were used during the trial period.

Housing, feeding and watering

The flock was placed in a broiler house (600 square meters) and reared on deep litter up to 42 days. Lighting was 24 hours per day. Temperature and ventilation were adjusted regarding the age of the broilers. The birds were fed ad libitum with standard food during entire rearing period. During the first three weeks of broilers life, food containing 22,0% of crude protein and 12,5 MJ/kg was used, whereas food containing 20,0% of crude protein and 13,0 MJ/kg was given after three weeks of life. Coccidiostat monensin in concentration of 100 ppm was added in food up to the 35th day of life. Watering of the birds was ad libitum as well. Automatic feeding system and bell drinkers were used.

Observations

The chickens were observed daily. Mortality was also recorded daily whereas necropsy of died birds was performed weekly. Trial birds were weighed weekly using random sample of 50 birds.

Serology

Blood for serology was taken on day 5, 12, 17, 26 and 40. Infectious bursal disease antibodies were assessed by indirect ELISA. Commercial FlockCheck kits (IDEXX, Maine, USA) and standard procedure were used. The working dilution of serum was 1:500.

Newcastle disease virus antibodies were assessed only in serum samples taken on day 40. Standard hemagglutination-inhibition (HI) method with

double serial dilutions, 1% chicken red blood cells and 4 hemagglutinating units was used (Beard and Wilkes, 1973). HI titres were expressed as log₂.

All serum samples were run in duplicate.

Control birds

Ten birds from the trial flock were taken prior to vaccination with GUMBOKAL® IM FORTE SPF and placed in isolator. Essential parameters for the control birds like feeding, watering, lighting and ambient temperature were same as for the trial birds. These birds were used as unvaccinated controls for histology of bursa of Fabricius.

Histology of bursa of Fabricius

Twenty-five trial birds and five control birds were killed on day 17 as well as on day 40 and the bursae were sampled for histology. The bursae were formalin fixed and paraffin embedded followed by standard procedure for histology using haematoxylin-eosin staining.

RESULTS

Observations

Observing the birds daily, no clinical signs of any disease or any other health disorder were recorded in either trial or control birds. Weekly mortality, necropsy records and body masses of trial birds are given in table 1.

Table 1

WEEKLY MORTALITY, NECROPSY FINDING AND BODY WEIGHT OF BROILERS VACCINATED WITH GUMBOKAL® IM FORTE SPF AT THE AGE OF 12 DAYS (n = 10,350)

Week of age	Mortality*	Necropsy finding	Average body weight**
1 st	87 (0.84%)	Omphalitis, uricosis	149 g
2 nd	61 (0.59%)	Enteritis, uricosis	397 g
3 rd	48 (0.46%)	Heart stroke, enteritis	767 g
4 th	65 (0.63%)	Heart stroke, enteritis	1,226 g
5 th	67 (0.65%)	Heart stroke, suffocation	1,675 g
6 th	70 (0.68%)	Heart stroke, suffocation	2,147 g

* Weekly mortality rates as a percentage of housed chickens are shown in parentheses.

Total mortality amounted to 3.85%.

** Average body weight of a random sample of 50 trial birds at the end of the week

Tablica 1

TJEDNO UGINUĆE, RAZUDBENI NALAZ I TJELESNE MASE TOVNIH PILIĆA VAKCINIRANIH GUMBOKALOM® IM FORTE SPF U DOBI OD 12 DANA (n = 10350)

Tjedni života	Uginuće*	Razudbeni nalaz	Prosječna tjelesna masa**
1.	87 (0,84%)	upala pupka, urikoza	149 g
2.	61 (0,59%)	enteritis, urikoza	397 g
3.	48 (0,46%)	srčana kap, enteritis	767 g
4.	65 (0,63%)	srčana kap, enteritis	1226 g
5.	67 (0,65%)	srčana kap, ugušenje	1675 g
6.	70 (0,68%)	srčana kap, ugušenje	2147 g

* U zagradama su prikazana tjedna uginuća kao postotak broja useljenih pilića. Ukupno uginuće je iznosilo 3,85%.

** Prosječne tjelesne mase nasumičnog uzorka od 50 pilića na kraju tjedna.

Serology

Results of ELISA for IBD virus antibodies are given in table 2. Mean HI titre for ND virus anti-

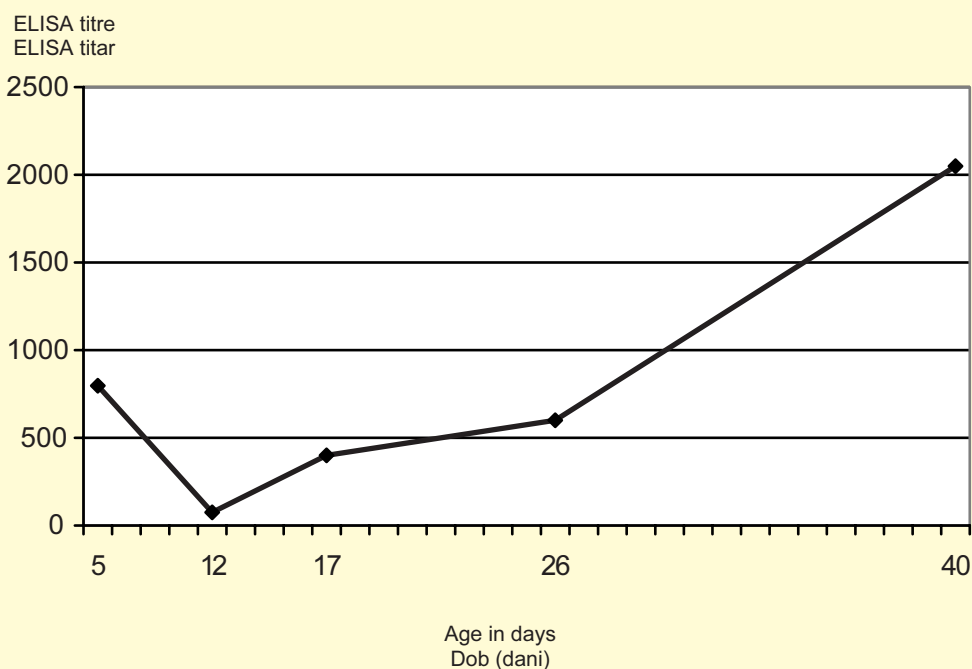
bodies in blood serum of trial birds on 40th day of life (23rd day post vaccination) was 4.12 log₂ with standard deviation of 0.73. IBD virus antibody curve is shown in figure 1.

Figure 1

IDEXX ELISA TITRES OF INFECTIOUS BURSAL DISEASE VIRUS ANTIBODIES IN BROILERS VACCINATED WITH GUMBOKAL® IM FORTE SPF AT THE AGE OF 12 DAYS

Slika 1

TITRI IDEXX ELISA PROTUTIJELA ZA VIRUS ZARAZNE BOLESTI BURZE U TOVNIH PILIĆA VAKCINIRANIH GUMBOKALOM® IM FORTE SPF U DOBI OD 12 DANA



Histology findings on bursae of Fabricius on the 5th day after vaccination (day 17)

Control group

Very mild atrophic alterations in mucosal epithelium and very mild or moderate lymphoid depletion and/or vacuolisation of bursa were found in all chickens of the control group (Figure 2).

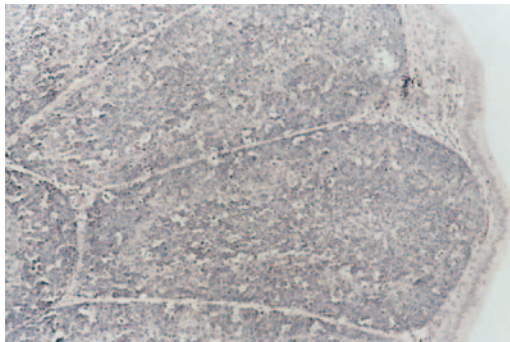


Figure 2
Folding of the mucosa of bursa of Fabricius in a chicken 5 days after vaccination (day 17) with vaccine GUMBOKAL® IM FORTE SPF (Control group). Very mild atrophic alterations in mucosal epithelium and moderate lymphoid depletion and vacuolisation of lymphoid follicles. HE, 50x.

Slika 2

Nabor sluznice burze u pileta 5 dana nakon vakcinacije GUMBOKALOM® IM FORTE SPF (kontrolna skupina). Vrlo blaga atrofija epitela sluznice i umjerena limfoidna deplecija i vakuolizacija limfoidnih folikula. HE, 50x.

Trial group

Mild atrophic alterations in mucosal epithelium and very mild, mild, moderate or pronounced lymphoid depletion and/or vacuolisation of lymphoid follicles and/or mild bursitis were found in most of the 25 examined bursae from the trial group. Moderate or pronounced bursitis and/or fibrosis of the bursa were found in 3 chickens.

Histology findings on bursae of Fabricius on 28th day after vaccination (day 40)

Control group

Very mild or mild atrophic alterations in mucosal epithelium and very mild or mild lymphoid depletion and/or vacuolisation were found in bursae of all chickens of the control group.

Trial group

Mild, moderate or pronounced atrophic alterations in mucosal epithelium and very mild, mild, moderate or pronounced lymphoid depletion

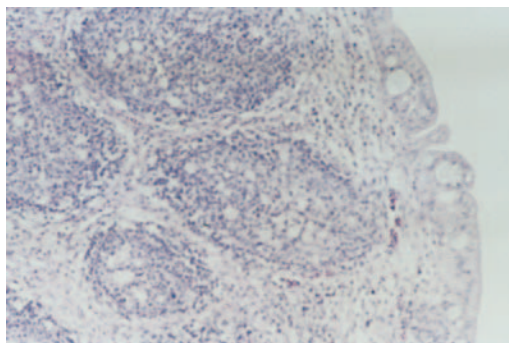


Figure 3
Folding of the mucosa of bursa of Fabricius in a chicken 28 days after vaccination (day 40) with vaccine GUMBOKAL® IM FORTE SPF (Trial group). Pronounced atrophic alterations in mucosal epithelium and pronounced lymphoid depletion and vacuolisation of lymphoid follicles and moderate proliferation of interstitial tissue with mild diffused compensatory lymphoid hyperplasia. HE, 50x.

Slika 3

Nabor sluznice burze u pileta 28 dana nakon vakcinacije GUMBOKALOM® IM FORTE SPF (pokusna skupina). Izražena atrofija epitela sluznice, izražena limfoidna deplecija i vakuolizacija limfoidnih folikula i umjerena proliferacija vezivnog tkiva s blagom difuznom kompenzatornom limfoidnom hiperplazijom. HE, 50x.

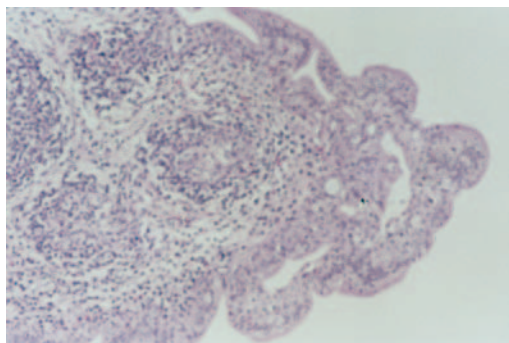


Figure 4
Folding of the mucosa of bursa of Fabricius in a chicken 28 days after vaccination (day 40) with vaccine GUMBOKAL® IM FORTE SPF (Trial group). Severe atrophy of mucosal epithelium and mild fibrosis with mild bursitis. HE, 50x.

Slika 4

Nabor sluznice burze u pileta 28 dana nakon vakcinacije GUMBOKALOM® IM FORTE SPF (pokusna skupina). Jaka atrofija epitela sluznice i blaga fibroza s blagim burzitisom. HE, 50x.

Table 2

IDEXX ELISA TITRES OF INFECTIOUS BURSAL DISEASE VIRUS ANTIBODIES IN BROILERS VACCINATED WITH GUMBOKAL® IM FORTE SPF AT THE AGE OF 12 DAYS

Age in days	5	12	17	26	40
Days post vaccination	-7	0	5	14	28
Mean titre	797	75	428	622	2049
± standard deviation	± 654	± 153	± 283	± 606	± 824

Tablica 2

TITRI IDEXX ELISA PROTUTIJELA ZA VIRUS ZARAZNE BOLESTI BURZE U TOVNIH PILIĆA VAKCINIRANIH GUMBOKALOM® IM FORTE SPF U DOBI OD 12 DANA

Dob pilića u danima	5	12	17	26	40
Dani poslije vakcinacije	-7	0	5	14	28
Srednji titar	797	75	428	622	2049
± standardna devijacija	± 654	± 153	± 283	± 606	± 824

and/or vacuolisation of lymphoid follicles and mild to moderate proliferation of interstitial tissue with mild diffused or nodular compensatory lymphoid hyperplasia were found in most of 25 examined bursae from the trial group (Figure 3). Moderate, pronounced or severe atrophy of mucosal epithelium and mild fibrosis found in 3 chickens, whereas mild bursitis was found in 3 chickens (Figure 4).

DISCUSSION

Vaccination is a major (Lukert and Saif, 2003) or the only successful (Lütticken, 1997) way for controlling the IBD infection, and therefore it is very important to pay particular attention to immunogenicity and safety of IBD vaccines. Wide range of live vaccine strains comprising various degrees of attenuation has been developed. It has to be taken into consideration that vvIBDV will break through immunity provided by highly attenuated vaccine strains. On the other side, it is well known that less attenuated strains ("hot vaccines") may cause lesions in the bursa follicles and, thus, immunosuppression even in vaccinated birds (Müller *et al.*, 2003). This usually leads to poor response to other vaccinations, apart from opportunistic bacterial or/and viral infec-

tions. Nevertheless, vvIBDV can be successfully controlled only if intermediate or hot vaccines are used (van den Berg, 2000).

In this trial we have vaccinated 12-day-old commercial broiler chickens via drinking water against IBD with intermediate vaccine GUMBOKAL® IM FORTE SPF to evaluate immune response as well as side effects under field conditions. The broilers originated from IBD vaccinated breeders, therefore maternally derived antibodies were found yet in age of 5 days, although in relatively low titres (mean titre = 797, standard deviation = 654). At the age of 12 days, when the vaccine was applied, most of the birds negative for IBDV antibody (mean titre = 75, standard deviation = 153). Five days post vaccination an increase in IBDV antibody titre was found (mean titre = 428, standard deviation = 283) whereas two weeks after vaccination further increased in antibody titres was recorded (mean titre = 622, standard deviation = 606). At the age of 40 days i.e. four weeks after vaccination, significantly higher and relatively uniform IBDV titres were found (mean titre = 2049, standard deviation = 824). These titres, according to the ELISA kit producer (FlockCheck Production guide), are considered to be at protective level. Therefore, it can be concluded that GUMBOKAL® IM FORTE SPF is an immunogenic vaccine for com-

mercial broilers. Further, no clinical signs of any disease or any other health disorder were recorded in trial birds neither any symptom that can be attributed to the vaccination was noticed. Mortality and average body masses were within the standards for the Ross 308 broilers. Histology on 5th as well as on 28th day after vaccination revealed that no significant fibrosis or inflammation of the bursae of Fabricius was found. Also, ND virus antibodies were at protective levels according to Alexander (1998). Twenty-two out of 25 samples with HI titre 4 log₂ or higher were found at the age of 40 days. This is in accordance with bursa histology findings that revealed no sig-

nificant post vaccinal bursa lesions. According to the results of bursa histology and satisfactory post-vaccinal ND virus antibody titres, it can be concluded that GUMBOKAL® IM FORTE SPF is a safe vaccine when used in a single dose under field conditions. Possibility that either IBD virus antibodies or ND virus antibodies were consequence of field outbreak(s) could be ruled out since no clinical signs, increased mortality or poor production results were recorded.

It can be concluded that GUMBOKAL® IM FORTE SPF is effective vaccine and suitable for vaccination of commercial broiler chickens against IBD.

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KLINIČKO ISPITIVANJE ŽIVE VAKCINE PROTIV ZARAZNE BOLESTI BURZE GUMBOKAL® IM FORTE SPF U TOVNIH PILIĆA

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IZVADAK. - Istraženi su imunogenost i popratni učinci žive vakcine protiv zarazne bolesti burze GUMBOKAL® IM FORTE SPF u terenskim uvjetima. Jato tovnih pilića Ross 308 od 10350 jedinki vakcinirano je pitkom vodom u dobi od 12 dana. Titri protutijela za virus zarazne bolesti određeni su u dobi pilića od 5, 12, 17, 26 i 40 dana pomoću FlockCheck ELISA kitova. U dobi od 12 dana, kada je primijenjena vakcina, većina pilića nije imala specifičnih protutijela za ovaj virus, no potom je zabilježen porast titara ovih protutijela koji su u dobi od 40 dana postigli relativno visoku srednju vrijednost uz ujednačene pojedinačne titre (srednja vrijednost = 2049, standardna devijacija = 824). Histološkom pretragom burza u dobi pilića od 5 i 28 dana nakon vakcinacije nisu nađene znatnije fibrozne ni upalne promjene. Nadalje, postvakcinalna protutijela za virus newcastleske bolesti bila su na zaštitnoj razini, što upućuje na činjenicu da nije bilo neželjenog učinka vakcine GUMBOKAL® IM FORTE SPF na vakcinaciju protiv newcastleske bolesti. Ni drugi nepoželjni učinci nisu uočeni tijekom ispitivanja. Uginuće (ukupno za 42 dana = 3,85%) i prosječna tjelesna masa (konačna masa 42. dana = 2147 g) su bili unutar standarda za ovaj hibrid.

GUMBOKAL® IM FORTE SPF može se smatrati imunogenom vakcinom protiv zarazne bolesti burze koja ne uzrokuje popratne pojave u tovnih pilića u terenskim uvjetima.

Ključne riječi: zarazna bolest burze, živa vakcina, tovni pilići