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Preparation and evaluation of kits for detection of antibodies of Pasteurella multocida

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Polyclonal hyperimmune serum against *Pasteurella multocida* type A:5, A:8 and A:9 was prepared in boskat rabbits. The indirect haemagglutination test (IHT) showed that such serum had an antibody titer of 1114. The immunoglobulins in the prepared antiserum were precipitated using saturated ammonium sulphate solution. Its concentration was adjusted to be 18mg/ml in normal saline then it was conjugated with horse radish peroxidase and evaluated through the application of double sandwich ELISA. It was successful to detect *Pasteurella multocida* antibodies in positive serum samples with strong positive reactions up to a dilution of 1:100 of the prepared conjugate.

Pasteurellosis or fowl cholera is a serious disease problem facing poultry industry in Egypt (Gergis, 1978). It is caused by *Pasteurella multocida* which has been distinguished by somatic capsular serotyping (Rhoad and Rimler, 1986). On the other side, fowl cholera represents a respiratory disease facing avian population in dramatic forms which may lead to 100% mortalities. The disease is characterized by septicemia with high morbidity and mortality rates causing great economic losses (Birggs and Skeeles, 1984; Schlink and Olson, 1987). Diagnosis of fowl cholera depends mainly on the symptoms which occur in three forms: acute, subacute and chronic inflammation of the air passages and lungs and often end with death (Anon, 1961). In addition, the postmortem and histopathological findings play an important role in the diagnosis of the disease as the presence of degenerative ultrastructural changes in epithelial and endothelial cells of the lungs of infected birds (Al-Haddawi *et al*., 2000). More recent techniques were arise for detection of *Pasteurella multocida* antigen or antibodies as shown by Borkoska-Opacka *et al*. (1997) who used ELISA to measure the level of specific IgG to *Pasteurella multocida* in the sera of birds vaccinated against pasteurellosis. The present study was planned to prepare peroxidase labeled antibodies against *Pasteurella multocida*; as a local product; to be used for detection of fowl cholera using ELISA saving the long time and cost which usually in need to provide a native conjugate.

Materials and Methods

Rabbits. Eight apparently healthy adult boskat rabbits of about 3 kg body weight were used for preparation of polyclonal antibodies against *Pasteurella multocida*. These rabbits were found to be free from *Pasteurella multocida* infection and antibodies as detected by IHA test.

Bacterial strains. *Pasteurella multocida* sero-groups 5-A, 8-A and 9-A standard strains were supplied by the Aerobic Bacteria Vaccine Research Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. The colony forming unit for these strains was $3.25x10^{10}$/ml.

Preparation of *Pasteurella multocida* antigen. *Pasteurella multocida* antigen was prepared according to (Higgins and Whithear,1985).

Preparation of *Pasteurella multocida* hyperimmune serum. It was carried out according to (Green and Manson,1990).

Precipitation of immunoglobulin. The prepared immunoglobulin was precipitated using Ammoniu sulphate (Hopkin and Williams LTD, Chad Well Health, Essex, England) according to the method described by (Vogt, 1969). The globulin content was determined by the method described by Henry (1974) using Beckman DU7400 spectrophotometer. The concentration was adjusted to be 18mg/ml in normal saline.

Conjugation of anti-*Pasteurella multocida* globulin with horse radish peroxidase. The obtained globulins were labeled with horse raddish peroxidase (Product No.P-8375 type VI, lot number 25C-9510, Sigma Chemical Com.) as described by Tijssen and Kurstak (1984) as

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Table (1): Absorbance values of the double antibody sandwich ELISA.

Tested serum	Dilution of the prepared conjugate			
	Undiluted	1:10	1:100	1:1000
Positive PM type 5-A antiserum	0.414	0.181	0.092	0.076
Positive PM type 8-A antiserum	0.419	0.250	0.110	0.090
Positive PM type 9-A antiserum	0.438	0.244	0.125	0.098

The absorbance values were read at 450nm wave length.
The limit of positive result is 0.0988.

follow: 5mg of horse radish peroxidase (HRP) were dissolved in 1ml double distilled water followed by addition of 0.4ml of freshly prepared sodium periodate solution (Win Lab laboratory reagents for fine chemicals) with gentle steering for 20 minutes at room temperature till a greenish color was obtained. The pH was adjusted to be 9.5 by adding 0.25ml of 0.2M $NaCO_3$. 18mg of *Pasteurella multocida* immunoglobulin in 1ml normal saline were added to such solution and placed on a shaker for 2 hours at room temperature. 0.1ml of 4mg/ml of sodium borohydrate (SD Fine Chemical LTD Scientific Company, Division Fair Lawn Jersey, USA) was added and the reaction was allowed to continue for 2 hours at 4^{o}C.The prepared conjugate was dialyzed against phosphate buffer solution and an equal volume of glycerol was added to it then stored at -20^{o}C till used.

Check board ELISA. It was done for titration of the prepared immuneperoxidase conjugated *Pasteurella multocida* immunoglobulin to detect the optimal antigen and antibody dilutions for plate coating according to (Rose *et al*.,1986).

Enzyme Linked Immunosorbent Assay (ELISA). Double antibody sandwich ELISA was performed according to Voller *et al*. (1982) It was applied for detection and estimation of *Pasteurella multocida* antigen. Imported anti-*Pasteurella multocida* horse radish peroxidase conjugate **(**Sigma Company, USA.) was used as positive control.

Positive sera against *Pasteurella multocida* type 5-A, 8-A and 9-A. These antisera were supplied by the Central Laboratory for Quality Control of Veterinary Biologics, Abbassia, Cairo.

Results and Discussion

Pasteurellosis or fowl cholera is a serious respiratory disease representing a problem facing poultry industry in dramatic forms in Egypt (Gergis, 1978).

Diagnosis of fowl cholera depends mainly on the symptoms; postmortem and histo-pathological findings (Al-Haddawi *et al*., 2000). More recent techniques were arise for detection of *Pasteurella multocida* antigen or antibodies as shown by Borkosa-Opacka *et al.* (1997) who used ELISA to measure the level of specific IgG to *Pasteurella multocida* in the sera of birds vaccinated against pasteurellosis. From this point of view a local ELISA kit saving time and cost, should be available.

The obtained results showed that the prepared *Pasteurella multocida* hyperimmune serum showed titer of 1114.

Accurate precipitation of immune globulin in such preparation revealed that its concentration was 12.5mg/dl which adjusted to be 18mg/ml as recommended by Tijssen and Kurstak (1984) for further conjugation with horse radish peroxidase. Periodate method for conjugation was used where there is no interference between the enzymatic activity of the serum and the periodate (Nakane and Kawaoi 1974;Tijssen and Kurstak, 1984; Zeidan *et al*., 2000).

It was found that the optimal dilution of both of the imported and prepared *Pasteurella multocida* immune globulins conjugated with horse radish peroxidase was 1:100.

Depending on the obtained results *Pasteurella multocida* immunoglobulin conjugated with horse radish peroxidase is available and could be used for production of local ELISA kit that

having the advantage of reduced cost with retaining sufficient sensitivity.

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تحضير وتقييم كيت خاصة لقياس الأجسام المناعية لميكروب الباستريلا مالتوسيدا

تم خلال العمل الحالى تحضير مصل عالى العيارية ضد البستريللا ملتوسيدا من النوع ٥-أ، ٨-أ، ٩-أ فى أرانب بوسكات وكان معيار هذا المصل طبقا لاختبار التلزن الدموى الغير مباشر هو ١١١٤ ٠ وقد تم ترسيب الجلوبيولين المناعى فى المصل المحضر باستخدام محلول سلفات الأمونيوم المشبع وتم ضبط تركيزه ليكون ١٨ مليجرام/مل من محلول الملح الفسيولوجى ثم تم إقرانه بإنزيم الهورس رادش٠ ولتقييم هذا المقترن تم إجراء اختبار ساندويتش الإليزا على عينات أمصال إيجابية للعترات المذكورة٠ وقد أعطى المقترن المحضر نتائج إيجابية حتى تخفيف ١ :١٠٠ مثل المقترن المستورد الأمر الذى يمكن معه القول بأن كيت محمل بالهورس رادش بيروكسيديز أصبح فى المتناول للكشف عن الأجسام المناعية للبستريللا مالتوسيدا من الأنواع ٥-أ، ٨-أ، ٩-أ على المستوى المحلى لا يقل فى جودته عن مثيله المستورد موفرا بذلك الوقت اللازم للاستيراد والثمن المرتفع٠