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Comparative studies on anti-avian sera conjugated with fluorescin isothiocyanate

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Rabbit antisera were successfully prepared against chickens; turkey; ducks; geese; pigeons and quails as antispecies and conjugated with fluorescein isothiocyanate (FITC). The ability to use these antisera as homologous and heterologous antispecies was studied through the application of indirect fluorescent antibody technique (FAT) using specific antigens and antibodies of Newcastle; infectious bursal disease virus; duck plague virus; duck hepatitis virus; fowl cholera and pigeon paramyxo virus. The results showed that Titration of the prepared anti avian sera conjugated with FITC induced strong positive FAT reactions up to dilutions of 1:10000; 1:1000; 1: 100000; 1: 1000; 1: 100000 and 1:1000 for anti- chicken; anti-Turkey; ant-duck; ant-goose; anti-pigeon and anti-quill sera respectively. It was found that homolgous species anti-sera showed strong positive FAT reaction (green apple fluorescing) scored as 4+ while hetrologous species anti-sera showed less degree of positive reactions ranged from 3+ to ± reaction showing that the usefulness of anti-sera as reagents in serological techniques is limited by the homogeneity and heterogeneity of such anti-sera. So, the present preparations could be used to low extent as heterologous anti-avian sera.

Specific antisera to individual immunoglobulin classes are valuable reagents in many immunological techniques such as indirect immunofluorescent technique (Kincade and Cooper, 1971-1973; Lawrence *et al.*, 1979). Specific, rapid and sensitive serological tests like fluorescent antibody technique (FAT) are required for accurate diagnosis of infectious agents to reach to well applicable control measures. FAT depends mainly on antigen-antibody reaction in the presence of a fluorescent dye (fluorescein isothiocyanate "FITC") which irradiates with ultraviolet light emitting apple green fluorescence (Tizard, 1996). The technique depends on the presence of specific antiserum conjugated with FITC. FAT has gained wide acceptance in virology serving for many purposes such as detection of cellular localization of viral antigens (Watson and Coons, 1954; Spendlove *et al.*, 1963); establishment of temporal sequence of antigens after infection appearance (Breitenfeld and Schafer, 1957); and detection of virus infected cells which fail to produce viral progeny and still synthesize virus coded antigens which continue to harbor viral genetic material (Pope and Rowe, 1964; Tevethia *et al.*, 1965).

The degree of specificity of the antiserum may have a considerable influence on the results. Commercially available antisera for use in avian serological assays are generally lacking in specificity (Nadaphan *et al.*, 1982). In addition, the use of heterogenous antibodies provides less potential for the immobilization to impact the antigen binding efficiency (Tumpey *et al.*, 2005).

The present work was designed to prepare and compare between different specific anti-avian sera (chicken; turkey; ducks; geese; pigeons and quails) conjugated with flourescin isothiocyanate concerning their specificity and the ability to use any of them instead of the other as heterologous agent in the absence of the homologous one.

Materials and methods

Birds. Five 2 months old of each of chickens; turkey; ducks; geese; pigeons and quails were used to collect blood and sera to be used for preparation of anti-species sera in rabbits. All birds were clinically healthy and free from Newcastle; infectious bursa disease; duck plague; duck hepatitis; fowl cholera and pigeon paramyxo antibodies and housed under hygienic measures in separated isolates receiving balanced ration and adequate water.

Rabbits. Thirty five healthy Bosket rabbits of average body weight 3 kg were used for preparation of different anti-avian sera whereas each antiserum was prepared in five rabbits leaving five rabbits as control. Each rabbit group was housed separately in hygienic isolates.

Preparation of species anti-sera. It was carried

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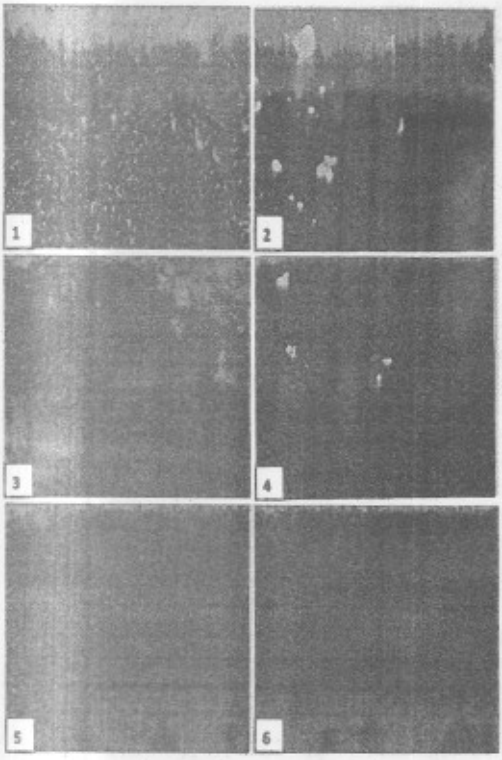

Fig. (1-6): 4+; 3+; 2+; +; ± and –ve FAT are shown in fig. 1,2,3,4,5 and 6 respectively

out following the procedure described by (Deutsch, 1967).

Precipitation of the immunoglobulin of the prepared antisera. It was carried out using ammonium sulphate according to (Narin and Marrack,1964). The globulin content was estimated according to (Weichselbaum, 1946) and adjusted to be 20mg/ml using phosphate buffer saline of pH 7.2.

Chemicals used for conjugation of the prepared species antisera with Fluorescein isothiocyanate (C_4 H_{11} NO_5 S) F. (C_4 H_{11} NO_5 S) E was supplied by Merck, Darmstadt for Microscopy (M.Gew.389.39).

Conjugation of the prepared species antisera with Fluorescein isothiocyanate. It was done according to the method of (Narin, 1969).

Antigens and antibodies. Newcastle; infectious bursa disease; duck plague; duck hepatitis; fowl cholera and pigeon paramyxo antigens and antibodies were supplied by the Central Laboratory for Quality Control of Veterinary Biologics, Abassia; Cairo.

Refernce anti-chicken conjugate. Fluorescein-labeled affinity purified antibody to chicken IgG (H+L), produced in goat, catalog No.02-24-06 was supplied by KPL Europe. It was used as a reference conjugate to evaluate the prepared anti-avian sera conjugated with FITC. It was used at a dilution of 1:100 according to the manufacturer directions.

Indirect fluorescent antibody technique (FAT). It was carried out on Newcastle; infectious bursa disease; duck plague; duck hepatitis; fowl cholera and pigeon paramyxo antigens and antibodies using the prepared species antisera conjugated with fluorescein isothiocyanate in homologous and heterologous manners. The technique was carried out according to (Soliman *et al.*, 1989).

Results and Discussion

Usually and especially in recent works, there is an increased demand to obtain rapid, sensitive and accurate diagnosis of infectious diseases especially those occur in the form of outbreaks. One of the most reliable techniques is the fluorescent antibody technique (FAT) which provides such requirements. Indirect FAT depends mainly on the presence of specific anti-specie serum conjugated with fluorescein isothiocyante (Brian and Hillor, 1996; Hala *et al.*, ,2003; Hanan *et al.*, 2003; Noura *et al.*, 2009).

The present work was mainly directed to prepare specific antisera against some avian species conjugated with FITC to investigate to any extent such antisera could be used instead of each other.

All prepared antisera showed higher levels of total protein level than in normal rabbit sera due to the increase in globulin levels form which species antibodies are formed (Table-1). These results are in accordance with those of Kataria and Sharma, (1993); Hala *et al.*, (2003); Hanan *et al.*, (2003).

Concerning titration of the prepared anti avian sera conjugated with FITC (Table-2), it was found that these preparations induced strong positive FAT reactions up to dilutions of 1:10000; 1:1000; 1: 100000; 1: 1000; 1: 100000 and 1:1000 for anti- chicken; anti-Turkey; ant-duck; ant-goose; anti-pigeon and anti-quill sera respectively. These values appear to be higher than that of the imported references anti-chicken conjugate which induced positive reaction only up to a dilution of 1:100 which was prepared according to the manufacturer directions. So, the present preparation could be considered preferable specific products than the non specific imported one whereas they provide larger amounts of the conjugate (when diluted on use)

Table (1): Serum proteins in the prepared rabbit anti-avian sera.

Tested antiserum	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Anti-chicken	5.57	2.35	3.22
Anti-Turkey	5.62	2.04	3.58
Anti-duck	5.79	2.37	3.42
Anti-goose	5.40	2.03	3.37
Anti-pigeon	5.12	2.25	2.87
Anti-quill	5.45	1.85	3.60
Normal rabbit serum	4.26	2.03	2.23

Table (2): Titer of FITC conjugated anti-avian sera.

Conjugate dilution	Tested conjugated antisera					
	Anti-chicken	Anti-Turkey	Anti-duck	Anti-goose	Anti-pigeon	Anti-quill
Undiluted	+	+	+	+	+	+
1:10	+	+	+	+	+	+
1:100	+	+	+	+	+	+
1:1000	+	+	+	+	+	+
1:10000	+	-	+	-	+	-
1:100000	-	-	+	-	+	-
1:1000000	-	-	-	-	-	-

NB: The reference antichicken conjugate was diluted as 1:100 according to the manufacturer directions.

Table (3): Positively reacted FITC conjugated homologous and heterologous anti-avian sera.

Tested anti-sera	Indirect FAT using					
	Chicken serum	Turkey serum	Duck serum	Goose serum	Pigeon serum	Quail serum
Anti-chicken	4+	3+	2+	2±	2+	+
Anti-Turkey	3±	4+	+	±	+	-
Anti-duck	3+	2+	4+	4±	±	-
Anti-goose	2+	+	4+	4+	±2	±
Anti-pigeon	3±	2±	+	2±	4+	3+
Anti-quill	2±	+	±	+	4±	4+

saving cost. Similar findings were recorded by (Hala *et al.*, 2003; Hanan *et al.*, 2003; Abd El-Wanis and Khodeir, 2004; Hussein *et al.*, 2006; Noura *et al.*, 2009) who prepared different local antisera conjugated with FITC of high quality saving time and cost.

Regarding the use of homologous and heterologous species anti-sera; it was found that homolgous species antisera showed strong positive FAT reaction (green apple fluorescence) scored as 4+ while hetrologous species anti-sera showed less degree of positive reactions ranged from 3+ to ± reaction as demonstrated in table (3). Such scores of positive FAT reactions were demonstrated in (Fig. 1-6). These findings confirm previous records of (MRC Working Shop, 1971; Hala *et al.*, 2003; Hanan *et al.*, 2003) showing that the usefulness of anti-sera as reagents in serological techniques is limited by the homogeneity and heterogeneity of such antisera.

It could be concluded that the prepared anti-avian sera conjugated with FITC are of good quality and could be used for detection of Newcastle; infectious bursa disease; duck plague; duck hepatitis; fowl cholera and pigeon paramyxo antigens and antibodies with high specificity and sensitivity saving the long time and high cost required for importation of such conjugates. In addition, the present preparations could be used to low extent as heterologous anti-avian sera.

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دراسات مقارنة على مضادات أمصال الطيور المحملة بالفلوريسين أيزوثيوسينات

تم خلال هذا العمل تحضير أمصال مضادة لأجناس الدجاج والرومى والبط والأوز والحمام والسمان ثم تم ترسيب الجلوبيولين منها وإقرانه بمادة الفلوريسين أيزوثيوسيانات. وقد تمت معايرة هذه المقترنات بإجراء اختبار الوميض الفلوريسنتى الغير مباشر على انتيجينات النيوكاسل والجمبورو والإلتهاب الكبدى الوبائى فى البط وطاعون البط وبارامكسو الحمام باستخدام أجسام مناعية مضادة لكل من هذه الأنتيجينات محضرة نوعيا فى طيور محصنة وذلك مقارنة بمقترن مرجعى مضاد للدجاج ١:١٠٠٠٠، ١:١٠٠٠٠، ١:١٠٠٠٠، ١:١٠٠٠٠، ١:١٠٠٠٠، ١:١٠٠٠٠ لمضادات الدجاج والرومى والبط والأوز والحمام والسمان على التوالى. وعند إجراء اختبار الوميض الفلوريسنتى الغير مباشر باستخدام المقترنات المحضرة نوعيا وتبادليا وجد أن استخدام النقترن النوعى يعطى تفاعل قوى (++) بينما الاستخدام التبادلى لمثل هذه المقترنات يعطى تفاعلاً أقل (+٣ إلى ±) ،وعلى ذلك يمكن القول بأنه يمكن استخدام أمصال الطيور المختلفة المقترنة بالفلوريسين أيزوثيوسينات فى أختبار الوميض الفلوريسنتى المناعى الغير مباشر تبادلياً ولكن بصورة محدودة والأفضل أن يستخدم مضاد جنس الطائر نوعياً للحصول على نتائج أفضل وأكثر دقة.