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SEROLOGICAL EVIDENCE OF THE OCCURRENCE OF BLUETONGUE IN IRAQ

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SUMMARY

Precipitating antibodies against bluetongue were detected in sheep and goat serum samples collected from animals slaughtered in Baghdad abattoir. Out of 294 *sheep serum samples and* 110 *goat serum samples examined,* 28 *and* 18 *samples respectively showed precipitating activity. In addition, examination of sheep serum samples collected from localities where clinical cases similar to bluetongue were previously reported revealed the presence of bluetongue precipitating antibodies in* 101 *sera out of* 198 *samples examined. This is the first report confirming the occurrence of bluetongue in Iraq.*

INTRODUCTION

During the past three decades, bluetongue (BT) has spread to, or had been recognised in, many countries outside its ancestral home in Africa. Recent serological studies in the Middle East have indicated that BT is widespread in Egypt (Hafez and Ozawa, 1973), Jordan (unpublished data) and Iran (Afshar and Kayvanfar, 1974). In Iraq, some field state veterinarians have reported clinical cases similar to BT among sheep. The purpose of this report is to record the detection of BT precipitating antibodies in sheep and goat serum samples collected from animals slaughtered in Baghdad abattoir as well as in sheep sera collected from localities where clinical cases similar to BT were previously reported.

MATERIAL AND METHODS

Antigens

BT precipitating antigen and the negative control antigen were prepared from cultures of monkey kidney stable (MS) cell line infected with type 10 BT virus or non-infected monolayers as described previously (Hafez and Ozawa, 1972).

Positive control serum

Immune serum against BT virus was prepared by inoculation of a Merino sheep with 2 ml of infected blood collected during viremia from another sheep infected with a virulent strain of type 10 BT virus. Convalescent serum collected 123 days after inoculation was stored at −20°C to be used as BT positive control serum.

Test sera

Sheep and goat serum samples were obtained from animals slaughtered in Baghdad abattoir in September and October 1976. In addition, sheep serum samples were collected in October 1976 from apparently healthy animals raised in Ramadi and Musaiyib where clinical cases similar to BT were reported in November 1975 and June 1976, respectively.

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Immunodiffusion test

The test was carried out in Petri dishes of 10 cm in diameter. Each plate contained 20 ml of diffusion medium, consisting of 1·5 per cent Noble Difco agar in physiological saline (pH 7·2) supplemented by 0·01 per cent sodium merthiolate as bacteriostatic reagent. Four sets of wells, 6 mm in diameter, were punched in every plate. Each set consisted of six wells arranged hexagonally around a central well and were 3 mm apart from each other. Peripheral wells were numbered clock-wise, Nos. 1–6, starting from the 12 o'clock position. Antigen was placed in the central well, reference positive sheep serum in wells Nos. 1 and 4 and the test sera in the remaining four wells. The plates were incubated at room temperature in a humidified atmosphere and examined daily for the precipitating lines using indirect illumination against a black background. The precipitating lines formed between the test serum samples and the antigen were compared with the lines produced by the control positive serum. All the reacting positive serum samples were retested using negative control antigen.

RESULTS

The precipitating lines formed by test sera against the BT antigen were found to be fused with those produced by the positive antiserum placed in wells Nos. 1 and 4, but the density of lines varied. Some sera formed complete lines of identity with those of the positive serum. Other sera did not form complete lines and only clear spurs originating from the ends of the adjacent precipitating lines produced by positive serum were seen, indicating recruiting effect by the reference antiserum. The length of the spurs varied from 1 to 3 mm. None of these reacting sera formed precipitating lines when tested again with a negative control antigen.

TABLE I

Detection of precipitating antibodies against bluetongue virus in sheep and goat sera collected from animals slaughtered in Baghdad abattoir

Animals	No. of sera examined	Samples with precipitating antibodies to bluetongue virus			
		Formation of complete lines		Spur formation (recruiting effect)	
		No.	%	No.	%
Sheep	294	7	2·4	18	6·1
Goats	110	6	5·5	12	10·9
Total	404	13	3·2	30	7·4

The sources, numbers of sera examined and the proportion of samples in which precipitating antibodies were detected either by the formation of complete lines or spurs are listed in Table I (for slaughter animals) and Table II (for sheep sera collected from localities where clinical cases similar to BT were previously reported).

DISCUSSION

The establishment and standardisation of the immunodiffusion technique for the detection of precipitating antibodies to BT virus in sera of infected animals have been described by several investigators (Klontz *et al.*, 1962; Jochim and Chow, 1969; Hafez and Ozawa, 1972). In the present study, the examination of the test sera in adjacent wells to known reference BT immune serum has led to the increase of the

TABLE II

Detection of precipitating antibodies against bluetongue virus in sheep sera collected from animals raised in localities where clinical cases similar to bluetongue were previously reported

District	No. of sera examined	Samples with precipitating antibodies to bluetongue virus			
		Formation of complete lines		Spur formation (recruiting effect)	
		No.	%	No.	%
Ramadi[1]	94	15	15·9	12	12·7
Musaiyib[2]	104	40	38·5	34	32·7
Total	198	55	27·8	46	23·2

[1] Clinical cases similar to bluetongue were reported in November 1975.
[2] Clinical cases similar to bluetongue were reported in June 1976.

sensitivity of the test by the recruiting effect of the positive serum (Klontz *et al*, 1962; Hazrati *et al*, 1968; Hafez and Ozawa, 1972). By this system it was also possible to compare the identity of each precipitating line produced with the individual test serum samples with those lines formed by the reference positive serum. The lines were completely coalescent and identical with the lines of the positive control serum and no reaction was observed when these sera were re-examined against the negative control antigen. These findings indicate that the lines formed by the test sera against the BT antigen are due to specific antigen–antibody reaction. The absence of any vaccination programme against BT in Iraq suggests that the occurrence of BT precipitating antibodies in the sera of local animals has arisen as a consequence of either natural infection with the virus or of passive immunisation with maternal antibodies.

BT has been reported in Iran (Afshar and Kayvanfar, 1974), Turkey (Tamer, 1949), Syria (Howell, 1963) and Jordan (unpublished data) four neighbouring countries to Iraq. Therefore, the confirmation of the prevalence of BT in Iraq is not surprising. However, studies are needed to increase our knowledge of the epizootiology and pathogenesis of BT virus infection among different animals in Iraq.

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PREUVE SÉROLOGIQUE DE L'EXISTENCE DE LA BLUETONGUE EN IRAK

Résumé—Des anticorps précipants contre la bluetongue ont été mis en évidence dans des échantillons de sérum de moutons et de chèvres recueillis sur des animaux abattus à l'abattoir de Bagdad. Sur 294 échantillons de sérum de moutons et 110 de chèvres examinés, 28 et 18 de ces échantillons se sont respectivement montrés précipitants. En outre l'examen d'échantillons de sérum de moutons recueillis dans des localités où existaient des cas cliniques pouvant faire soupçonner la bluetongue a montré la présence de sérums précipitants de la maladie dans 101 des 198 échantillons examinés. C'est là le premier report confirmant l'existence de la bluetongue en Irak.

EVIDENCIA SEROLÓGICA DE LA OCURRENCIA DE LENGUA AZUL EN IRAK

Resumen—Se detectaron anticuerpos precipitantes de la enfermedad de la Lengua Azul en el suero de ovejas y cabras beneficiadas en el matadero de-Bagdad. La prueba fue positiva en 28 de 294 sueros ovinos y en 18 de los 110 sueros caprinos examinados. Posteriormente, se examinaron 198 sueros ovinos colectados en áreas en donde se sospechaba la existencia clinica de la enfermedad, encontrandose 101 positivos. Este es el primer informe de la existencia de Lengua Azul en Irak.