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

**Zeinab M. Sror[1*], Anhar Abd El-Moety[1], Hanan, M. Ibrahim[1], M. L. Sayed, A. R. Mahmoud[2], S. M. Shafei[1], M. H. Khodeir[3]**

*[1]Central Laboratory for Quality Control of Veterinary Biologics, [2]Animal Health Research Institute, [3]Veterinary 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 allover 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 harmful effects have been reported (Cox and Forsyth, 1972). Massive overdoses from inappropriate use of thimerosal-containing products have resulted in toxicity (Axton, 1972).

The major toxicity of organic mercury compounds is expressed in the central nervous system, though the kidneys and the immune system also may be affected (Yess, 1993 and Clarkson, 1997). Organic mercury readily crosses the placenta and blood-brain barrier and it is readily absorbed by ingestion, inhalation and through skin and is distributed in all tissues but concentrate in blood and brain. It was indicated that the break down of thiomersal releases ethyl mercury that can penetrate cell membranes and bind to intracellular enzymes and proteins inhibiting their biological functions causing cell injury and death (Haley, 2008).

Distribution of thiomersal was examined in squirrel monkeys (400-900g bw) after dosing different concentrations of thiomersal. Increased concentration was seen after single subcutaneous

* 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.

**Detrmination 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 of thimerosal 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 thimerosal residues and histo-pathological 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 amintransferase 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 .**

| Tested vaccine | Newcastle disease vaccine | Avian influenza vaccine | Infectious bursal disease vaccine |
|---|---|---|---|
| **Thiomersal content1 (µg / ml)** | 0.03 | 0.02 | 0.03 |

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

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

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

**Table (3): Mercury content in vaccinated chicken muscles**

| WPV* | 1WPV | 2WPV | 3WPV | 4WPV |
|---|---|---|---|---|
| **Mercury (mg/kg)** | 0.72 | 0.52 | 0.046 | 0.00 |

*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%.

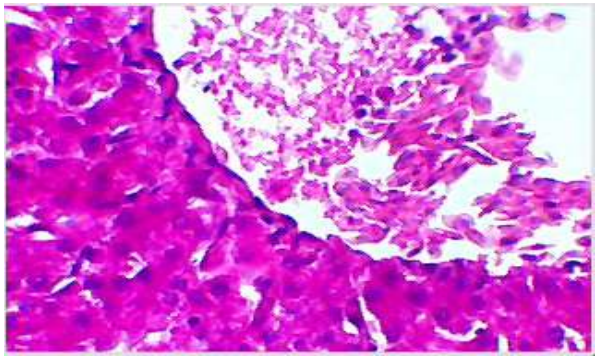

**Photo (1): Chicken liver, 2 weeks post vaccination showing degeneration of hepatic cells and congestion of the central vein (H&E, X400).**

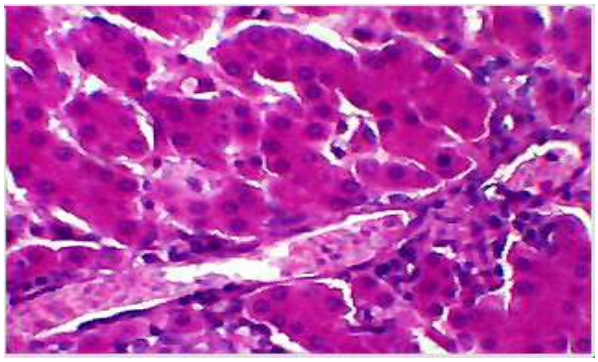

**Photo (2): Chicken liver, 4 weeks post vaccination showing degenerated hepatic cells with inflammatory cell infiltration and congested blood vessels (H&E, X400).**

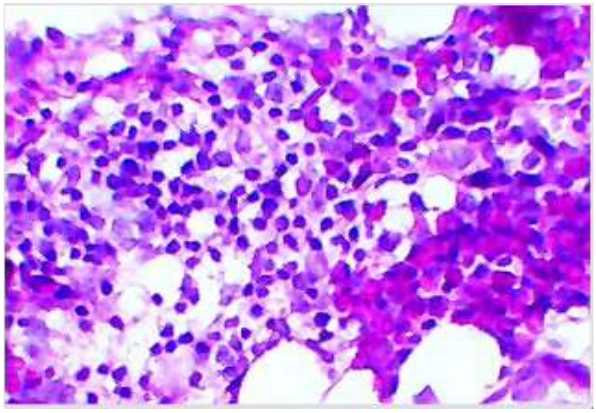

**Photo (4): Chicken spleen, 5 weeks post vaccination showing depletion of lymphocytes (H&E, X400).**

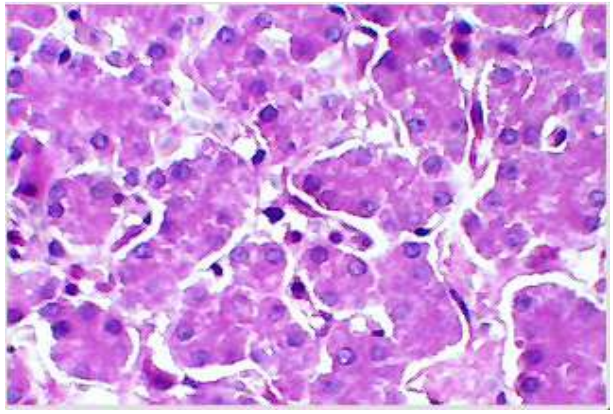

**Photo (3): Chicken liver, 5 weeks post vaccination showing coagulative necrosis of hepatic cells (H&E, X400).**

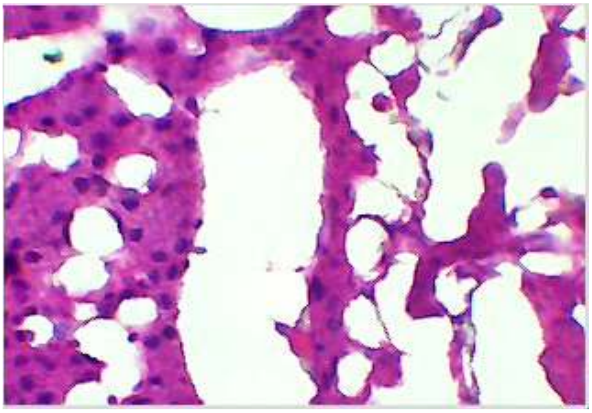

**Photo (5): Chicken lung, 5weeks post vaccination showing thickening of the alveolar wall by proliferation of the septal cells (H&E, X400).**

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

**Anon (1985):** Effect of thiomersal concentration on the efficacy of inactivated Newcastle disease oil-emulsion vaccines. Avian Dis., 29 (4): 1030-1035.

**Anon (1985):** Mercury quantitative determination of the total content in food by atomic absorption spectrometry-cold vapour technique. J. Assoc. Off. Anal. Chem. 68(5): 891-893.

**Anon (1996):** Committee for Veterinary Medicinal products, thiomersal and timerfonate. Summary Report. The European Agency for the evaluation of Medicinal products, Veterinary Medicines Evaluation Unit. EMEA /MRL/ 140/96-Final, October 1996.

**Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996):** Theory and practice of histological technique, 4th Ed.

**Cox, N.H. and Forsyth, A. (1988):** Thimerosal allergy and vaccination reaction. Cont.Derm.18: 229-233.

**Haley, E. (2008):** General vaccine issues: Mercury-Thiomersal and neurodevelopment. Vaccine Risk Awareness Network- "Affidative of Boyd E.Haley. VRAN: A Canadian Vaccination Information Network.

**Henry, J.; Todd, B. and Davidsohn, S. (1974):** Clinical diagnosis and measurement by laboratory methods. 16th Ed. W.B.Saunders and Co., Philadelphia, P.A.P.

**Keith, L. H. and Walters, D. B. (1992):** The National Toxicology Program's Chemical Data Compendium, 1-VIII. Boca Raton, FL: Lewis Publishers, Inc; 1992.

**Petrie, A. and Watson, P. (1999):** Statistics for veterinary and animal science. 1st ed .pp:90-99. The Black well science Ltd, United Kingdom.

**Quality Control of Vaccines (1983):** Rijksinstitutlit Voor Volksgezondheid En Milieuhygiene Bilthoven. The Netherlands Egyptian Organization for Biological Products and Vaccines Library, Agouza, Giza, Egypt.

**Reitman, S. and Frankel, S. (1957):** A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-58.

**Teger-Nilsson, A.C. (1961):** Quantitative colorimetric determination of creatinine in serum or urine. Scand. J. Clin. Lab. Invest., 13: 326.

## دراسات على التأثير التراكمى لمادة ثيومرسال الصوديوم على دجاج التسمين المحصن بلقاحات الدواجن المثبطة

**يعتبر الثيوميرسال من المواد المستخدمة كمضادات للميكروبات فى اللقاحات المثبطة وقد صممت الدراسة الحالية لاستبيان تأثير هذه المادة فى لقاحات النيوكاسل والالتهاب الشعبى وانفلونزا الطيور المثبطة على وظائف الكبد والكلى والتأثير السمى فى هذه الأعضاء إضافة إلى تحديد الوقت الملائم لذبح الطيور بعد التحصين بهذه اللقاحات ، وقد أوضحت نتائج قياس الثيوميرسال أن محتوى هذه المادة هو 0.03 ميكروجرام/مل فى كل من لقاح النيوكاسل وانفلونزا الطيور ، 0.02 ميكروجرام/مل فى لقاح الإلتهاب الشعبى ، وأظهرت نتائج وظائف الكبد ارتفاعاً معنويا حتى الأسبوع الرابع بعد آخر التحصين بينما أظهر معدل اليوريا مستوى أقل من الطبيعى ثم بدأت هذه الوظائف فى الاعتدال بعد ذلك، كما أظهر الفحص المجهرى لعينات من الكبد والطحال والرئة من الطيور المحصنة تغيرات باثولوجية شملت تنكرز وتحلل مع وجود خلايا التهابية ، أما بالنسبة لمحتوى العضلات من الزئبق (ناتج تحلل الثيوميرسال بالجسم) فقد وجد أنه يتناقص تدريجيا ليصل إلى حد الصفر فى الأسبوع الرابع بعد آخر تحصين ، وعلى ذلك يمكن التوصية بعدم ذبح الطيور قبل فترة أربعة أسابيع على الأقل من آخر تحصين مع استبعاد الكبد والكلى إضافة إلى تفضيل استخدام اللقاحات المركبة لتقليل نسبة الثيوميرسال بها.**