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A serologicalsurveillance of bluetongue disease in sheep and goats in Iraq by using acompetitive ELISA Technique

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Summary

In this study, a sero-survillance on ovine and caprine serum samples from all over Iraq except the governorates of Iraqi Kurdistan region was conducted. It aimed for the detection of specific antibodies directed against VP7 protein - the sero-group specific antigen of blue tongue virus – by using a competitive ELISA technique.The results showed that among a total of 3277 serum samples, 1436 samples were found to be positive forming 43.82% of total samples, while 1687 samples were negative with 51.48 % and 154 sample were suspicious forming 4.69 %. The highest positive serum samples were found in Al Basrah Province (southern of Iraq) with 60.41% and in Al Anbar Province (Western of Iraq) with 61%, while the lowest of positive samples were found in Nineva (Northern of Iraq) with 22.35%.
The results also showed a closely ratio between positive percentage of goats and sheep samples with the positive goats serum samples (in five provinces) were 45 of 114 samples representing 39.47 %,while the positive sheep serum samples (in fifteen provinces) were 1391 of 3163 samples representing43.97%. Our study is complementary to the previous works held in this field and it represents the first field serological survey for bluetonguein sheep and goats in Iraq.

التحري المصلي عن مرض اللسان الازرق في الاغنام والماعز في العراقباستخدام التقنية التنافسية لتفاعل الانزيم المناعي الممتز

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الخلاصة

تم في هذه الدراسة اجراء التحري المصلي على عينات مصل جمعت من الاغنام والماعز من جميع انحاء العراق بأستثناء محافظات اقليم كردستان العراق، حيث هدفت الدراسة الى تحديد وجود الاجسام المناعية المتخصصة والموجهة نحو البروتين VP7 والذي يمثل المحدد المستضدي الخاص بفايروس اللسان الازرق من خلال استخدام الطريقة التنافسية لاختبار الانزيم المناعي الممتز.اظهرت نتائج الفحوصاتوجود 1436عينة موجبة وبنسبة 43.82 % من اصل 3277 عينة،بينما كان عدد العينات السالبة للفحص هو 1687 عينة وبنسبة 51.48 % والعينات ذات المعيارالمناعيالمشكوك به هو 154عينة وبنسبة 4.69 % من مجموع العينات الكلي. ظهرت اعلى نسبة للعينات الموجبة في محافظةالبصرة جنوبي العراق حيث وصلت نسبتها الى 60.41% وكذلك في محافظة الانبار غربي العراق حيث كانت نسبتها 60.11 % ، بينما كانت اوطأ نسبة للعينات الموجبة في محافظة نينوى شمالي العراق 22.35 %.اشارت النتائج ايضاً الى تقارب نسب العينات الموجبة بين الماعز والاغنام ، حيث كان عدد العينات الموجبة للماعز (في خمسة محافظات) هو 45 عينة من مجموع 114 عينة ممثلاً مانسبته 39.47 % ، بينما كان عدد العينات الموجبة للاغنام (في خمسة عشرمحافظة) هو1391 عينة من مجموع 3163 عينة ممثلاً مانسبته 43.97 %. تعد هذه الدراسة مكملة للبحوث السابقة في هذا المجال كما انها تمثل اول مسح مصلي حقلي لمرض اللسان الازرق في الاغنام والماعز في العراق.

Introduction

Bluetongue is an economically important, non-contagious, vector-borne, viral disease of domestic and wild ruminants, including sheep, cattle, buffaloes, deer, goats and camelids,

caused by Orbivirus belonging to the family Reoviridae,transmitted by Culicoides biting midges,and the virus has 26 serotypesreported all over the world(3,14,16).
The severity of the disease varies among different susceptible ruminant species and breeds.The disease is considered as one of the most feared diseases in sheep-producing countries. Although sheep are most severely affected, cattle and buffaloes are the main mammalian reservoirs of BTV and are very important in the epidemiology of the disease (3).

Many records had indicated the presence of the disease in the whole middle-east region and in the Iraqi neighboring countries since 1951 involving both clinical and subclinical manifestations. Recently the disease was recorded in the region, and the disease was reported in Iran, Jordan, Oman, Saudi Arabia, Syria, Turkey ,Yemen and Israel (10,15).
The first serological evidence of the occurrence of the disease in Iraq was recorded during 1978 when a precipitating antibodies technique was used to detect specific antibodies to bluetongue virus in sheep and goat serum samples collected from animals slaughtered in Baghdad abattoir, and alsobluetongue specific antibodies was detected from clinical cases in Al-Ramadi and Al-Musaiyib districts during November 1975 and June 1976 from animals that shows clinical signs similar to the bluetongue (6,7).

Serological diagnosis of the disease can be done through three techniques as recommended by The World Organization for Animal Health (OIE) which are: Complement fixation test (CFT), Agar gel immunodiffusion (AGID) and Competitive Enzyme Linked Immunosorbent assay (c-ELISA) which was prescribed for international trade (11).
Many research results has declared that The competitive ELISA technique that detect antibodies directed against the VP7 core protein can be applied to all 26 BTV serotypes, and this technique is highly sensitive and specific.The specificity is gained by the use of the monoclonal anti-VP7, which is the protein that distinguishes the bluetongue serogroups from other Orbivirus serogroups, representing the preferred method to determine and monitor BTV circulation (1,2,3,4,5,13).

The diagnostic sensitivity and specificity of c-ELISAreach 87.8 % (85.1-91.1) and 98.2 % (96.3-99.6), and it considers as the first choice for the serological surveillance of BT in susceptible animals/herd (3,17).

Materials and Methods

Random sampling concepts was used depending on the live stock population of 2008.A multi stage sampling method advised by the OIE was used which is consist of 4 stages (Districts, Villages, livestock owners and Individual animals).

The OIE represent that Iraq has 300 villages; the total number of each animal species was divided on the total number of villages. The random numbers are selected using a random table and a computer using survey toolbox.Once the numbers are produced, each village on the list with the corresponding number is selected.The Samples were testing by using a serological diagnosis of specific antibodies to the VP7 core protein of the bluetongue virus through the using of competitive ELISA technique designed by Institute Pourquire/ France.

The steps of the test was followed as the manufacturer instructions, in which serum samples were added to the specific microtiter plate that are pre-coated with the VP7 protein. After an incubation period, a monoclonal anti VP7 antibodies conjugated with Horse redish Peroxidase enzyme HRP was added directly without emptying the plates. The reaction plates were incubated at room temperature for 45 minutes, and then the plates were washed to remove any unbound reagents and a substrate solution was added. The plates were incubated for 10 minutes in dark place at room temperature, then the reaction was stopped by adding a stop solution H_2SO_4, and the plates were read in a spectrophotometer at 450 nm.

Validity of the tests was checked prior to the analyzing of the results depending on the criteria that the test is consider valid when the optical density of the negative control is between 0.700 – 3.000 and the ratio of the optical density between the positive to the negative control should not exceed 20%.

Results were calculated by using the specific formula provided by the manufacturer: Percentage of Sample to Negative = (Optical density of sample at 450nm/ Optical density of negative control at 450nm) X 100.

Later, any sample give a ratio ≥ 80% consider negative, samples that have a ratio ≤ 70% are consider positive and the samples with ratio between 70 – 80% are considered suspected.

Results

Validity of the tests

According to the criteria provided by the kit manufacturer that detect the validity of the tests, the mean optical density (OD)of negative control was 2.493 which is lower than the maximum value (3.000) and higher than the minimum value (0.700)at OD of 450nm. The mean optical density of the positive control was 0.376 so that the positive to negative ratio will be 15% which is lower than the maximum ratio value (20%).

Analyzing of results

The sample to negative percentage ratio was calculated by the specific formula, and the results found that from a total of 3277 serum samples , 43.82% found positive which represent 1436 sample, 51.48% of the samples found negative that represent 1687 samples , while 4.69% that represent 154 samples found suspicious(Table 1).

The sero-positive samples of sheep were 1391 samples that form 43.97 % of the total sheep samples 3163, and the total negative sheep samples were 1622 samples that form 51.28 % and the total suspected samples were 150 samples forming 4.74 % (Table 2).

The sero- positive goats samples were 45 of 114 samples forming 39.47 %, while the total negative samples were 65 samples forming 57.01%, and the suspected samples were 4 samples forming 3.50 % (Table 2).

Table 1 Represent the total number of samples tested, positive, negative and suspected sample for each provenance.

Province	Tested samples	+ve samples	-ve samples	Suspect samples	Percentage of positive samples
Baghdad	**155**	**76**	**74**	**5**	**49.03**
Nineva	**407**	**91**	**293**	**23**	**22.35**
Al-Basrah	**48**	**29**	**18**	**1**	**60.41**
Missan	**208**	**92**	**100**	**16**	**44.23**
Diyala	**351**	**139**	**206**	**6**	**39.60**
Wasit	**360**	**158**	**194**	**8**	**43.88**
Al Ta'amim	**368**	**112**	**226**	**30**	**30.43**
Salahaldin	**270**	**136**	**125**	**9**	**50.37**
Al- Anbar	**336**	**202**	**120**	**14**	**60.11**
Babil	**180**	**88**	**81**	**11**	**48.88**
Karbala	**29**	**17**	**11**	**1**	**58.62**
Al-Najaf	**45**	**25**	**18**	**2**	**55.55**
Al-Diwania	**171**	**85**	**80**	**6**	**49.70**
Thiqar	**229**	**123**	**85**	**21**	**53.71**
Al-Muthana	**120**	**63**	**56**	**1**	**52.50**
Total	**3277**	**1436**	**1687**	**154**	**43.82**

Table 2 Represent the total numbers of sheep and goats serum samples and the test results for each species

Province	Sheep					Goats				
	Total Number	+ve samples	-ve samples	Suspect samples	Percentage of positive samples	Total Number	+ve samples	-ve samples	Suspect samples	Percentage of positive samples
Baghdad	155	76	74	5	49.03	-	-	-	-	-
Nineva	407	91	293	23	22.35	-	-	-	-	-
Al-Basrah	48	29	18	1	60.41	-	-	-	-	-
Missan	208	92	100	16	44.23	-	-	-	-	-
Diyala	317	123	189	5	38.80	34	16	17	1	47.05
Wasit	318	143	168	7	44.96	42	15	26	1	35.71
Al Ta'amim	354	109	217	28	30.79	14	3	9	2	21.42
Salahaldin	270	136	125	9	50.37	-	-	-	-	-
Al- Anbar	336	202	120	14	60.11	-	-	-	-	-
Babil	180	88	81	11	49.00	-	-	-	-	-
Karbala	29	17	11	1	58.62	-	-	-	-	-
Al-Najaf	42	22	18	2	52.38	3	3	0	0	100
Al-Diwania	171	85	80	6	49.70	-	-	-	-	-
Thiqar	229	123	85	21	53.71	-	-	-	-	-
Al-Muthana	99	55	43	1	55.55	21	8	13	0	38.09
Total	3163	1391	1622	150	43.97	114	45	65	4	39.74

Discussion

In spite of the absence of typical clinical disease, positive serological evidence has been recorded (6, 7); but Bluetongue disease was never been confirmed in Iraq by a reference laboratory and the virus was never been isolated.

The positive results of c-ELISA test can be explained to the following causes(12):

1- Positive results due to the lack of specificity of the test.

2- Vaccination against BTV.

3- Maternal Antibodies.

4- Natural Infection with BTV.

The positive results due to the lack of specificity of the test or vaccination was abandoned due to the performance of C-Elisa test which was confirmed by many researchers as well as the recommendation of the OIE organization to be the most specific test for the detection of BTV specific antibodies especially when using the VP7 core protein while the positive results due to vaccination is also unacceptable because to our knowledge there are no vaccination strategies or vaccination programs as well as the availability of commercial vaccines due to the lack of identification of the specific serotypes of bluetongue virus that present in Iraq(1,2,5,11,13).

Positive results due to maternal antibodies is unclear because many animals that were bleed were mature and with the lack of vaccination , even maternal antibodies should came from natural infection which is the most acceptable explanation of the positive results gained by this study.

Many evidence are supporting the theory of the natural infection, among of them the geographic nature of Iraq and the presence of rivers, wet lands such as the marshes, lakes, oasis and pools, and the humid hot climate especially in the southern parts of Iraq which provided a very suitable condition for the propagation of the biting midges the main transport vector of the virus especially *Culicoidesimicola*(Kieffer, 1913) that was recorded in Iraq by (4,8).

Also the recording of the disease in the neighboring countries especially in the recent years when the disease was recorded in Iran, Saudi Arabia and Israel in 2008, Oman and The Palestinian Autonomous Territories in 2009 and Kuwaitin 2010when some clinical cases was mentioned in the Abdali region closely to the Iraqi borders in the early of 2010 (a few months prior to our sero-survillance) which was diagnosed later as a novel serotype of bluetongue

virus infection that may have a relationship to the results gained by our study and disease situation in Iraq (9,10).

The positive ratios between the Iraqi provinces are variable with the higher value in Al Basrah province (60.41%) and in Al-Anbar province (60.11%) and that may belong to the different animal population in each Iraqi province and the continuous movement of animals from south to north (especially Nineva) for grazing during the harvesting season (May and June) which may play an essential role in increasing the morbidity ratio due to the exposure to large area of land which are form a suitable environment for the raise and survival of the arthropod vector.

The highly positive ratio in Al Basrah may be a cause or a result to the outbreak of the disease that occur in Kuwait during February 2010.

On the other hand the lowest ratio found in Nineva with 22.35% and that may be explained by the nature of animal husbandry and the following of the scientific routes by animal owners especially the using of preventive anti parasitic doses against internal and external parasites that minimize the exposure of the animals to the biting midges the main transmittedcause of the disease.

The results also indicated that there is a smallvariation in the percentage of the presence of bluetongue specific antibodies between goats and sheep samples, with 43.97% of positive sheep samples against 39.47 % of positive goats samples. These results did not agree with the results of the previous study that showed among 294 of sheep samples only 28 serum sample were positive that represent 9.52%, while among 110 of goats serum samples 18 samples were positive representing 16.36% (7). This variationmay be a result to the low number of collected goat samples in comparison with sheep samples because the criteria of the study was concerned on the random sampling from small ruminants without focusing on the ovine or caprine origin of sample, and that also explain the 100% of positive samples in Al-Najaf province from only 3 samples which cannot represent the actual population of goats population in the province, and also the previous studies were followed the technique of precipitating antibodies which is less sensitive than competitive ELISA technique we used in this study (13).

The results also showed that 154 serum samples found to be suspicious to the infection (150 samples belong to sheep and 4 samples belong to goats), and that positive antibody titer may reflect a very recent infection at time of samples collection or those titers may be at decline stage after a previous infection.

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