ASSESSMENT OF THE EFFICIENCY OF APPLYING METHISOPRINOL AND VACCINATION OF TURKEYS AGAINST THE HAEMORRHAGIC ENTERITIS VIRUS USING IN OVO METHODS

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Abstract

The aim of the research was to determine the efficiency of in ovo immunisation of turkeys against the haemorrhagic enteritis virus, while simultaneously applying a synthetic immunomodulator – methisoprinol – by the same route of administration. Dindoral SPF vaccine, in a dose of 0.1 ml of the solution prepared ex tempore after dissolving the vaccine in 100 ml of water for injection, and methisoprinol in a dose of 5 mg per egg, were administered in ovo on the 26th d of incubation. The control groups consisted of turkeys hatched from eggs into which only the methisoprinol or the vaccine was administered, as well as birds hatched from eggs that were not interfered with. The susceptibility of turkeys to HE virus infection was determined on the basis of the presence of HE antibodies in serum, the evaluation of a splenic index, and attempts to register the virus presence in the spleen 120 h after the control infections. The research proved the effectiveness of immunising turkeys against the haemorrhagic enteritis virus by administering Dindoral SPF vaccine in ovo. It was also demonstrated that the simultaneous application of methisoprinol, showing an antiviral effect, and the vaccine inhibited the development of post vaccinal immunity against this virus.

Key words: turkeys, haemorrhagic enteritis virus, methisoprinol, in ovo vaccination, immunomodulation.

Understanding the mechanisms of a bird’s immune system, and especially showing that bird embryos in the final period of embryogenesis are immunocompetent (20, 22), provided the basis for developing and implementing an in ovo vaccination (10). This method is widely used in broiler chickens as a prophylaxis for Marek’s disease and Gumboro disease, but it remains in the research stage as regards a specific prophylaxis for turkeys (1). The advantage of in ovo vaccination is its high efficiency (up to 60,000 embryos per hour). Additionally, it provides an opportunity to obtain a high and uniform immunity, while eliminating unfavourable factors (stress, technical errors in vaccination), which accompany the classical methods of immunisation (10). Besides, in ovo immunoprophylactics, a further innovative direction seems to be immunomodulation through the same route of administration. This is significant, insofar as the immunity system of embryos is not affected by external factors, which very frequently result in immunosuppression in birds reared in intensive production systems (4, 21). Preliminary research regarding the application of a synthetic low toxic immunomodulator, namely methisoprinol, administrated in ovo, showed that it increased the humoral immune response against the ND virus in turkeys (12, 19), and stimulated 5-day-old turkey poults hatched from such eggs to produce mainly unspecific mechanisms of humoral immunity response, demonstrated by a higher percentage of subpopulation of T CD3+ and CD4+ lymphocytes in peripheral blood and in the spleen (23).

Considering the above facts, a study was carried out to determine the efficiency of immunising turkeys against the haemorrhagic enteritis virus (HEV) using the in ovo method, with a simultaneous administration of methisoprinol by the same route.

Material and Methods

Four groups of BUT 9 type turkeys were examined (15 birds in a group), hatched from eggs into which the following were applied on day 26 of incubation: methisoprinol (inosin–[(1-dimethyloamino-2-propanol)-4-acetamidobenzoat]) (VetAgro Lublin, Poland) in a dose of 5 mg per egg (group 1); methisoprinol in the same dose as above, together with the vaccine against HEV (Dindoral SPF, Merial (France)
in a dose of 0.1 ml of solution prepared ex tempore after dissolving a vial of vaccine in 100 ml of water for injection (group II); or which were immunised against the HEV using the in ovo method of administrating Dindoral SPF in the same dose as above (group III). The turkeys in group IV constituted a control group (without immunisation or methisoprinol administration). All the birds were reared in accordance with current technology.

On days 5, 21, and 47 of their life, blood samples were taken from 10 turkeys of each group for serologic examinations. The AGP test to check the presence of HEV antibodies was used (11). On day 42 of rearing, 10 turkeys in each group were infected by per os application (to the crop) of 1 ml of HEV suspension, in a dose of 10^4.3EID_{50}. Five days after infection, the turkeys of each group were subjected to euthanasia, weighed, and their spleens were removed in order to determine macroscopic changes and the splenic index (IS) (the spleen to body weight ratio as a percentage share), and to register the virus presence using the AGP method with a HEV specific serum (11). The control group consisted of five turkeys from each group, which were not infected with the virus.

The results obtained were statistically analysed using a variance analysis and determining the mean, standard deviation, and significance of differences.

**Results**

The results of the effectiveness of immunising turkeys against the HEV using the in ovo method with a simultaneous application of an immunomodulator, methisoprinol, by the same route of administration, are presented in Table 1. As it is evident from the Table, HEV antibodies were found in the serum of 5-day-old turkeys from group IV (control), and group III (immunised against the HEV using the in ovo method). Turkeys from this group also revealed the presence of HEV antibodies on days 21 and 47 of their life.

An anatomicopathologic examination of the turkeys’ spleen (increased IS and marble pattern, characteristic for HEV infection), as well as its viral analysis proved that turkeys hatched from eggs in the groups I, II, and IV were susceptible to challenge with the HEV. A lack of susceptibility to such an infection was found in the turkeys of the group III.

**Discussion**

The study concerned the determination of the susceptibility of turkeys hatched from eggs to the HEV infection compared to those immunised in ovo against HEV, with the simultaneous application of methisoprinol in a dose of 5 mg per embryo. It must be emphasised that the immunisation of turkeys against HEV in field conditions brings many problems. This procedure is most commonly performed on the 28 d of rearing, by administering the vaccine with drinking water. The earlier application of the vaccine is not effective inasmuch as the spread of infections of the HEV field strains in reproductive herds of turkeys is common, and hatched poults possess maternal antibodies (11), which, on one hand, protect turkeys against infection but, on the other, prevent the development of post-vaccinal immunity (3, 5).

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>HEV AGP antibodies</th>
<th>Response to infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age (d)</td>
<td>IS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>*</td>
<td>2.17^A ±0.30</td>
</tr>
<tr>
<td></td>
<td>(Methisoprinol in ovo, 5 mg/egg)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0/10 0/10 0/10 0/10</td>
<td>2.05^A ±0.36</td>
</tr>
<tr>
<td></td>
<td>(Methisoprinol 5mg/egg + Dindoral SPF in ovo)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2/10 6/10 5/10 5/10</td>
<td>1.11^B ±0.12</td>
</tr>
<tr>
<td></td>
<td>(Dindoral SPF in ovo)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>2/10 0/10 0/10 0/10</td>
<td>2.16^C ±0.23</td>
</tr>
<tr>
<td></td>
<td>(control)</td>
<td></td>
</tr>
</tbody>
</table>

IS - splenic index in birds non-infected with HEV ranged from 0.68 to 0.96±0.04-0.39
* - numerator indicates the number of positive sera (spleen), denominator indicates the number of examined sera (spleen)
AB - P≤0.01
± - standard deviation
In such a case, turkeys remain susceptible to infections with field strains of this virus, which, regardless of their virulence degree, causes immunosuppression (8, 9, 16, 17, 24). However, a fact worth emphasising is that, in the light of the results obtained by Neumann et al. (18) and Guiro (8), Dindoral SPF, a commercial vaccine, is safe, immunogenic, and does not cause immunosuppression. Taking the results of own research demonstrating an immunomodulating effect of methisoprinol administered in ovo into account (12, 19), as well as the proven possibility of using this method to immunise turkeys against HEV (1), this study was undertaken with the aim of demonstrating the degree of effectiveness of immunising turkeys by using in ovo administration, with the simultaneous application of methisoprinol. The results obtained (Table 1) confirmed that immunisation by this route, and the application of methisoprinol at the same time, has no negative effects on the hatching ability or the health of reared turkeys (2). It was also shown that the Dindoral SPF vaccine administered in ovo effectively protects 6-week-old turkeys against field infection with the HEV. It was proven by the demonstration of AGP antibodies in the turkeys’ serum, non-increased splenic index, and lack of virus reisolation from the spleen of infected turkeys. Whereas, turkeys hatched from the eggs into which Dindoral SPF vaccine and methisoprinol were applied in ovo at the same time, as well as turkeys hatched from eggs receiving only methisoprinol, were fully susceptible to HEV infection.

The above results confirm the anti-viral effect of methisoprinol (6, 7, 13-15), and they show that this preparation administered in ovo at the same time as Dindoral SPF inactivates the vaccine virus, and inhibits the development of active post-vaccinal immunity.

Reference