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Original article

Production and characterization of egg yolk antibodies against bovine alimentary tract pathogens

O. Sitnik[1], P. Jawor[1], W. Kopeć[2], T. Skiba[2], T. Stefaniak[1]

[1] Department of Immunology, Pathophysiology and Veterinary Prevention
[2] Department of Animal Products Technology and Quality Management,
Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wrocław, Poland

Abstract

Aim of the study was to evaluate the effect of immunization of hens with bovine vaccines (C, R, T) on the course of IgY antibodies production against selected bovine *E.coli* strains, rota- and coronaviruses in egg yolk in farm conditions. The hens (40 individuals per group) were vaccinated twice, subcutaneously in four week interval and eggs were harvested once a week. Control group consisted of eggs sampled from non-vaccinated hens located in neighbouring cages. The antibody activity was measured by ELISA. All used vaccines induced the rise of IgY antibody in egg yolks. Based on the duration and the highest level of IgY antibody against bovine alimentary tract pathogens C vaccine was further used in next two trials for vaccination of 1000 hens each time. Double immunization seems to be enough in mounting response against examined pathogens for several weeks. Immunization with C vaccine allowed to harvest eggs with satisfactory levels of *E.coli*, rotavirus and coronavirus IgY antibodies which may be used to evaluate their protective effect by oral administration in calves.

Key words: vaccination, hens, calf diarrhoea, Escherichia coli, rotavirus, coronavirus, IgY

Introduction

Neonatal calves' diarrhoea is one of major problems in dairy calves from birth to 90 days of life (Svensson et al. 2003). Economic importance of diarrhoea is associated with significant costs of treatment and decreased weight gain, increased susceptibility to other diseases and lowered number of heifers available to reproduction (Haława and Stefaniak 2002, Furman et al. 2011). Alimentary tract infections in calves are caused mostly by type A rotaviruses in about 60% of cases, less commonly by enterotoxic *E.coli,* coronaviruses and other pathogens. *Cryprosporidum parvum* is identified commonly, but rarely as the primary cause (Thomson et al. 2007, Bartels et al. 2010).

Vaccination of pregnant cows against the most common bacterial and viral pathogens of neonatal calves' alimentary tract induce several fold increase of antibody (Ab) concentration in colostrum (Acres et al. 1979, Snodgrass et al. 1980). Unfortunately, their concentration decreases rapidly in milk and after few

Correspondence to: T. Stefaniak, e-mail: tadeusz.stefaniak@up.wroc.pl

days is too low to protect locally calves' gastrointestinal tract against infections (Snodgrass et al. 1980). Because of high costs of vaccination of pregnant cows and treatment of calves with diarrhoea other methods of specific protection are searched. The supplementary oral application of antibodies for specific protection of alimentary tract after colostral period seems logical (Stefaniak 2006). One of low-cost and efficient methods is oral application of egg yolk (IgY) antibodies (Ikemori et al. 1992, Mine and Kovacs-Nolan 2002, Stefaniak 2006).

In former study of our group the oral application of lyophilized IgY preparation from egg yolk of non-immunized hens to chickens resulted in dose-dependent antibody activity against *Klebsiella pneumoniae*, *Escherichia coli* O157, *Salmonella* Enteritidis and *S*. Typhimurium in feces measured by ELISA (Stefaniak et al. 2004) and protected them against oral challenge with *S.* Enteritidis.

Production of IgY by hens is one of the most efficient method of immunoglobulins production. The only Ab isotype present in hen egg yolks is IgY. Approximately 1500 mg of IgY may be harvested every month from each laying hen (5-25 mg/ml egg yolk), with between 2 and 10% being antigen-specific IgY, representing a faster and cheaper way of polyclonal Ab production than other sources (Vega et al. 2011). It exceeds several times the yield of serum Igs from rabbits, pigs and from cow colostrum (Hatta et al. 1993, Mine and Kovacs-Nolan 2002, Stefaniak 2006). Large-scale production of eggs and their processing is easier than other sources of Igs. This encourages to apply the IgY antibodies as the alternative to antibiotics in control or prevention of diarrhoea in young farm animals.

Aim of the study

Aim of the study was to evaluate the influence of immunization of the laying hens with three commercial bovine vaccines (C, R, T) on the course of IgY antibodies production against selected bovine *E.coli* strains, rota- and coronaviruses in egg yolk.

Materials and Methods

The study was accepted by II Local Ethical Committee in Wrocław (permission No. 37/2009).

Vaccines

Three vaccines present on Polish market (C, R, T) were used, licensed for immunization of pregnant cows and heifers. Vaccines contained antigens of bovine rota- and coronaviruses and *Escherichia coli* K99.

The content of vaccines used differed. They contained:

– bovine coronavirus (inactivated strain Mebus – R vaccine; inactivated INRA bovine coronavirus strain ≥1.5 SN.U/5 ml – T vaccine; inactivated enteropathogenic bovine coronavirus, at least 10^5 $TCID_{50}$ – C vaccine);

– bovine rotavirus (inactivated strain, strain UK – Compton, serotype G6 P5, $10^{7.6}$ – $10^{7.9}$ TCID50/2 ml – R vaccine; inactivated bovine rotavirus ≥2.0 HAI.U/5 ml – T vaccine; bovine rotavirus, at least 10^5 $TCID_{50}$ before inactivation/2 ml – C vaccine);

– purified antigens of *Escherichia coli* (F5 (K99) adhesin – R vaccine; K99, Y, 31A and F41 antigens – T vaccine; strains O8:K35, K99; O9:K35, K99; O101:K30, K99 at least 1.71 x 10^9 CFU before inactivation – C vaccine);

– adjuvants: 0.85 – 1.15 mg aluminium hydroxide and 1.40 ml light mineral oil/emulsifier/ 2 ml – R vaccine; aluminium hydroxide (Al+++) 3.5 mg; saponin 1.5 mg/5 ml – T vaccine; oil adjuvant/ 2 ml – C vaccine).

Lohmann Brown hens on the beginning of laying period were used in one production farm. Animals were kept at the same environmental and nutritional conditions in cages for 6-8 heads. In one row there were three floors of cages.

Immunization of hens

Three experiments (1 – spring 2010, 2 – spring 2011, 3 – autumn 2011) were carried out on different groups of animals.

The aim of first experiment was to select the vaccine that induce the highest level of antibodies against enterotoxic *E.coli*, rota- and coronaviruses. Eggs obtained at 2nd and 3rd experiment were used to produce spray-dried egg yolk preparations.

First experiment

Forty hens in each group were immunized subcutaneously two times, at lower part of the neck, with 4 week interval using 0.4 ml of respective vaccines (C, T and R). The health status of hens was supervised during study. Eggs were harvested once a week until 11th week in groups C and T and until 8th week after first immunization in group R. Control eggs were sampled at the same time from non-immunized hens in neighbouring cages.

Second and third experiment

For large scale production of IgY Ab against bovine alimentary tract pathogens C vaccine was selected. In both experiments each time one thousand of hens was immunized subcutaneously, two times at lower part of the neck, with 4 week interval using vaccine C in volume 0.4 ml/head. Control group were eggs from hens kept in neighbouring cages. Eggs for laboratory examination were harvested from C and control group once a week until 16th week (in second trial) and until 12th week (in third trial) after first immunization.

Sampling and processing the harvested eggs

For laboratory examinations 30 eggs from each experimental and control groups were sampled randomly once a week. From thirty eggs from each group 23 eggs were selected randomly. Each time individual 6 samples of yolk were taken, diluted 1:4 with PBS pH 7.3 and kept frozen in three replicates in -20°C until analysis. Additionally, 23 yolk eggs were pooled, mixed and diluted 1:4 with PBS pH 7.3. From this three samples were made in volume 1 ml and kept in -20°C until analysis. Pooled egg yolk samples were used for detection *E. coli* antibodies in ELISA. Individual egg yolk samples were tested for rotavirus and coronavirus antibodies by ELISA.

Activity of IgY antibodies against selected *E.coli* bovine pathogenic strains

NUNC maxisorp F microplates (cat. no. 439454 F96) were coated with 1 x 10^8 cfu/ml strain *Escherichia coli* O157 isolated from cattle, or with *E.coli* strain isolated from small intestine of five days-old calf which died with signs of watery diarrhoea (strain no. 1). Bacterial suspensions dissolved in 0,05M carbonate buffer pH 9.6 were added (50 µl/well) and incubated for 3 hours at 37°C and at 4°C overnight. The microplates were washed using BIOTEC ELx50 microplate washer, 3 times x 150 µl/well with PBS pH 7.3, containing 0.05% Tween20 (Fluka) (PBS-T). Thawed yolk samples (two pooled samples from each experimental and control groups in different weeks) were diluted 1:1000 with PBS-T and added to wells of microplate (50 µl/well). Samples were incubated for 2 hours at room temperature with stirring (Elpan, laboratory shaker type 358S). Next, the microplates were washed as mentioned above. After washing, 50 µl/well (1:10000) of rabbit anti-chicken IgG antibodies conjugated with HRPO (SIGMA cat. No. A9046) was added, incubated for 2 hours and washed again. Substrate solution in volume 100 µl per well (o-phenylenediamine 5 mg, 10 ml of 0.05 M citrate-phosphate buffer pH 5.0, 3 µl 30% H_2O2_2) was added to wells and incubated at room temperature in the dark for 30 minutes. Reaction was stopped by addition of 25 µl of 1 M H_2SO_4. Optical density was measured at λ = 492 nm (BIOTEC MQx200).

In experiment 1 the Ab activity against *E. coli* strains was examined weekly for 11 weeks in control, T and C vaccines group and in R vaccine group for 8 weeks.

In experiment 2 the Ab activity against *E. coli* strains was examined weekly for 8 weeks.

In experiment 3 the Ab activity against *E. coli* strains was examined weekly for 9 weeks.

Evaluation of yolk bovine rotavirus and coronavirus antibodies

Activity of IgY antibodies against bovine rota- and coronavirus was measured using ELISA (Idexx test) in Laboratory of Veterinary Diagnostic (Weterynaryjna Diagnostyka Laboratoryjna, Gietrzwałd). Yolks for coronavirus antibody testing were diluted 1:160 and 1:320 for rotavirus Ab. In experiment 1 the Ab activity against bovine rota- and corona-viruses strains were examined for 10 weeks in control, T and C vaccines group and in R vaccine group for 8 weeks. In experiment 2 the Ab activity against bovine rota- and coronaviruses strains were examined for 16 weeks. In experiment 3 the Ab activity against bovine rota and coronaviruses strains were examined for 12 weeks.

Statistical analysis

The rota- and coronavirus antibody levels measured by absorbance in ELISA showed abnormal distribution. To estimate the influence of vaccination on the course of Ab production in successive weeks, pairs from all vaccinated groups (R, C, T) with control group using the Mann-Whitney U test at p-value less than 0.05 and 0.01, were compared.

Results

In majority of immunized animals local swelling of 5-6 mm diameter was seen at the site of injection which decreased stepwise to 3 mm diameter fibrous nodule during the following 4 weeks.

First experiment

Moderate fluctuation of *E.coli* O157 IgY Ab level was observed in control group, until 8th week and later the activity reached similar level as in experimental groups (Fig. 1). Pooled yolks from C group showed gradual increase of Ab level during whole observation period except 6th week. Slightly lower increase occurred in T group, although in the 4th week the concentration increased about 2 times and decreased in the 5th week. Moderate, gradual increase of *E.coli* O157 antibody occurred in yolks from R group.

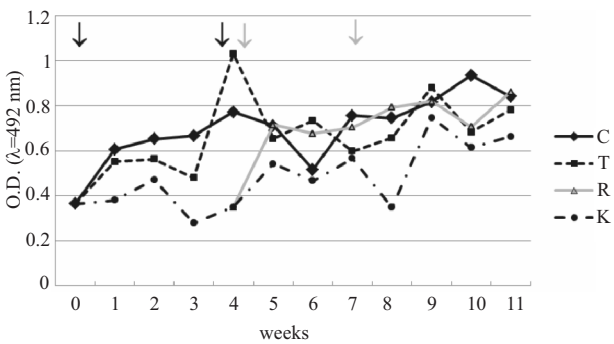

Fig. 1. The course of IgY antibody production against *E. coli* strain O157 in control (K) and experimental groups (C, T, R). Arrows – dates of vaccinations (black colour – T and C, grey – R vaccine).

E. coli strain 1 IgY Ab in egg yolks from control hens as well as from T and R groups showed no significant changes in their level until 7th week of experiment (Fig. 2). In the 8th week 3 – fold increase of Ab activity occurred in groups K and T, and slightly higher in R group. Only in group C the Ab amount increased from 1st week up to 10th week of experiment, except 5th week when the decrease of the Ab activity was noted. The absorbance in C group in week 10th exceeded the pre-vaccination level about 6 times.

At the start of study relatively strong reactivity of IgY rotavirus Ab was found in experimental and control groups (Fig. 3). The highest reactivity was found in C group (the difference was statistically significant from 6th to 10th week after start of immunization, $p<0.05$ in 6th and 10th, $p<0.01$ in 8th week). The absorbance was significantly lower in T group, as compared to control group in 2nd week ($p<0.05$). In T group the Ab reactivity exceeded significantly the control group only in 8th week of experiment ($p<0.01$). The highest mean Ab level occurred in group C at 10th week of study, but high differences in reaction intensity of individual samples occurred.

At the start of study IgY antibody level was slightly lower (statistically not significant) in egg yolks from experimental than in control group (Fig. 4). In the

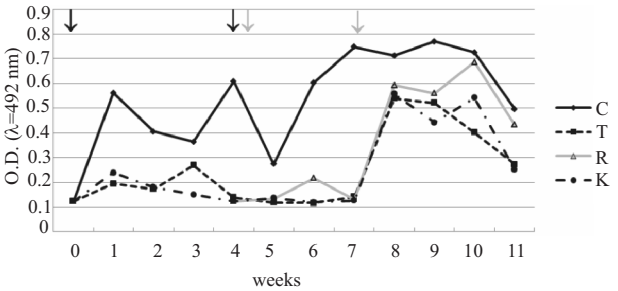

Fig. 2. The course of IgY antibody production against field *E. coli* (strain 1) in control (K) and experimental groups (C, T, R). Arrows – dates of vaccinations (black colour – T and C, grey – R vaccine).

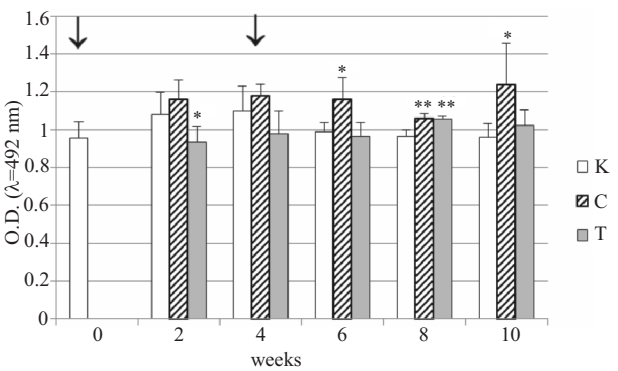

Fig. 3. IgY antibody levels against bovine rotavirus in eggs from control hens (K) and immunized with C or T vaccine (C and T respectively). Arrows – dates of vaccinations, *$p<0.05$, **$p<0.01$.

following weeks the reactivity of IgY rotavirus antibodies was higher in experimental group, but the difference was statistically significant only in 8th week of experiment ($p<0.05$).

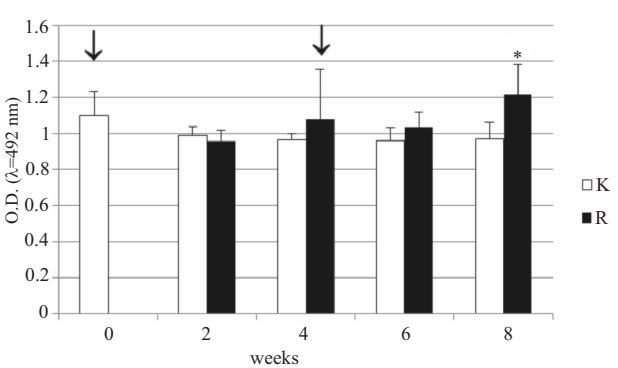

Fig. 4. IgY antibody levels against bovine rotavirus in eggs from control hens (K) and immunized with R vaccine (R). Arrows – dates of vaccinations. * $p<0.05$.

The highest intensity of IgY coronavirus antibody reaction was found in eggs from hens immunized with C vaccine at 6th week of study ($p<0.05$) (Fig. 5). In the following weeks the Ab activity in this group de-

creased (no statistically significant difference to control group). Reactivity of Ab in T group was similar to control group for first six weeks of experiment, at week 8th it decreased significantly below the control group level ($p<0.01$), but in 10th week absorbance increased significantly ($p<0.01$).

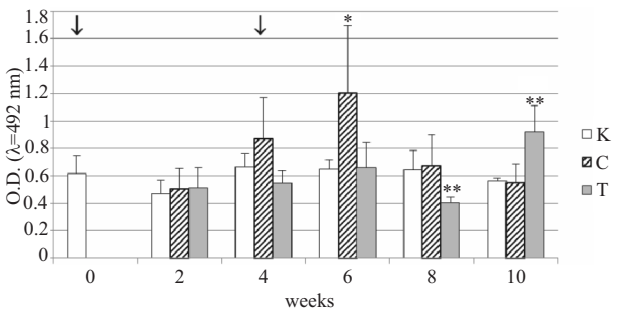

Fig. 5. IgY antibody levelsy against bovine coronavirus in eggs from control hens (K) and immunized with C or T vaccine. Arrows – dates of vaccinations, *$p<0.05$, **$p<0.01$.

In egg yolks from hens vaccinated with R vaccine (Fig. 6) stepwise increase of coronavirus antibody reactivity was observed from 2nd week up to the end of observation. The difference was significant in 6th and 8th weeks of the study ($p<0.01$).

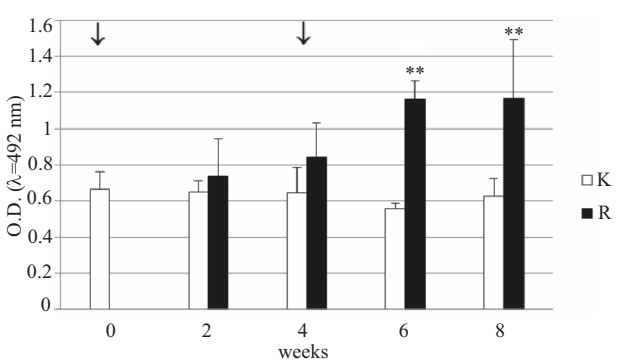

Fig. 6. IgY antibody levels against bovine coronavirus in eggs from control hens (K) and immunized with R vaccine (R). Arrows – dates of vaccinations, **$p<0.01$.

Second experiment

Due to the best preliminary results (high level of immune response and lower price) for large scale production of IgY antibodies against bovine alimentary tract pathogens C vaccine was selected. After the first immunization there was decrease in egg laying rate up to 50% for 10 days. From the first week after the first vaccination reactivity of yolk Ab from hens immunized with C vaccine was higher than in control group except to weeks 4-5th where Ab level of experimental group decreased to the level similar to that in control group (Fig. 7).

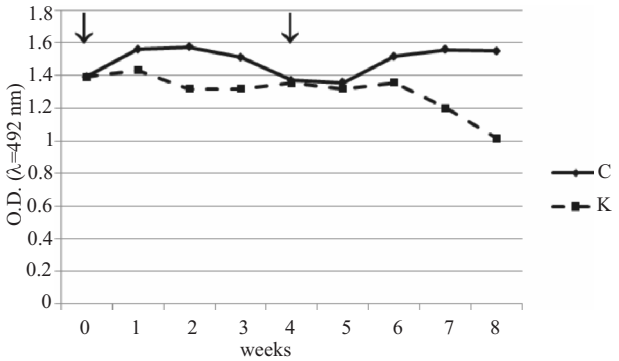

Fig. 7. The course of IgY antibody production against *E. coli* strain O157 in control (K) and C vaccine group. Arrows – dates of vaccinations.

During the experimental period higher absorbance levels were noted in C group from first week until 8th week with exceptions for 3nd and 6th week (Fig. 8). The largest difference and the highest absorbance for C group when compared to control group was seen in 8th week after the first immunization.

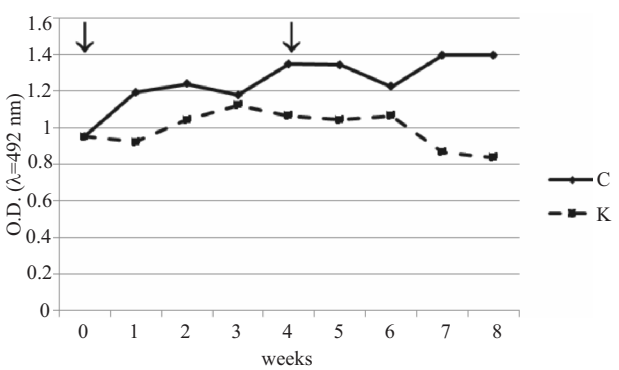

Fig. 8. The course of IgY antibody production against field *E. coli* (strain 1) in control (K) and C vaccine group. Arrows – dates of vaccinations.

In the second experiment the intensity of rotavirus IgY Ab reactivity in eggs from vaccinated hens significantly increased in 2nd week ($p<0.01$), but large individual differences between samples occurred (Fig. 9). High level of Ab remained up to 8th week of experiment ($p<0.05$), but later decreased to the level similar to the control group.

The increase of coronavirus Ab reactivity occurred in 2nd week of experiment and remained at the elevated level up to the end of observation period ($p<0.01$, except for week 12th $p<0.05$) (Fig. 10). The highest reactivity was detected in 6th week and decreased stepwise up to the 12th week.

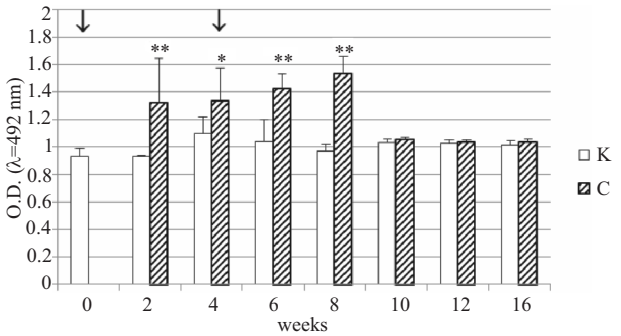

Fig. 9. IgY antibody levels against bovine rotavirus in eggs from control hens (K) and immunized with C vaccine. Arrows – dates of vaccinations, *p<0.05, **p<0.01.

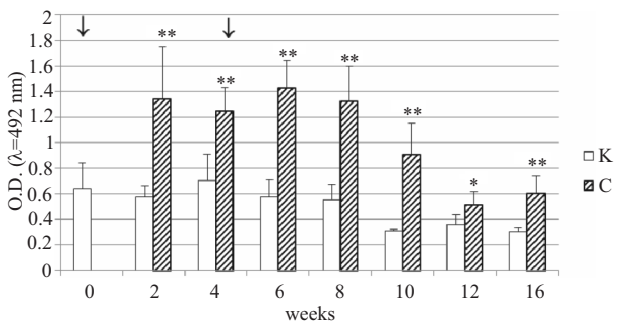

Fig. 10. IgY antibody levels against bovine coronavirus in eggs from control hens (K) and immunized with C vaccine. Arrows – dates of vaccinations, *p<0.05, **p<0.01.

Third experiment

From 1st up to 5thweek of immunization reactivity of IgY antibodies from vaccinated hens exceeded that of the control group (Fig. 11). At 6th week decreased of IgY Ab level was found in C group and at the same time increased in the control group. The intensity of IgY *E.coli* O157 antibody production was similar in both groups between 7th and 8th weeks and in the last week of observation again increased in vaccinated and decreased in control group.

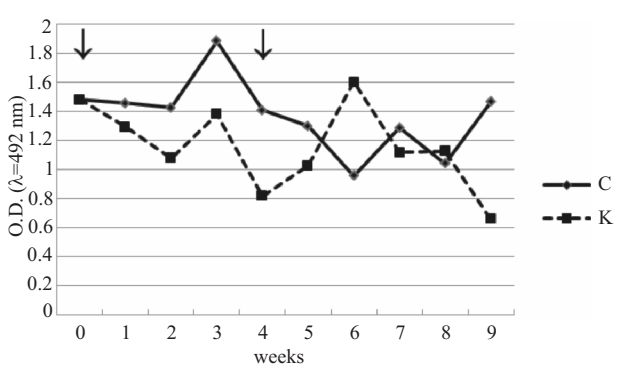

Fig. 11. The course of IgY antibody production against *E. coli* strain O157 in control (K) and group immunized with C vaccine. Arrows – dates of vaccinations.

For the first two weeks of the experiment reactivity of *E.coli* strain 1 IgY antibody in the control group slightly exceeded that in the vaccinated hens (Fig. 12). In the 3rd week the Ab level of the vaccinated group increased considerably and stayed elevated up to the end of the study.

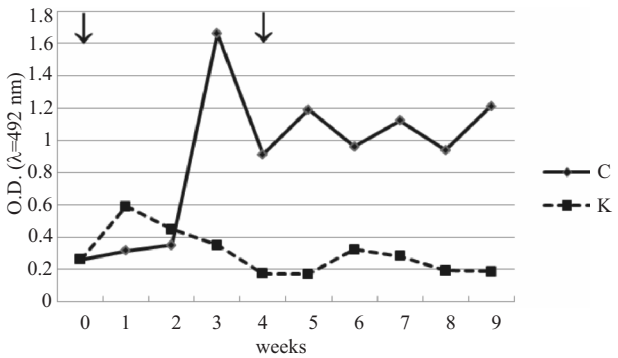

Fig. 12. The course of IgY antibody production against field *E. coli* (strain 1) in control (K) and group immunized with C vaccine. Arrows – dates of vaccination.

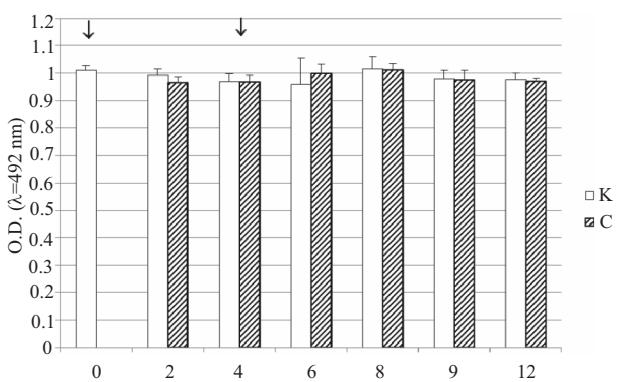

Fig. 13. IgY antibody levels against bovine rotavirus in eggs from control hens (K) and group immunized with C vaccine. Arrows – dates of vaccination.

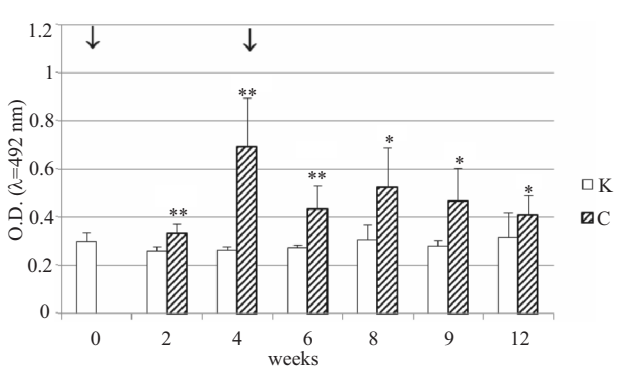

Fig. 14. IgY antibody levels against bovine coronavirus in eggs from control hens (K) and group immunized with C vaccine. Arrows – dates of vaccination, *p<0.05, **p<0.01.

Similarly as in the second experiment (Fig. 9) high level of rotavirus antibodies was found before start of immunization (Fig. 13). Vaccination had not mounted higher level of immune response in C group as compared to control hens.

From 2nd week IgY coronavirus antibody level of experimental group increased and was significantly different from control hens ($p<0.01$ in 2nd, 4th and 6th weeks; $p<0.05$ in 8th, 9th and 12th week) (Fig. 14). The highest absorbance was noted in 4th week of experiment.

Discussion

Double immunization of laying hens with bovine vaccines induced no important side effects except of local swelling in site of injection that disappeared within four weeks. In the second trial we also noticed decreased egg laying rate for ten days after first vaccination. The effect of immunization on lying capacity is inconsistent between studies and even in the case of using the same adjuvant (Freund's complete adjuvant) the results may be contradictory (Bollen and Hau 1996, Chalghoumi et al. 2008). In field conditions it should be recommended to avoid the Freunds' complete adjuvant that contains mycobacterial antigens which may cause diagnostic problems associated with control of avian tuberculosis and presents tissue-damaging potency (Schade et al. 2005).

The increase of antibody reactivity against three examined pathogens in egg yolks from vaccinated hens was shown in the first experiment. Among three examined vaccines the weakest increase of Ab level occurred in the case of T vaccine. When compared C and R groups the increase of *E.coli* and rotavirus antibody activity was more distinct in C group, but coronavirus antibodies remained for longer time at elevated level in R group. Higher Ab activity against two of three pathogens and the significantly lower price of the C vaccine in comparison to R resulted in choosing C vaccine in later studies.

An increase of Ab level after immunization was shown for examined *E.coli* strains and coronavirus antigens in all three trials. In the case of rotavirus we observed significant increase of antibody level in vaccinated group in the first and the second trial. No significant differences were found in last trial. Moreover the initial level of rotavirus Ab was high already in yolks before immunization. Yolken et al. (1988) demonstrated that antibodies to rotaviruses were widely prevalent in eggs which were available for consumption by humans in United States. This was most likely related to a high rate of natural infection with strains of rotaviruses that share antigenic determinants with human rotaviruses. Since some avian rotaviruses are antigenically related to mammalian group A rotaviruses (McNulty 2003) this is very probable that in our study elevated Ab level in egg yolks from non-vaccinated hens was due to those similarities between avian and bovine rotaviruses.

One of the aims of this study was to evaluate if this vaccination method induce the immunity against major pathogens of the calves' gastrointestinal tract. Vaccination should not negatively influence the health and laying capacity of hens. Therefore, subcutaneous route and use of widely available bovine vaccines seemed logical. Immunization with mixture of different antigens at the same time is not a new idea. Chalghoumi et al. (2008) showed increase of IgY antibody against two *Salmonella* serovars in the same yolk. Moreover, IgY obtained from hens immunized simultaneously with various pathogens could have various functions against these pathogens. Sugita-Konishi et al. (1996) immunized hens with twenty-six strains of different bacteria and investigated yolk activity against *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Staphylococcus aureus*. Despite the very high reaction in ELISA for all those three pathogens, the growth inhibition was noted only for *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Although in our study there was evident reaction with strain isolated from calf with diarrhea, the effectiveness in preventing diarrhea must be verified *in vivo*.

Any differences in the degree of Ab reactivity after immunization with C, T and R vaccines may be associated with different adjuvants as well as different *E.coli* and virus preparations. In our study the vaccines were given to the hens subcutaneously, whereas majority of former experiments used intramuscular application. Therefore, it is difficult to compare our results to other studies because of different routes of administration of the vaccines, different antigens and adjuvants used as well as different intervals between antigen injections (Schade et al. 2005, Pauly et al. 2009).

Ikemori et al. (1992) vaccinated hens with 1 mg of crude pili preparation from *E. coli* strains 431 (0101:K30; K99; F41.NM,ST) and B44 (09:K30; K99; F41:NM,ST) given i.m. in emulsion with 5% mannide monooleate to breast muscles 3 times (booster injections after 6 and 14 weeks). The eggs were harvested every 2 weeks after booster immunization based on plate agglutination testing. Agglutination titer in egg yolks reached 1:1024 – 1:2048.

Doses of rotavirus used in that study were similar or lower from utilized in study of Sarker et al. (2001) where hens were immunized intramuscularly with 1×10^7 FCFU of human rotavirus (Wa, RV5, RV3 and ST3) twice at the interval of 1 week using

Freund's complete adjuvant. Hatta et al. (1993) immunized hens four times intramuscularly with 1 x 10^7 FCFU of human rotavirus (HRV) strains Wa and Mo and produced elevated neutralization titer of IgY Abs (13000 against Wa strain and 15000 against Mo strain). Also in that study Freund's complete adjuvant was used to enhance the immune response. One year production of anti-HRV antibodies was at least 15 times (anti-Wa strain) and 120 times (anti-Mo strain) more effective than those in immunized rabbits, but during this period booster immunizations were repeated several times. No data were given about decrease of laying yield in experimental hens. In our former study hens immunized intramuscularly with human haptoglobin (0.2 ml FCA/0.2 ml of antigen dissolved in saline given i.m., 4 times in intervals of 2 weeks) decreased the laying yield about four times (not published).

In hens immunized with human rotavirus Bogstedt et al. (1996) found significant differences of neutralization titer between strains. Although they found the correlation between ELISA and neutralization titres, they indicated that serotype-dependent differences may affect the outcome of clinical trials. Therefore, the clinical effectiveness of produced IgY Ab applied in the field conditions may be affected by several pathogens- and animals- dependent factors.

In the available literature scarce data were found about immunization of hens with bovine coronavirus. Ikemori et al. (1997) vaccinated 10 white Leghorn hens with NCDC antigen containing about $10^{8.5}$ $TCID_{50}$/ml with 0.3% formalin. The antigen was mixed with an equal volume of oil adjuvant with 5% mannide monooleate and 1.0 ml of the mixture was injected intramuscularly. Six weeks after the initial injection booster inoculation was administered in a similar manner and eggs were harvested two weeks later. The vaccination doses of coronavirus were higher than in our study (available data only for C vaccine). Harvesting eggs two weeks after 2nd vaccination seems to be the optimal time since in our study the peak of coronavirus antibody activity occurred two weeks after second vaccination in experiment 1st and 2nd, but not in 3rd. The vaccination increases the specificity of antibody and although is laborious, cost more money and could result in temporary decrease of lying rate, may be more effective in disease control. Immunoglobulins isolated from unimmunized chickens failed to prevent the development of rotavirus gastroenteritis in mice infected with murine rotavirus (Yolken et al. 1988). Antibodies from hens vaccinated with *Salmonella* Enteritidis, among others, significantly suppressed *in vitro* production of the toxin compared to non-immunized yolk (Sugita-Konishi et al. 1996). Vaccination of hens could be better solution than vaccination of cows, because large scale production is easier and there is not much difference because of similar antibody response (neutralization titers 1:5120) in vaccinated hens and cows (Ikemori et al. 1997).

In conclusion, all used vaccines mounted the humoral immune response in laying hens. Double immunization seems to be enough in mounting response for several weeks against examined pathogens. C vaccine chosen for 2nd and 3rd trial allowed to harvest egg yolks with satisfactory levels of *E.coli*, rotavirus and coronavirus IgY antibodies which may be used to evaluate their protective effect by oral administration in calves. This was first, to our knowledge, trial of simultaneous vaccination of the hens with three major bovine gastrointestinal tract pathogens.

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References

Acres SD, Isaacson RE, Babiuk LA, Kapitany RA (**1979**) Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins. Infect Immun 25: 121-126.

Bartels CJ, Holzhauer M, Jorritsma R, Swart WA, Lam TJ (**2010**) Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev Vet Med 93: 162-169.

Bogstedt AK, Johansen K, Hatta H, Kim M, Caswall T, Svensson L, Hammarstrom L (**1996**) Passive immunity against diarrhoea. Acta Paediatr 85: 125-128.

Bollen LS, Hau J (**1996**) Freund's complete adjuvant has a negative impact on egg laying frequency in immunized chickens. In Vivo 13: 107-108.

Chalghoumi R, Théwis A, Portetelle D, Beckers Y (**2008**) Production of hen egg yolk immunoglobulins simultaneously directed against *Salmonella* Enteritidis and *Salmonella* Typhimurium in the same egg yolk. Poult Sci 87: 32-40.

Furman-Fratczak K, Rzasa A, Stefaniak T (**2011**) The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. J Dairy Sci 94: 5536-5543.

Haława W, Stefaniak T (**2002**) The economic evaluation of the *Haemophilus somnus* passive-active immunoprophylactic programme in field conditions (in Polish). In: Stefaniak T (ed) Problemy zdrowia narządu oddechowego młodych zwierząt gospodarskich. Akademia Rolnicza, Wrocław pp. 121-137.

Hatta H, Tsuda K, Akachi S, Kim M, Yamamoto T (**1993**) Productivity and some properties of egg yolk antibody (IgY) against human rotavirus compared with rabbit IgG. Biosci Biotech Biochem 57: 450-454.

Ikemori Y, Kuroki M, Peralta RC, Yokoyama H, Kodama Y (**1992**) Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K99-piliated enterotoxigenic *Escherichia coli*. Am J Vet Res 53: 2005-2008.

Ikemori Y, Ohta M, Umeda K, Icatlo FC Jr, Kuroki M, Yokoyama H, Kodama Y (**1997**) Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. Vet Microbiol 58: 105-111.

McNulty MS (**2003**) Rotavirus Infections in Viral Enteric Infections. In Saif YM (ed) Diseases of Poultry. Blackwell Publishing Company, Iowa, pp. 308-319.

Mine Y, Kovacs-Nolan J (**2002**) Chicken egg yolk antibodies as therapeutics in enteric infectious diseases: a review. J Med Food 5: 159-169.

Pauly D, Dorner M, Zhang X, Hlinak A, Dorner B, Schade R (**2009**) Monitoring of laying capacity, immunoglobulin Y concentration and antibody titer development in chickens immunized with ricin and botulinum toxins over a two-year period. Poult Sci 88: 281-290.

Sarker SA, Casswall TH, Juneja LR, Hoq E, Hossain I, Fuchs GJ, Hammarstrom L (**2001**) Randomized, Placebo-Controlled, Clinical Trial of Hyperimmunized Chicken Egg Yolk Immunoglobulin in Children With Rotavirus Diarrhea. J Pediatr Gastroenterol Nutr 32: 19-25.

Schade R, Calzado EG, Sarmiento R, Chacana PA, Porankiewicz-Asplund J, Terzolo HR (**2005**) Chicken Egg Yolk Antibodies (IgY-technology): A Review of Progress in Production and Use in Research and Human and Veterinary Medicine. ATLA 33: 1-26.

Snodgrass DR, Fahey KJ, Wells PW, Campbell I, Whitelaw A (**1980**) Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. Infect Immun 28: 344-349.

Stefaniak T (**2006**) Control of intestinal diseases by dietary supplementation with antibodies. In: Mosenthin R, Zentek J, Żebrowska T (eds) Biology of nutrition in growing animals. Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto pp. 285-309.

Stefaniak T, Wieliczko A, Kuczkowski M, Kopeć W, Jamroz D (**2004**) Effect of hen's egg yolk immunoglobulin (IgY) additive to the fodder of broiler chickens on experimental Salmonella Enteritidis infection and production results. Med Weter 60: 432-436.

Sugita-Konishi Y, Shibata K, Yun SS, Hara-Kudo Y, Yamaguchi K, Kumagai S (**1996**) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. Biosci Biotechnol Biochem 60: 886-888.

Svensson C, Lundborg K, Emanuelson U, Olsson SO (**2003**) Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev Vet Med 58: 179-197.

Thompson HP, Dooley JS, Kenny J, McCoy M, Lowery CJ, Moore JE, Xiao L (**2007**) Genotypes and subtypes of Cryptosporidium spp. in neonatal calves in Northern Ireland. Parasitol Res, 100: 619-624.

Vega C, Bok M, Chacana P, Saif L, Fernandez F, Parreño V (**2011**) Egg yolk IgY protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. Vet Immunol Immunopathol 142: 156-169.

Yolken RH, Leister F, Wee SB, Miskuff R, Vonderfecht S (**1988**) Antibodies to rotaviruses in chickens' eggs: a potential source of antiviral immunoglobulins suitable for human consumption. Pediatrics 81: 291-295.