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## **EFFICACY OF SEROLOGICAL AND ALLERGIC DIAGNOSIS OF *A.fumigatus* INFECTION IN CHICKENS**

*By*

**A.A. BASSIYONI, F.E. SAAD, M. RAFAI & A.M. EL BATRAWI**

### **SUMMARY**

Counter immunoelectrophoresis and intradermal allergic tests provide new rapid means of diagnosing *A. fumigatus* infection in chickens. Precipitins and allergic reactions could be detected as early as the clinical symptoms of the disease began to appear. It was found that a strong relationship was noticed between the presence of pathological lesions in the lung and the detection of precipitins in the sera and between the re-isolation of *A.fumigatus* from the respiratory organs and the allergic skin reaction.

### **INTRODUCTION**

In recent years fungal diseases in poultry have been found to be of much more importance than was expected. The diagnosis of such infections has been based only on mycological examinations, which are laborious and need much time and experience. Moreover, it is a real problem to determine if the isolated fungi are commensal or pathogenic colonists.

Serological tests, as a recent trend in the diagnosis of mycotic diseases, have made significant advances in the evaluation of various forms of aspergillosis in humans, but have not been used for the diagnosing aspergillosis in birds (Beneke, 1975).

This study was planned to evaluate the serological and allergic methods as rapid and sensitive methods in diagnosing aspergillosis in chickens.

### **MATERIAL AND METHODS**

#### **Media:**

Sabouraud's glucose agar (Cruickshank *et al.*, 1975) was used for isolation of fungi.

Czapek's solution agar (3%) (Raper and Fennell, 1977) was used for identification of *Aspergillus*, and Czapek's solution medium (40%) was used to grow *A.fumigatus* for antigen preparation.

Medium and buffer for counter immunoelectrophoresis test (CIE) were made according to Corkill (1977).

Faculty of Vet. Medicine-Cairo University

**Aspergillus fumigatus strain:**

A strain of *A.fumigatus* (resenius) isolated from lung nodules in a 2 month-old chicken was used in experimental infection and preparation of antigens for CIE and allergic skin tests.

**Experimental birds:**

One hundred and fifty, one-day old Fayoumi chicks, one hundred (2-week-old) Fayoumi chicks and twenty four, (5-month old) Nicols chickens were used. Five (5-month old) Nicols chickens were additionally used for the production of *A.fumigatus* hyperimmune serum.

**Preparation of spore suspension:**

The spores of well-sporulated *A.fumigatus* culture were washed off with physiological saline to which a few drops of tween 80 were added to ensure a homogenous suspension. The doses were estimated by counting the spores using the haemocytometer.

**Preparation of A.fumigatus antigens:**

Acetone precipitated, x8 concentrated culture filtrate of 5-week-old *A.fumigatus* culture (Palmer et al., 1977) was used in CIE.

**Preparation of A.fumigatus hyperimmune serum in chicken:**

This Five, (5-month-old) Nicols chickens were used for the production of *A.fumigatus* hyperimmune serum according to the schedule shown in Table (1). The birds were bled 2 weeks after the last injection and the serum was preserved with sodium azide in a final concentration of 0.1%.

**Mycological examination:**

Tracheal swabs were streaked on the surface of Sabouraud's glucose agar plates. Tissue specimens were cultured on Sabouraud's agar plates by inoculating the media with small pieces of the tissue. The plates were incubated at 37°C for 3 to 8 days.

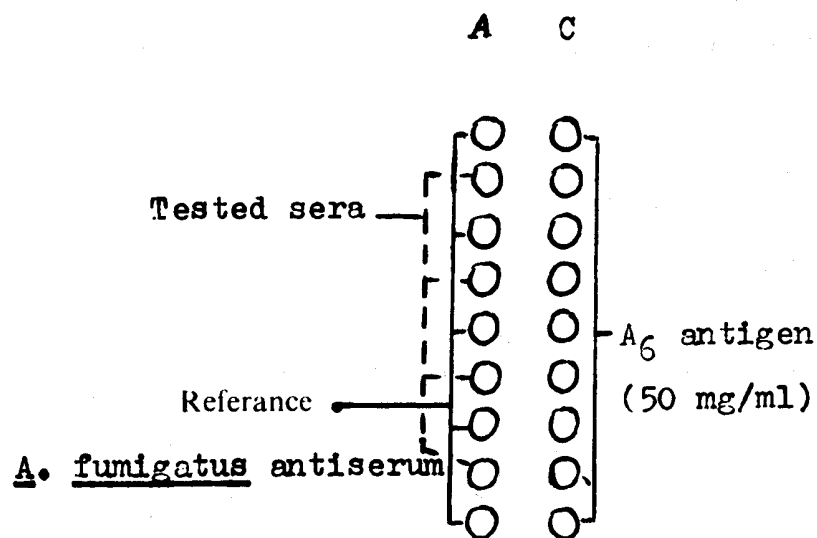
Colonies suspected to be *A.fumigatus* were cultured on Czapek's solution agar (3%) plates and identified according to Raper and Fennell (1977).

**Serological test:**

CIE (Corkill, 1977) was used for the detection of *A.fumigatus* precipitins in the sera of experimentally infected birds in the pattern showed in Figure 1.

(Table 1) Preparation of anti-*A.fumigatus* hyper-immune serum in chickens.

Injection No.	Interval between the next injection	Route	Dose	Inoculum
1	3 days	subcutaneous	$10 \times 10^6$	<i>A. fumigatus</i> spores
2	3 days	subcutaneous	$10 \times 10^6$	
3	3 days	subcutaneous	$20 \times 10^6$	
4	1 week	subcutaneous	$20 \times 10^6$	
5	1 week	subcutaneous	$40 \times 10^6$	
6	----	subcutaneous	$40 \times 10^6$	



(Fig 1) CIE for routine testing.

**Allergic skin tests:**

The test was made by inoculation of 0.1 ml. of the antigen in the wing web of baby chicks, or in the wattles of adult chickens. The results were read 1 hour after testing, where congestion in wing web or swelling in the wattles were interpreted as positive.

**Experimental design:**

One hundred and fifty one-day-old chicks (group I) were divided into 3 equal subgroups (A, B and C). Those of subgroups A and B were infected intranasally with  $4 \times 10^6$  *A.fumigatus* spores. Birds of subgroup B received, 2 weeks later, a second similar dose intranasally. Those of subgroup C were kept as control.

One hundred 2-week-old chicks (group II) were divided into 2 equal subgroups (A and B). Birds of subgroup A were infected intranasally with  $4 \times 10^6$  spores of *A.fumigatus*, while those of subgroup B were kept as control.

Twenty four, 5-week-old chickens (group III) were divided into 4 equal groups (A, B, C and D). Birds of subgroups A, B and C were infected, with  $4 \times 10^7$  spores of *A.fumigatus*, intratracheally, via abdominal air sac and subcutaneously respectively. Birds of subgroup D were kept as control.

All birds in groups I and II were kept under observation for a period of 6 weeks during which any clinical symptoms were recorded and 3 chicks were taken from each subgroup at intervals of 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38 and 42 days after infection and subjected to skin testing and blood was individually collected, for CIE, before being sacrificed for post mortem and mycological examinations.

Birds of group III were kept under observation for a period of 8 weeks where individual tracheal swabs, for mycological examination, and blood samples, for CIE, were taken every week. For allergic testing, 3 birds from each subgroup were alternatively tested, with the other 3 birds, each 2 weeks.

**RESULTS**

In group I, birds of subgroups A and B showed symptoms of depression, ruffled feathers, nasal discharge, sneezing and gasping on the 10th day after infection. Five and seven birds died during the experiment in subgroups A and

**B** respectively. On post mortem examination the lesions were confined only to the respiratory organs, in the form of catarrhal exudate in respiratory passages and congestion of the lungs on the 3rd day after infection. By the 7th day after infection, the exudate became purulent, the lungs were pneumonic and the air sacs were turbid. Yellowish white hard nodules of various sizes were observed in the lungs and air sacs on the 10th and 14th days after infection. On the 17th and 21st days after infection the lungs of birds of subgroup A were congested or pneumonic, while those of subgroup B had greyish white fleshy nodules about 1 cm in diameter. From the 24th day after infection and later on, the lung appeared normal. Birds of subgroup C appeared normal throughout the observation period. The results of skin test, CIE and reisolation of **A.fumigatus** from the lungs are shown in Table (2).

In group II, some birds of subgroup A showed nasal discharge and frequent shaking of the head on the 10th day after infection. Three birds died during the experiment. On post mortem examination, there were purulent respiratory exudate and congestion or pneumonia in the lung on the 7th day and up to the 24th day after infection, however, the lungs of a bird died on the 15th. day as well as of 2 sacrificed birds on the 17th and 21st days after infection revealed yellowish-white hard nodules. Birds of subgroup B were apparently normal throughout the observation period. The results of skin test, CIE and reisolation of **A.fumigatus** from the lungs are shown in Table 3.

In group III, birds of subgroups A and B were apparently normal throughout the observation period. In birds of subgroup B, sneezing, coughing and gasping were observed on the 3rd week after infection, which became milder on the 5th week and began to subside on the 6th week after infection. One bird died on the 26th day after infection. On post mortem examination, the dead bird and four of the five birds, sacrificed at the end of the experiment revealed purulent exudate in trachea, congestion or haemorrhages on the lungs and cheesy material in the inoculated air sac from which **A.fumigatus** could be reisolated. The fifth bird was apparently normal. Birds of subgroup C showed subcutaneous swellings about 3-4 cm in diameter on the 2nd week after infection from which a yellowish transparent gelatinous fluid could be aspirated and yielded **A.fumigatus** when cultivated. By the 3rd week after infection, the swellings became hard and shrank and tended to disappear on the 7th week after infection. One bird which died on the 19th day after infection showed on post-mortem examination greenish yellow caseated material at the site of inoculation which was loosely attached to the

(Table 2) Results of skin test, counter immunoelectrophoresis, and post-mortem and mycological examinations in one-day-old chicks.

days after infection	3	7	10	14	17	21	24	28	31	35	38	42
Group No. 1	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
ST	-	-	+	+	+	+	+	+	+	+	+	+
CIE	-	-	+	+	+	+	+	+	+	+	+	+
L	c	n	p	p	p	c	c	c	c	c	c	c
R	+	+	+	+	+	+	+	+	+	+	+	+
Group No. 2	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
ST	-	-	-	-	-	-	-	-	-	-	-	-
CIE	-	-	+	+	+	+	+	+	+	+	+	+
L	c	c	c	c	c	c	c	c	c	c	c	c
R	+	+	+	+	+	+	+	+	+	+	+	+
Group No. 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
ST	-	-	-	-	-	-	-	-	-	-	-	-
CIL	-	-	-	-	-	-	-	-	-	-	-	-
L	n	n	n	n	n	n	n	n	n	n	n	n
R	-	-	-	-	-	-	-	-	-	-	-	-

ST: Skin test CIE: Counter immunoelectrophoresis  
 L : Lung lesion (n: normal C: congestion P: pneumonia N: the characteristic nodules. F.N.: Flesh nodules). R: Re-isolation of *A.fumigatus*.

(Table 3) Results of skin test, counter immunoelectrophoresis and post-mortem and mycological examination in 14-day-old chicks.

days after infection	3	7	10	14	17	21	24	28	31	35	38	42
group chick No.	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3
ST	- - -	- - -	- + -	+ + +	++	+ - +	+ - +	- - -	- - -	- - -	- - -	- - -
CIE	- - -	- - -	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	- - -	- - -	- - -	- - -
L	n n n	n c c	c o n	p c c	n p n	n n c	p p n	n n n	n n n	n n n	n n n	n n n
R	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	- - -	- - -	- - -	- - -	- - -
ST	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
CIE	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
L	n n n	n n n	n n n	n n n	n n n	n n n	n n n	n n n	n n n	n n n	n n n	n n n
R	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -

ST: Skin test CIE: Counter immunoelectrophoresis  
 L: Lung lesion (n: normal C: congestion P: pneumonia N: nodules) R: Re-isolation of *A. fumigatus*.

surrounding tissue and **A.fumigatus** could be re-isolated from it and not from any other organs. In addition to the subcutaneous lesion, the dead bird revealed congestion and marked enlargement of the liver and spleen. The survived birds, when sacrificed at the end of the observation period revealed yellowish white nodules at the site of inoculation which were firmly attached to the surrounding tissue and from which **A.fumigatus** could be reisolated but not from other internal organs. The results of skin test, CIE and isolation of **A.fumigatus** from tracheal swabs are shown in Table (4).

### DISCUSSION

In this investigation, the authors turned their attention to the efficacy of serological and allergic tests in early diagnosis of **A.fumigatus** infection in chickens. It was known that one-day old chicks were highly susceptible to **A.fumigatus** infection, while 2 week old chicks were generally resistant (Klimês and Rosa, 1964 and Faubleé and Boller, 1975). Moreover, the infection is sporadic and of chronic form in adult birds (Chute, 1978). Therefore it was beneficial to study the efficacy of serological and allergic tests in diagnosing aspergillosis in one-day, 2-week and 5-month old chickens.

The infective dose for young chicks was estimated to be  $4 \times 10^6$  spores, however, in order to establish the infection in adult birds a tenfold increase of the dose ( $4 \times 10^7$ ) was chosen. Furthermore, different routes of infection of adult chickens were tried.

In this study, one-day as well as 2-week-old chicks showed the clinical symptoms of the disease in the 10th day after infection and all of the infected groups had birds with the characteristic lung nodules. However, in one day old chicks which received double infection, some birds had greyish white fleshy nodules in the lungs which resembled those reported by Savage and Isa (1933). The presence of such uncommon nodules can be attributed to the variation in the defense reactions to **A.fumigatus** following the first infection, where the defense reaction in young birds to *Aspergillus* was only of oesinophilic granulocytes, while the histocytes and giant cells proliferation did not develop until few days after infection (Kardevan, 1967).

The intratracheally infected birds were apparently normal throughout the observation period, while those infected in the abdominal air sac or subcutaneously contracted the disease. These findings are in agreement with Gauger (1941) and Battelli (1944) who could not observe symptoms in fowls



(Table 4) Results of skin test, counter immunoelectrophoresis and mycological examination in 5-day-old chickens.

Weeks after infection	1	2	3	4	5	6	7	8
Group No. 1 Chick. No. 1-6	1	2	3	4	5	6	7	8
	2	3	4	5	6	1	2	3
	3	4	5	6	1	2	3	4
	4	5	6	1	2	3	4	5
	5	6	1	2	3	4	5	6
	6	1	2	3	4	5	6	1
Group No. 2	1	2	3	4	5	6	7	8
	2	3	4	5	6	1	2	3
	3	4	5	6	1	2	3	4
	4	5	6	1	2	3	4	5
	5	6	1	2	3	4	5	6
	6	1	2	3	4	5	6	1
Group No. 3	1	2	3	4	5	6	7	8
	2	3	4	5	6	1	2	3
	3	4	5	6	1	2	3	4
	4	5	6	1	2	3	4	5
	5	6	1	2	3	4	5	6
	6	1	2	3	4	5	6	1
Group No. 4	1	2	3	4	5	6	7	8
	2	3	4	5	6	1	2	3
	3	4	5	6	1	2	3	4
	4	5	6	1	2	3	4	5
	5	6	1	2	3	4	5	6
	6	1	2	3	4	5	6	1

ST: Skin test CIE: Counter immunoelectrophoresis  
 R: Re-isolation of *A. fumigatus* from tracheal swabs.  
 D: Chickens died during the experiment.

infected intranasally or intratracheally. On the other hand, Chute et al. (1962) found that air sac inoculation was the most suitable route for screening the pathogenicity of fungi, while, Bory (1937), Chute et al. (1955) and Barbesier and Samsó (1961) reported *A.fumigatus* skin infection following skin injury.

It is of epidemiological importance to mention here that in chickens infected via the abdominal air sac, although the respiratory symptoms subsided on the 5th week after infection, however, still in the air sacs active lesions that yielded *A.fumigatus* cultures. This may question whether a chronic form of aspergillous may follow an acute one as reported in ducks by Dvorkin (1970) or there is an inapparent infection or a carrier state in *A.fumigatus* infection in chickens.

In this work skin testing of adult birds provided an indication that the period between each testing of the same bird was 14 weeks. This will ensure that the tested wattle would be desensitized before the application of the second test as it had been already shown that the half-life of persistence of reagins in human skin was of the order of 13 days (Augustin et al., 1966).

In the present studies, it seemed that a relationship between the presence of *Aspergillus* lesions, whatever their nature, and the presence of precipitins in the sera do existed. This observation was reported also by Taylor and Burroughs (1973) in *A.fumigatus* infected chicks and reported in humans by Longbottom and Pepys (1975) who found that precipitins rapidly became weaker or even disappeared in these patients from which aspergillomas were removed. On the other hand, a correlation between the isolation of *A.fumigatus* and allergic skin reactivity was observed.

Both the CIE and the allergic skin tests were found to be rapid in diagnosing *A.fumigatus* infection in chickens as early as the clinical symptoms of the disease just appeared. Field applications of these tests are needed before establishing any testing programmes.

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