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Seroprevalence of bluetongue virus antibodies in sheep and goats in Kerala State, India

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Summary
The results presented here record the first confirmation of bluetongue virus (BTV) antibody in sheep and goats in Kerala State. A total of 1,010 sera collected from the 14 districts within the state were screened for the presence of group-specific BTV antibodies by dot enzyme-linked immunosorbent assay (dot ELISA). Positive samples were obtained from 12 of the 14 districts. The overall BTV antibody prevalence was 5.1 ± 1.9% (at 95% confidence level) although the prevalence levels were consistently higher in organised farms than in the field population. Comparative tests carried out using the dot ELISA and competitive ELISA (C ELISA) showed a good agreement for all the positive sera. The dot ELISA was simple to perform, economic and rapid, and is therefore ideally suited for routine screening for BTV antibody at the farm level.

Keywords
Bluetongue – Competitive enzyme-linked immunosorbent assay – Dot enzyme-linked immunosorbent assay – India – Kerala State – Seroprevalence.

Introduction

Bluetongue (BT) is an arthropod-transmitted viral disease affecting domestic and wild ruminants between approximate latitudes 35°S and 40°N, and is designated as a List A disease by the OIE (World Organisation for Animal Health). The clinical form of the disease is usually only seen in sheep. Infection in cattle and goats is generally not noticeable, although clinical BT has been reported in both species (8, 13). The clinical signs of the disease range from a mild febrile illness to extensive erosions of the oral mucosa, which can be confused with the symptoms of foot and mouth disease (FMD) (4). BT was first reported in India in 1961 in the state of Maharashtra (19). Since then, the disease has been recorded in 11 states in India, either on the basis of virus isolation or by the detection of group-specific antibodies against the virus (16).

Kerala, which has a tropical climate, is situated at the southernmost tip of the Indian Peninsula between latitudes 8°18' N and 12°48' N, and between longitudes 72°22' E and 74°52' E. The state is bounded to the west by the Arabian Sea, and to the east by the Western Ghats (and their extensions, the Anamalai and Cardamon hills). The livestock population consists mainly of cattle and goats, although sheep are reared in some areas of Central Kerala.

There have been no confirmed reports of BT in the state, although field veterinarians have previously reported a BT-like disease in some of the northern districts. Similarly there have been reports of a FMD-like disease in animals from Kerala that had been previously immunised against FMD. Furthermore, there is an unrestricted flow of livestock into Kerala State from the neighbouring south Indian states of Tamil Nadu and Karnataka, where bluetongue virus (BTV) is endemic.

This study was conducted to assess the seroprevalence of BTV in sheep and goats in Kerala State, using a commercially available competitive enzyme-linked immunosorbent assay (C ELISA) and a dot ELISA that was developed in-house.

Materials and methods

Antigen

For the agar gel immunodiffusion (AGID) test and dot ELISA, a baby hamster kidney-21 cell line based BTV antigen was used (marketed commercially by the Vaccine Research Centre [VRC], Centre for Animal Health Studies, Tamil Nadu University of Veterinary and Animal Sciences, Chennai, India). The total protein concentration of the antigen, estimated by a procedure described in an earlier study (12), was 3.39 mg/ml.

Positive sera

A reference AGID-positive sheep serum obtained from the VRC was used as the control when testing sheep sera by dot ELISA. A goat serum sample that gave a strong positive reaction in the AGID test was used as a positive control when assaying caprine sera by dot ELISA.

Negative sera

AGID-test-negative sheep or goat sera were used as negative controls in the respective dot ELISAs.

Collection of sera

In all 1,010 sera – 109 (10.8%) from sheep (two districts) and 901 (89.2%) from goats (14 districts) – were collected from local owners and farmers, organised farms and slaughterhouses throughout the state (Table I). The samples were collected between October 2001 and July 2002. The numbers to be collected from each district were calculated from the number of sheep and goats in each district as a percentage of the total population of small ruminants in Kerala State. For example, Thiruvananthapuram district contained 10.3% of the small ruminants in the state, so a minimum of 103 samples were collected from the district (Table I).

Agar gel immunodiffusion test

The test was performed as described by Jochim and Chow (10) with slight modifications. Between 3 ml and 4 ml of melted agarose (1% agarose in normal saline) was poured onto pre-coated glass slides and allowed to set. One central well and five peripheral wells, each 3 mm in diameter, were cut in such a way that the distance between the central well

Table I
Seroprevalence of bluetongue in sheep and goats in Kerala

District	Sheep			Goats			Total prevalence (%)
	Samples collected	Samples positive	Prevalence (%)	Samples collected	Samples positive	Prevalence (%)	
Thiruvananthapuram[a]	–	–	–	104	6	5.8	5.8
Kollam[a]	–	–	–	95	5	5.3	5.3
Pathanamthitta	–	–	–	46	2	4.3	4.3
Alappuzha	–	–	–	40	0	0	0
Kottayam	–	–	–	79	3	3.8	3.8
Idukki	–	–	–	81	4	5.0	5.0
Ernakulam	–	–	–	80	1	1.3	1.3
Thrissur[b]	30	5	16.7	79	10	12.7	13.8
Palakkad[a]	79	4	5.1	57	3	5.3	5.1
Malappuram	–	–	–	49	3	6.1	6.1
Kozhikode	–	–	–	68	4	5.9	5.9
Wayanad	–	–	–	20	0	0	0
Kannur(a)	–	–	–	57	3	5.3	5.3
Kasaragode[a]	–	–	–	46	4	8.7	8.7
Total	**109**	**9**	**8.25**	**901**	**48**	**5.3**	**5.1 ± 1.9[c]**

a) Districts in which goat farms are located
b) District in which both sheep and goat farms are located
c) At 95% confidence level

and any peripheral well was 3 mm. The distance between the adjacent peripheral wells was kept equal. The central well was filled with 20 µl of BTV antigen. One of the peripheral wells received 20 µl of positive serum, and another received the same amount of negative serum. The remaining wells were filled with 20 µl each of test sera. The slides were incubated at room temperature in a humid chamber for 48 hours and were examined in diffuse light for the presence of precipitin lines.

Dot enzyme-linked immunosorbent assay

The test was performed as described by Gupta *et al.* (6) with minor modifications. A plastic template was made, with eight 5-mm-wide prongs set 4 mm apart. Nitrocellulose membrane (NCM) squares (5 mm × 5 mm) were attached to the tip of each prong. Two microlitres of undiluted antigen was added to each NCM and air-dried. Unbound sites on the NCMs were blocked for one hour at 37°C with phosphate-buffered saline (PBS) containing 0.1% PBS Tween 20 (PBST) and a 5% solution of skim-milk powder. The membranes were rinsed three times in PBST, blotted dry and then incubated at 37°C for one hour with 200 µl of 1:20 positive, negative or test sera diluted in bovine serum albumin (BSA)-PBST (1% BSA). The membranes were rinsed as before and then incubated at 37°C for 30 min with 200 µl of anti-sheep or anti-goat IgG-horseradish peroxidase (HRP) conjugate, diluted 1:1000 in BSA-PBST. The NCMs were rinsed as before and then immersed in diaminobenzidine (DAB) solution (5 mg DAB in 10 ml PBS pH 7.4 + 10 µl of 30% H_2O_2) for two to three minutes. The reaction was stopped by rinsing the membrane in PBS. Formation of a brown spot at the site of antigen deposition was regarded as positive. Incomplete brown spots or brown rings were regarded as possibly positive and warranted further investigation.

Competitive enzyme-linked immunosorbent assay

Sera that recorded complete or incomplete dot formation by dot ELISA were cross-checked, using a monoclonal antibody-based C ELISA kit (a test recommended by the OIE). The test was carried out as described in the protocol supplied by the manufacturers, and the percentage inhibition (PI) values were calculated as described by Afshar *et al.* (1). Samples with PIs equal to or greater than 50% were considered to be positive, and those with PIs of less than 50% were taken as negative.

Results

The initial screening of all the sera by AGID revealed two positive samples. These AGID-positive sera together with randomly selected AGID-negative sera were subsequently used as controls for dot ELISA. Of the 1,010 samples tested by dot ELISA, 57 samples (including AGID-positive samples) recorded a clearly identifiable brown spot (proportion of positive samples = 0.05643; confidence interval 0.0437 to 0.0726 at 95% confidence). A further 22 sera recorded either faint colour development, an incomplete spot or a brown ring. All these 79 samples were subsequently confirmed as BTV positive by C ELISA. The dot ELISA-negative controls were also confirmed by C ELISA.

A prevalence of BTV antibody was detected in 12 of the 14 districts of Kerala State (Table I, Fig. 1), the highest being recorded in sheep and goats from Thrissur, where 5 of 30 sheep (16.7%) and 10 of 79 goats (12.7%) were positive. The next highest prevalence was recorded among goats from the Kasaragode district, where 4 of the 46 samples (8.7%) were positive. The prevalences of BTV antibodies in both species in Thrissur and Palakkad districts were 13.8% and 5.1% respectively. The prevalence values for the 12 affected districts ranged from 1.3% to

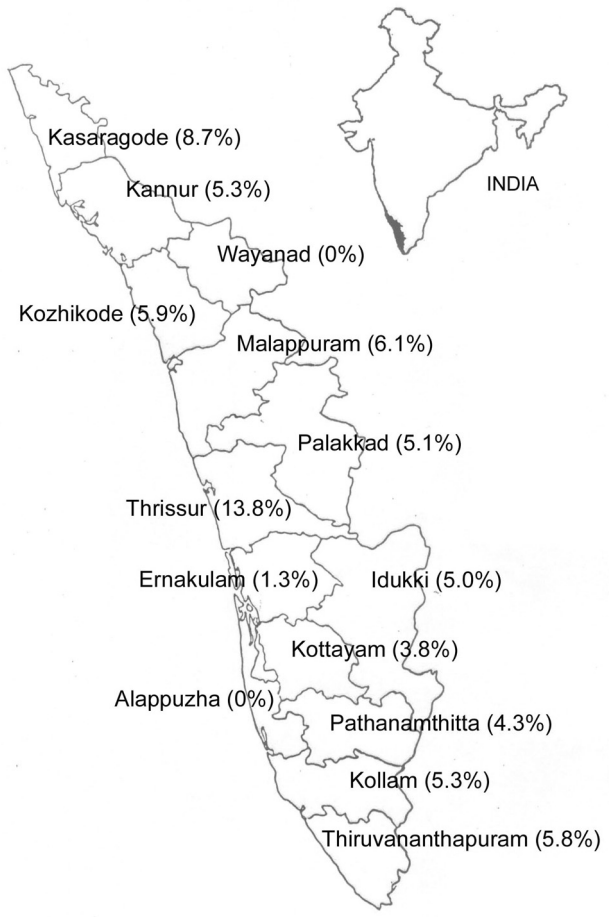

Fig. 1
Seroprevalence of bluetongue in sheep and goats in different districts of Kerala

13.8% with an overall prevalence of 5.1 ± 1.9% (at 95% confidence level).

Discussion and conclusion

The results presented here record the first confirmation of BTV antibody in sheep and goats from Kerala. The overall prevalence of the BTV antibodies in sheep and goats in the state was found to be 5.1 ± 1.9%, and sero-positive animals were detected in 12 of the 14 districts sampled. As the goat population in Alappuzha and Wayanad districts was smaller than that in other districts, the samples collected from these two districts were proportionately fewer. Failure to detect seroreactors in these districts may be due to the relatively low number of animals screened. The absence of overt disease in sheep is probably a reflection of the relatively small numbers present in Kerala State together with the susceptibility of the breed present, a factor that remains unknown. In addition, mild disease may go unnoticed and/or unreported.

The highest proportion of seropositive sheep and goats came from Thrissur district, and the second highest rate was in goats from the Kasaragode district. This may be attributed to the nearness of these districts to the states of Tamil Nadu and Andhra Pradesh, where BTV is considered endemic, and to the unrestricted movement of relatively large numbers of cattle, sheep and goats from these 'endemic' regions into the Thrissur and Kasaragode districts. A similar situation has been reported in Andhra Pradesh State, where the highest number of BT cases occurred in districts lying in close proximity to BTV-affected areas of neighbouring states (21). Most of the districts in Kerala State that had a lower seroprevalence are close to either Tamil Nadu or Karnataka; however, their geographical isolation by the Western Ghats makes transport and movement of animals difficult (Fig. 2).

The prevalence of BTV antibodies in sheep and goats was 8.3% and 5.3% respectively. Previous reports in other states of India have recorded BTV antibody prevalence levels of between 1.9% and 57.6% in sheep (2, 3, 5, 7, 9, 11, 14, 15, 17, 18, 21) and between 0.8% and 28.0% for goats (3, 5, 11, 18). The highest prevalence levels of BTV antibodies were in animals on organised farms. Similarly high prevalence levels have been reported previously in flocks maintained on organised sheep farms elsewhere in India (15, 20). In the present study, the high prevalence levels of BTV virus antibodies on the farms may to some extent have influenced the percentage of seroreactors in Thrissur, as two organised farms are located in the district. One of the factors that might contribute to these higher levels in farms could be the high concentration of animals in close proximity to each other, which would favour vector transmission. *Culicoides oxystoma* is thought to be a

Fig. 2
Map showing geographical isolation of Kerala by the Western Ghats

potential vector for BTV in India. Although midges have been trapped from various districts of Kerala State, especially in the north, their identity and role in BTV transmission remain unclear.

The dot ELISA described here detected positive BTV antibody in sera from sheep and goats in 12 of the 14 districts of Kerala. All 79 sera that were identified as having reacted in the dot ELISA were confirmed as positive by the C ELISA. The dot ELISA is economical, easy to perform, specific and rapid. This assay has proved reliable for the detection of BTV antibody at the farm level and could be used with confidence as an alternative to the C ELISA.

Further studies are being undertaken to determine the prevalence of BTV antibody in cattle and to determine the BTV serotypes that are and have been circulating in Kerala.

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Confirmation de la présence d'anticorps sériques vis-à-vis du virus de la fièvre catarrhale du mouton chez des ovins et des caprins de l'État de Kerala, Inde

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Résumé

Cet article décrit la première confirmation de la présence d'anticorps vis-à-vis du virus de la fièvre catarrhale du mouton (VFC) chez des ovins et des caprins de l'État de Kerala en Inde. Au total, 1 010 prélèvements effectués dans les 14 districts de Kerala ont été soumis à l'épreuve immuno-enzymatique « dot » (dot-ELISA) pour la recherche d'anticorps spécifiques à un groupe du VFC. Des résultats positifs ont été obtenus dans des prélèvements provenant de 12 des 14 districts. La prévalence globale d'anticorps vis-à-vis du VFC était de 5,1 ± 1,9 % (intervalle de confiance à 95 %), avec des taux significativement plus élevés dans les élevages commerciaux. Les dosages obtenus en dot-ELISA aussi bien qu'en ELISA de compétition étaient cohérents pour tous les prélèvements séropositifs. Le dot-ELISA, facile à réaliser, rapide et peu onéreux constitue le test de dépistage idoine pour la recherche d'anticorps vis-à-vis de la FC au niveau des élevages.

Mots-clés

Épreuve immuno-enzymatique de compétition – Épreuve immuno-enzymatique « dot » – État de Kerala – Fièvre catarrhale du mouton – Inde – Prévalence sérologique.

Seroprevalencia de anticuerpos contra el virus de la lengua azul en ovejas y cabras del estado de Kerala (India)

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Resumen

Los resultados que aquí se presentan constituyen la primera confirmación de la presencia de anticuerpos contra el virus de la lengua azul en ovejas y cabras del estado de Kerala. Se analizaron un total de 1.010 sueros procedentes de los 14 distritos del estado para detectar en ellos, mediante un ensayo inmunoenzimático "de manchas" (dot ELISA), un grupo de anticuerpos específicos del virus de la lengua azul. En 12 de los 14 distritos se encontraron muestras positivas. La prevalencia total de anticuerpos se elevaba a 5,1 ± 1,9% (con un nivel de confianza del 95%), aunque la prevalencia era sistemáticamente mayor en las explotaciones industriales que en los rebaños nómadas. Las pruebas comparativas realizadas con dot ELISA y ELISA de competición pusieron de manifiesto un alto grado de coincidencia en todos los casos de sueros positivos. La técnica de dot ELISA resultó sencilla y rápida de ejecución, amén de económica, por lo que es idónea para realizar las pruebas sistemáticas de detección de anticuerpos en las explotaciones.

Palabras clave

Ensayo inmunoenzimático de competición – Ensayo inmunoenzimático de manchas (dot ELISA) – Estado de Kerala – India – Lengua azul – Seroprevalencia.

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