Studies on the cumulative effect of sodium thiomersal on broilers vaccinated with inactivated poultry vaccines

Zeinab M. Sror1*, Anhar Abd El-Moety1, Hanan, M. Ibrahim1, M. L. Sayed, A. R. Mahmoud2, S. M. Shafei1, M. H. Khodeir3

1Central Laboratory for Quality Control of Veterinary Biologics, 2Animal Health Research Institute, 3Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.

Merthiolate (Thiomersal) is known to be used as antimicrobial agent in inactivated vaccines without affecting vaccine potency. The present work investigated the effect of thiomersal contents in ND, AI and IBD inactivated vaccines on liver and kidney functions of vaccinated birds. The histopathological effect and the withdrawal time of such mercurial product from vaccinated chicken muscles were also investigated. Results revealed that residual thiomersal contents in ND, AI and IBD were 0.03; 0.02 and 0.03mg/ml respectively. Liver and kidney function parameters showed significant increases in serum activities of alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) up to the 4th week post vaccination. Serum urea was significantly decreased on the 3rd week in vaccinated birds compared to control ones. Significant increase in serum creatinine in vaccinated chickens was recorded on the 5th week post vaccination. Liver and kidney functions' parameters remain high all over the experimental period (8 weeks). The histopathological examination of liver specimens revealed degeneration of hepatic cells and congestion of the central vein with inflammatory cell infiltration and congestion of blood vessels as well as coagulative necrosis. The spleen of vaccinated chickens showed depletion of lymphocytes while lungs showed thickening of the alveolar. Mercury contents in muscle were 0.72, 0.52; 0.046 and 0.00 mg/kg on the 1st, 2nd, 3rd and 4th week post last vaccination that considered safe to the consumer. It is recommended that vaccinated poultry with inactivated vaccines containing thiomersal should not be slaughtered before at least 4 weeks and it is preferable to use combined inactivated vaccines to reduce the thiomersal contents as possible.

Thimerosal has been used as an additive to biologics and vaccines since 1930s because it is very effective for killing bacteria. It was used in several vaccines for prevention of contamination, particularly in opened multidose containers (Keith and Walters, 1992).

Thimerosal (Merthiolate) is an ethylmercury-containing pharmaceutical compound (49.55% mercury) that is developed in 1972. It is metabolized to ethyl mercury and thiosalicylate and both forms of organic mercury are associated with neurotoxicity in high doses. Definitive data regarding the doses at which developmental effects occur are not available. When vaccines containing thimerosal have been administrated in the recommended doses, hypersensitivity has been noted, but no other

* Corresponding author. Tel.: +20 23424406; fax: +20 23428321. (Zeinab M. Soror).
and /or ophthalmic application of thiomersal in all tissues except blood with highest levels of the element detected in the kidney, followed by liver and brain. It was stated that the levels in the blood and liver decreased with time while in the kidneys the residues mostly is in the form of inorganic mercury which persisted even 14 days after the exposure. The half-life of methyl mercury in man is about 70 days as demonstrated by Anon (1996).

Regarding the effect of thiomersal on the vaccine potency, it was found that different quantities of thiomersal in inactivated Newcastle oil-emulsion vaccines affected the HI response in vaccinated broilers with stored vaccines for 1,21 and 52 weeks. HI serology was conducted at 2,4 and 6 weeks post vaccination. Mean HI titers 4 weeks after vaccination decreased significantly with increasing concentrations of thiomersal. In addition, HI titers 4 week after vaccination with 1-week-old vaccine were significantly higher than those after vaccination with 52-week-old vaccine at all thiomersal concentrations. It was concluded that at the recommended dose of thiomersal, there is no significant decrease in vaccine efficacy (Anon, 1985).

The present work was planned to investigate the effect of thiomersal content in some inactivated poultry vaccines (AI; ND and IBD) on liver and kidney functions; histopathological findings in the liver and kidneys in addition to determination of the muscle content of mercury post vaccination to determine its suitable withdrawal time.

**Material and Methods**

**Chickens.** A group of sixty specific pathogen free (SPF) chickens were vaccinated with the recommended vaccines for broilers. So, these chickens were vaccinated with inactivated avian influenza (AI) vaccine at 7 days of age and inactivated Newcastle disease (ND) and infectious bursal disease (IBD) vaccines at 14 days of age.

Another group of sixty SPF chickens was kept without vaccination as control. Each chicken group was kept separately under hygienic measures.

**Vaccines.** Inactivated AI vaccine batch number: B390508-31; ND vaccine batch number: 15065G/C and IBD vaccine batch number: 15064D/D were supplied by CEVA Company and used for vaccination of experimental chickens.

**Determination of thiomersal contents in used vaccines.** The thiomersal content in the used vaccines was estimated by the Central Laboratory for Control on Veterinary Biologics (CLCVB), Abbasia, Cairo, Egypt; following the directions of Quality Control of Vaccines (1983).

**Sampling.**

**Blood sampling.** Blood samples were obtained from experimental birds at one week intervals post vaccination to separate serum for determination of thiomersal and serum biochemical parameter.

**Tissue sampling.** Scarification of randomly selected vaccinated and control chickens was carried out at one week intervals post vaccination. Specimens from the breast muscles, kidneys, livers and spleen were obtained for determination of thiomersal residues and histopathological examination.

**Histopathological examination.** The collected tissue specimens were prepared for histopathological examination and stained with Haematoxylin and Eosin stains according to (Bancroft et al, 1996).

**Biochemical assays.** Determination of serum alanine and aspartate aminotransferase activities (ALT and AST) was carried out according to the method described by Reitman and Frankel (1957). Determination of blood urea nitrogen value was carried out according to Henry et al (1974).

Determination of serum creatinine concentration was done following the method of Teger-Nilsson (1961).

Determination of mercury in chicken muscles.

Determination of mercury in the muscles of vaccinated chickens was carried out by atomic absorption spectrometry- cold vapour technique as described by Anon (1985) in the Central Laboratory of Residue Analysis of Pesticides and Heavy metals in Food; Agriculture Research Center, Giza, Egypt.

**Statistical analysis.** The obtained results of serum biochemical analysis were statistically analyzed and the significant differences between the obtained values were determined by conducting F-test and least significant difference (LSD) according to Petrie and Watson (1999).

**Result and Discussion**

Although live attenuated vaccines induced high levels of immunity, inactivated vaccines were found to be preferable to avoid the possible hazard which may be induced in vaccinated hosts (due to less attenuation of the included agent) or in contact individuals (due to excretion of the live agent). Merthiolate (Thiomersal) is
Table (1): Thiomersal content in the used inactivated vaccines.

<table>
<thead>
<tr>
<th>Tested vaccine</th>
<th>Newcastle disease vaccine</th>
<th>Avian influenza vaccine</th>
<th>Infectious bursal disease vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiomersal content (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Parameters of liver and kidney functions in vaccinated chickens.

<table>
<thead>
<tr>
<th>Weeks post the 1st vaccination</th>
<th>ALT (IU/ml)</th>
<th>AST (IU/ml)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3±2.00</td>
<td>16±5.00</td>
<td>2±0.6</td>
<td>0.8±0.18</td>
</tr>
<tr>
<td>1</td>
<td>3±1.41</td>
<td>16±4.00</td>
<td>0.97±0.5</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>2</td>
<td>4±0.00</td>
<td>15±4.00</td>
<td>0.97±8</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>3</td>
<td>6±2.0</td>
<td>16±3.0</td>
<td>0.82±0.2</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>4</td>
<td>7±1.15</td>
<td>18±4.00</td>
<td>1.5±0.5</td>
<td>0.8±0.4</td>
</tr>
<tr>
<td>5</td>
<td>5±1.4</td>
<td>20±4.0</td>
<td>1.93±0.7</td>
<td>1.35±0.76</td>
</tr>
<tr>
<td>6</td>
<td>4±1.0</td>
<td>18±5.5</td>
<td>3.02±0.3</td>
<td>1±0.85</td>
</tr>
<tr>
<td>7</td>
<td>4±1.0</td>
<td>21±1.0</td>
<td>4.06±0.3</td>
<td>1.4±0.25</td>
</tr>
<tr>
<td>8</td>
<td>4±0.2</td>
<td>16±0.02</td>
<td>1.93±0.66</td>
<td>1.29±0.06</td>
</tr>
</tbody>
</table>

ALT= Serum alanine aminotransferase activity
AST= Serum aspartate aminotransferase activity

Table (3): Mercury content in vaccinated chicken muscles

<table>
<thead>
<tr>
<th>Mercury* (mg/kg)</th>
<th>1WPV</th>
<th>2WPV</th>
<th>3WPV</th>
<th>4WPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.72</td>
<td>0.52</td>
<td>0.046</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

*WPV = Weeks post vaccination.

The limit of quantification (LOQ) of mercury is 0.03mg/kg. The estimated relative standard deviation of this method is < 15%.

Photo (1): Chicken liver, 2 weeks post vaccination showing degeneration of hepatic cells and congestion of the central vein (H&E, X400).

Photo (2): Chicken liver, 4 weeks post vaccination showing degenerated hepatic cells with inflammatory cell infiltration and congested blood vessels (H&E, X400).

Photo (3): Chicken spleen, 5 weeks post vaccination showing depletion of lymphocytes (H&E, X400).

Photo (4): Chicken liver, 5 weeks post vaccination showing coagulative necrosis of hepatic cells (H&E, X400).
known to be used as antimicrobial agent in inactivated vaccines (Keith and Walters, 1992 and Anon, 1996) without affection of the vaccine potency (Anon, 1985).

The present work was planned to investigate the effect of thiomersal contents in some inactivated poultry vaccines (ND; AI and IBD) on liver and kidney functions; histopathological findings in the liver and kidneys in addition to determine the muscle contents of mercury post vaccination to determine its withdrawal time.

The experimental results revealed that the merthiolate contents in ND; AI and IBD inactivated vaccines used for chicken vaccination in the present work (Table-1) were 0.03; 0.02 and 0.03mg/0.5ml respectively. These contents appear to be within the recommended limits as recorded by Anon (1996) who stated that such contents should not exceed 0.04 - 0.1µg / ml.

Estimation of liver and kidney function parameters (Table-2) showed that GPT and GOT were mild significantly higher while serum urea was significantly decreased on the 4th week in vaccinated birds than in control ones. There was a significant increase in serum creatinine in vaccinated chickens on the 5th week post vaccination. All estimated liver and kidney function parameters were still high allover the experiment period (8 weeks post the first vaccination). Parallel to and confirming these finding, the histopathological examination of liver specimens of vaccinated chickens revealed degeneration of hepatic cells and congestion of the central vein (Photo-1) on the 2nd week; hepatic cell degeneration with inflammatory cell infiltration and congested blood vessels (Photo-2) on the 4th week and coagulative necrosis of hepatic cells (Photo-3) on the 5th week post vaccination. The spleen of vaccinated chickens showed depletion of lymphocytes (Photo-4) on the 5th week post vaccination. Also the lungs revealed thickening of the alveolar wall by proliferation of the septal cells on the 5th week post vaccination (Photo-5). These findings could be attributed to the effect of thiomersal where it could be detected in all tissues except blood with highest levels of the element detected in the kidney, followed by liver and brain followed vaccination with inactivated vaccines Anon (1996). Also Yess (1993) and Clarkson (1997) showed that chronic exposure to inorganic and organic forms of mercury leads to renal damage.

Regarding the chicken muscle contents of mercury, it was found that these contents were 0.72, 0.52; 0.046 and 0.00 mg/kg on the 1st, 2nd, 3rd and 4th week post last vaccination (Table-3). These observations clarified that the muscles contents of thiomersal decreased gradually to reach 0-level by the 4th week post last vaccination. So, these muscles could be considered safe to the consumer. Quality Control of Vaccines (1983) recommended that content of mercury should not exceed 0.03mg/kg. Moreover, Clarkson (1997) found that the average half-life for mercury in blood is 40-50 days for adult and breastfeeding infants. It could be recommended that vaccinated poultry with inactivated vaccines containing thiomersal should not be slaughtered before 4 weeks at least with elimination of the liver. Also it is spear to be preferable to use combined inactivated vaccines to reduce the thiomersal content as possible.

References


**Arabic section:**

دراسات على التأثير التراكمي لمادة ثيوميرسل الصوديوم على دجاج التسمين المحسن بلقاحات الدواغين المثبتة

يعتبر الثيوميرسل من المواد المستخدمة كمضادات للفيروسات في اللقاحات المثبتة وقد صممت الدراسة الحالية لإثبات تأثير هذه المادة في لقاحات النيوكاول والالتهاب الشعبي والفأرة زлага الطيور المثبتة على وظائف الكبد والكلى والتأثير السمعي في هذه الأعضاء إضافة إلى تجديدوقيق الحالات ذات الخطر الزائد بعد التحصين بهذه اللقاحات. وقد أوضحت نتائج قياس الثيوميرسال أن مستوى هذه المادة هو 0,03 ميكروجرام/مل في كل من لقاح النيوكاول والفأرة زلاط الطيور، 0,02 ميكروجرام/مل في لقاح الالتهاب الشعبي، وأظهرت نتائج وجود مادة مثيرة للالتهاب عند هذه الظروف في الاعتدال بعد ذلك، كما أظهر الفحص المجهري لعينات من الكبد والثدي والرئة من الطيور المحسنة تغيرات هندسية شملت تأكيز وتحلل مع وجود خلايا التهابية، أما بالنسبة للنسبة المئوية من الزنقي (ناتج ناقل الثيوميرسال بالجسم) فقد وجد أنه يتقلص تدريجيًا ليصل إلى حد المقرر في الأسبوع الرابع بعد آخر تحقين، وعلى ذلك يمكن التوصية بدعم نهج الطيور قبل فترة أربعة أسابيع على الأقل من آخر تحقين مع استعداد الكبد والكلى إضافة إلى تحسين استخدام اللقاحات المركبة لتقليل نسبة الثيوميرسال بها.