Diagnostic & immunological study on *Aspergillus fumigatus* of camels in Thi-Qar province

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Abstract

Fungi have an important role as an etiological factor in respiratory tract disorders in farm animals. In camels, this study was conducted to discover the immune status against *Aspergillus fumigatus* ; the most common fungi in the world. 90 camels were inspected and the blood was sampled from herds in Thi Qar governorate. Camels ranked in different ages, 1-10 years, and both male and female were included. The study dialed with camels suffering respiratory disorder. Serum was examined by passive hemagglutination test using the antigen of *Aspergillus fumigatus* isolated and prepared by (1). 16 camels were positive which present 17.8% , this result informs an important role of *Aspergillus fumigatus* as a cause of respiratory tract disorder in Iraqi camels.

Introduction

The genus *Aspergillus* is extraordinary as exemplified by the diversity of its disease manifestations, almost all termed "aspergillosis". All forms of aspergillosis are sapronoses, that is transmissible form an abiotic environment, and not communicable from person to person, or zoonosis (2). Genus *Aspergillus* is a wide spread fungi around the world. Among its types; *A. fumigatus* is the most pathogenic type causing diseases in human & animals. *A. fumigatus* is an opportunistic fungus affecting respiratory system (3). Elevated pathogenic manifestations of *A. fumigatus* belong to several factors; it's ability to growth faster than other types in wide range of temperature (20-50) C^{o}, *A. fumigatus* is highly sporelating fungus for which it has found in about (1-100) spore/m^{3} in the atmosphere (4). Besides, spore ability to combine using fibrinogen & laminine which facilitate the spore adhesion to the

epithelium of respiratory tract (5). Other factor is that *A. fumigatus* secrets the complement inhibitor (6), seven polypeptide allergens responsible for cases of asthma & inflammation of the mucous membranes. Other secretions have reported are β-1-3 glucan which is an immunostimmulant (7), protease & elastease. It's thought that protease is responsible of the pathogenecity of the fungus in the lung tissue when an infection had happened (3).

A. fumigatus is a zoonotic fungus (5), In animals it causes bronchitis when the spores have been inspired or ingested with contaminated diet, the case may develop to abortion in pregnant females (8). Consequently, regarding to the economic losses in animal husbendary caused by this type of pathogen & its risk on human health. This study merged as the first in Iraq & camels were the target animal because they present alarge part of the animal population in our country.

Materials & methods

1- Solutions:

- Tannic acid solution 1:20000

2- The fungus *Aspergillus fumigatus*: diagnosed & isolated by (1) from human suffering respiratory disorders. Antigen (Aspergillin) was extracted according to (9). Mycelium antigen was used in this study.

3- Blood samples: 90 samples of 5ml whole blood were collected from the jugular vein collected from camels at different sexes & ages. The camels show signs of bronchitis, mucopurulent discharge, inappetite, fever, pulmonary consolidation, on auscultation; there were rales; & cough is a frequent symptom. Using the centrifuge; 3000rpm for 10 minutes to separate the serum. The complement was inhibited by putting the serum in 56 Cº water bath for 30 minutes & left to cool & then frozen up to use.

4- Sheep blood: suspending the antigen on the red blood cells as an essential part of the passive hemagglutination test.

5- Passive hemagglutination test used to measure the titer of antibody in the serum sampled from the suspected camels. In this test; we need sheep blood to sensitize the antigen on the red blood cells (RBCs). According to Boyden (1951) RBCs were separated & washed. The 2nd step was to use tannic acid to prepare Tanned RBCs (TRBCs). The best titration of the aspergillin was found 1:8 which used to sensitize the antigen on the TRBCs to become sensitized RBCs (SRBCs). 0.02ml of 1% natural serum of the suspected camels were poured in each small pits of microtiter plates. These pits were diluted according to two fold serial dilution way, next step was to add 0.02 ml of SRBCs to each pits & then the plate was incubated in 37 C°. Primary results was read after 2 hr while the final results to be read after 24 hr.

Results

Passive hemagglutination test has resulted positive immune response in different levels. The largest titer presented in 1:32 in 7 camels, 1:16 in 7 camels & one camel positive at titer of 1:8 & 1:4as in table (1). These results prove that these camels have infected with respiratory aspergillosis in 17.8. The rest samples didn't show any response which means they are not infected. 12 females & 4 males were positive to the serological test, passive hemagglutination test, as illustrated in table (2). The calf camels in the first year old has the highest percentige (37.5%). Four calves aging 2 years were infected, in the same time, camels aging 3,4,7 & 8 have reported one case apart. Finaly, 2 cases were diagnosed in camels eld 5 years.

Statistically; it was significant, according to (10), that there is an effect of age on the infection of aspergillosis as demonstrated above (Chi^2=26.7), but there was no effect of sex on this infection (Chi^2=0.06) because there were 12 positive females to 54 negative (18.2%) against 4 positive males to 20 negative (16.7).

Table (1) titeration of antibodies against *A. fumigatus* in camels

Titer	No. of positive camels	Percentage
1:4	1	1.1
1:8	1	1.1
1:16	7	7.8
1:32	7	7.8

Table (2) the effect of age & sex on infection of aspergillosis

Age	1 year		2 years		3 years		4 years		5 years		6 years		7 years		8 years	
Positive camels	F*	M*	F	M	F	M	F	M	F	M	F	M	F	M	F	M
	4	2	3	1	1	0	1	0	2	0	0	0	0	1	1	0
Tested camels	20		10		10		10		10		10		10		10	
Percentage	0.3		0.4		0.1		0.1		0.2		0.0		0.1		0.1	

***F: female**
***M: male**

Discussion

A. fumigatus is the only fungal agent thus far associated with systemic diseases in camels. The fungus was isolated from a five-year-old camel with chronic rhinitis, characterized by bilateral mucopurulent nasal discharges and mild inspiratory dyspnea. *A. fumigatus* was incriminated in the aetiology of an outbreak of bronchopneumonia and gastroenteritis that affected a large number of racing camels in the Emirates (3). *A.fumigatus* was isolated from samples of sputum from persons suffering respiratory disorders in order to gain a pathogenic type of *A.fumigatus* differs from that isolated from environment because that the last one don't contain the pathogenic gene responsible of the infection (4). The antigene used in this study has been used previously by Al-Ameed (2008) in immunizing the rabbits, the highest titer of antibody in their serum were measured 128-2048 & so it was used in this study to measure the antibody in suspected camels with infection of *A.fumigatus*. These results make this antigen as an indicator in diagnosis of this infection. Yaseen (2006) has

done a similar study on persons suffering respiratory disorders & found titers 8-256 using the same test & antigen.

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دراسة تشخيصية ومناعية على عفن الرشاشيات الدخناء في الإبل العراقية

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تلعب الفطريات دورا مهما كعامل مسبب لإصابات الجهاز التنفسي في حيوانات المزرعة، وقد جاءت هذه الدراسة للكشف عن الحالة المناعية ضد عفن الرشاشيات الدخناء الأكثر انتشارا في العالم. تم جمع عينات الدم من ٩٠ حيوان من الإبل العراقية في محافظة ذي قار. تراوحت أعمار الإبل المفحوصة دون سن العاشرة ومن كلا الجنسين والتي تعاني من اضطرابات تنفسية. ولغرض إجراء فحص التلازن الدموي غير المباشر تم عزل المصل من الدم باستخدام الطرد المركزي واعتمادا على المستضد المحضر من العفن الذي تم عزله من قبل العميد (٢٠٠٨). اظهر ١٦ حيوانا من الإبل المفحوصة نتيجة موجبة والتي تمثل نسبة ١٧,٨% وهذا ما يظهر الدور المهم لهذا العفن في الإصابات التنفسية في الإبل.