Bluetongue Virus Status in Papua New Guinea

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Abstract

At about the same time (1977–78) that Australia was conducting a massive serological survey for bluetongue virus, after the isolation of the CSIRO 19 strain (BLU20), a similar but smaller-scale survey was being carried out in Papua New Guinea. At the National Veterinary Laboratory, group-specific testing by agar gel immunodiffusion (AGID) of both survey sera and stored sera dating back as far as 1974 demonstrated seroconversion in cattle, deer, goats, buffalo and sheep. Subsequent type-specific serum neutralisation tests demonstrated positives in buffalo for CSIRO 19 (BLU20), and in cattle for CSIRO 154 (BLU21) and CSIRO 156 (BLU1). In Papua New Guinea, BLU2 to BLU17 and BLU20 have never been recorded in any ruminants. In 1989, the Northern Australian Quarantine Strategy surveillance and monitoring of sentinel herds and border surveys was established in Papua New Guinea in cooperation with Australia. So far, neither border surveys near Irian Jaya in 1991, 1992 and 1993, nor sentinel herd sera sampled up to 1994, have detected seroconversion in sheep.

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An Outbreak of Bluetongue in Cattle in Japan

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Abstract

An outbreak of Ibaraki-like disease swept over the eastern parts of Japan between August and the end of October 1994. The clinical and pathological findings were very reminiscent of Ibaraki disease. The pathological changes were confined to the mucous membrane of the digestive tract and its musculature, with the lesions being essentially hyperaemic. The most conspicuous changes were in the oesophagus, larynx and pharynx with degeneration of striated muscle. An attempt to isolate the causative virus directly in HmLu-1 or BHK21 cells failed. Bluetongue viral RNA was detected in blood samples from affected cattle by polymerase chain reaction (PCR) tests, carried out by the method of McColl and others. Antibodies to bluetongue group virus were detected in the serum of all affected cattle. However, all sera were negative for Chuzan virus while a few had antibodies to Ibaraki virus. In the epidemic area, bluetongue antibodies, as determined by agar gel immunodiffusion (AGID) were detected at a high prevalence, whereas they were found in few or none of the serum samples collected in non-epidemic areas. Among the animals, there was a correlation between bluetongue infection and difficulty in swallowing.

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Bluetongue History, Serology and Virus Isolation in China

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Abstract

This paper reports the history of bluetongue disease, serology and virus isolation in China. Bluetongue disease was first diagnosed in Yunnan Province in 1979, an event which initiated bluetongue research in China. Extensive serological surveys were carried out among domestic animals such as sheep, goats and buffalo, using agar gel immunodiffusion (AGID). Antibodies have been found in most animals in most provinces, while clinical bluetongue has occurred in six provinces. Strains of bluetongue virus have been isolated from sheep, goats or *Culicoides* midges in Yunnan, Hubei, Sichuan, Shanxi, Shandong and Gansu Provinces, and in Xinjiang and Inner Mongolia.

BLUETONGUE is an infectious, non-contagious, arthropod-borne viral disease, transmitted by midges of the genus *Culicoides* which feed on sheep as well as other ruminants. Bluetongue disease has had an epidemic history on the African continent since 1852, when Merino and other European sheep were imported to South Africa. The history of bluetongue as a distinct entity in the veterinary world spans the past hundred years (as described elsewhere in these Proceedings). The presence of bluetongue in China was not confirmed until 1979. This paper reports the history, serology and isolation of bluetongue virus (BLU) in China.

The Discovery and Diagnosis of Bluetongue in Yunnan Province

In May 1979, there was an outbreak of a bluetongue-like disease in the sheep farms of Shizong county and the surrounding area in the Qujing region of Yunnan Province. The investigation and study of bluetongue in China began shortly thereafter, in July 1979.

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Epidemiological Survey

Natural environment of the area and brief epidemiology

Shizong County, the epidemic area, is located in a mountainous region in the eastern part of Yunnan, at 24°40'N, 104°11'E. The average altitude is 1987 m above sea level, although the surrounding area ranges from 1750 m to 2000 m. The four seasons are not clearly defined but there are obvious dry and wet seasons each year. According to (incomplete) climate records, the average annual temperature is 10.5°C, with an average maximum of 26.4°C in May and an average minimum of –1.4°C in January. The average relative humidity is 71.2%, with general average precipitation of 1465.8 mm per year. On the grazing area of some 6070 hectares, the majority of livestock are ruminants (1206 sheep, 483 goats, 559 cattle and buffalo, and a few horses and pigs).

The first outbreak of bluetongue occurred in two flocks of sheep on Shizong Farm, followed by outbreaks in three flocks of sheep in neighbouring villages. Of a total of 1206 sheep at risk in the five epidemic sites, 430 (35.7%) became sick, and 170 (31.9%) of these died. One sick goat was also suspected to have bluetongue. No other ruminants on the farm were affected.

Analysis of the disease's origin

Sheep have been kept on the Shizong farm since 1958. Although some regional breeds had been introduced (including Xinjing and Caucasus breeds from Gongnaisi, Xinjing and the Yunnan Provincial Farm, and Australian breeds such as Polwarth and Romney introduced through Xundian farm in 1977; Table 1), there was no significant relationship between the introduced breeds and the incidence of bluetongue. There were fragmentary reports about suspected cases in 1966 but no detailed records. The first infection was identified in July 1974. From 1974 to 1977 only four sheep died out of 10 that were ill (Table 2). In May 1978 there was an acute outbreak of bluetongue. While the origin of the disease on Shizong Farm is not clear, the other epidemic sites had either taken sheep from Shizong Farm or had crossgrazed flocks of sheep within the farms themselves. For example, 46 sheep were introduced from Shizong Farm to the Tuoluo village of Longqing Primitive Commune in May 1980. Subsequently, sick sheep were found in local flocks. There was a higher mortality rate among local sheep than among introduced animals.

Susceptible animals

In Yunnan, only sheep are susceptible to bluetongue. The survey in Shizong county showed differences in the breeds, age and gender of infected animals: the latter comprised 69% (101/146) of all sheep, 22.6% of lambs aged 6 months to 1 year, and 46.6% of animals aged one to four years (most aged one to two years). The prevalence in Xinjiang sheep was higher than in crossbreeds (Table 3) while the prevalence in females was higher than in males.

Epidemic season

According to records from the five epidemic areas, the first sheep was diagnosed with bluetongue on 18 May 1978, with the terminal date of epidemic disease being 14 October 1978, a duration of 150 days. Prevalence was highest at 60.2% in July, with a decline to 33% in August. The mortality rate in the epidemic period was also highest in July at 35.1% (Table 4). Only one sheep relapsed, on 20 February 1980. No incidence or other relapse was found after the outbreak.

Table 2. Outbreaks of suspected bluetongue disease, Shizong Farm, 1974–1978.

Epidemic years	No. of sick animals	No. of animals that died	Mortality	Remarks
1974	5	3	3/5	veterinary records
1975	2	1	1/2	from incomplete records 1974
1977	3	1	1/3	no records before 1977
1978	22	6	6/22	from incomplete veterinary records
Total	32	11	11/32	

Clinical Signs

Through systematic observation of 48 sheep, and more general observations on 144 naturally-infected sheep, the following clinical signs were noted. The initial sign was an elevated body temperature, about 40.5–41.5°C, quite often followed by a greyish-white lesion on the upper gums and corners of the mouth, and oedema of the gums, lips, muzzle and ears.A watery discharge from the nostrils then occurred, becoming mucocatarrhal and hardening to form pinkish crusts.

Table 1. Introduction of sheep breeds to Shizong.

Farm	Date	Breed	Source	No. of sheep
Tuoluo village	May 1979	Xinjiang	Shizong 5.7 Farm	46
Longqing Commune	1958	Local	Fuyuan County	358
	1960	Caucasus	Qujing County	20
	1964	Xinjiang	Xinjiang Gongnaisi Farm	153
Shizong Farm	1966	Xinjiang	Xinjiang Gongnaisi Farm	158
	1972	Xinjiang	Xundian Breeding Farm	3
	1972	Caucasus	Xundian Breeding Farm	2
	1977	Xinjiang	Xundian Breeding Farm	2
	1977	Half-Polwarth	Xundian Breeding Farm	1
	1978	Romney	Xundian Breeding Farm	2

Table 3. Relationship of morbidity and mortality rates for bluetongue disease with age and breed of sheep.

Breed	Age (years)	No. of sick sheep (%)	No. of sheep that died (%)
Xinjiang	0.5–1	20 (27)	10 (45.4)
	1–2	23 (31)	6 (27.3)
	3–4	17 (22.9)	3 (13.6)
	5–6	9 (12.1)	1 (4.5)
	>7	5 (6.7)	2 (9.1)
	Total	**74**	**22 (29.7)**
Cross-breeds	0.5–1	14 (18.4)	4 (25)
	1–2	28 (36.8)	6 (37.5)
	3–4	16 (21)	3 (18.7)
	5–6	13 (17.1)	2 (12.5)
	>7	5 (6.5)	1 (6.2)
	Total	**76**	**16 (21)**
Total	0.5–1	34 (22.6)	14 (36.8)
	1–2	51 (34)	12 (31.6)
	3–4	33 (22.9)	6 (15.8)
	5–6	22 (14.6)	3 (7.9)
	>7	10 (6.6)	3 (7.9)
	Total	**150**	**38 (35.3)**

Sloughing of the epithelium of the inner mouth followed, with anorexia and depression. In some cases diarrhoea occurred, occasionally haemorrhagic. Difficulty in swallowing and aspiration of ruminal contents resulted in the secondary pneumonia that was the proximal cause of death.

In some cases, the skin above the hooves became swollen, hyperaemic and haemorrhagic in the early stages, followed by sloughing of the shell of the hooves. The infected animal became recumbent and was reluctant to rise. Wool condition suffered, with substantial permanent breaks in the fibre and shedding over a wide area of the back and groin, although new wool grew after shedding. Needle-point spots of hyperaemia could be seen on the skin, particularly in the areas of shedding (base of tail, elbow and groin).

Serology tests on 48 sheep showed that there was a significant decrease of leucocytes in the blood: 33/48 declined to 8000/mm^3 (average 6386/mm^3). The lowest total number of leucocytes was 4150/mm^3, with 15/48 having counts of 8000–10000/mm^3 (average 10700/mm^3). The average number of leucocytes of the 48 sheep was 7736/mm^{3}3, whereas the normal level was 10700/mm^3. The most obvious decrease of leucocytes occurred in the early stages of infection, but continued in cases of secondary infection.

Postmortem Examinations

Postmortem examinations were conducted on six of the infected sheep. The major pathological changes associated with bluetongue related to the vascular system. The pathological lesions due to the virus were characteristic of an inflammatory process with increased vascular permeability of the mucous membranes of the digestive tract. Hyperaemia, oedema and haemorrhage were found in mucous membranes in the mouth, with severe lesions in the top of tongue papillae. Whitish pseudo-membranes, from 1–2 cm wide by 3 cm long were found on the upper gums in the mouth: an irregular erosion was seen after the pseudo-membrane was removed. A dried membrane occurred on the muzzle. Viscid reddish-brown secretions were seen around the nose, and erosions and ulcers on the mucosae of the mouth and hard palate. Oedema had developed in the muscles around the throat, and gelatinous infiltration was found in the fatty tissues. Mucosal haemorrhages were seen on the trachea and lungs. A few haemorrhage spots were found on the external cardiac membranes, with speckles of haemorrhage around the ventricle and atrium. The colour of the myocardium varied, the coronary fatty tissue was gelatinous and atrophied, and giant focal haemorrhages were seen in cardiac muscles. Scattered haemorrhages were found on the forestomach (muscular pillars and oesophageal groove). The older haemorrhage spots resembled black sesame seeds. The mucosa of the intestines and forestomachs was haemorrhaged and came off easily. The liver and kidneys were congested.

Diagnosis

Sheep inoculation

The first experiment was carried out from 17 to 30 July 1979. Nine healthy animals in three groups were injected with 65–100 mL of whole blood (citric acid anticoagulant) drawn from naturally-infected sheep. The dose was repeated once. Three to five days after the first injection, a slightly elevated body temperature (41–41.5°C) occurred in all animals and lasted for four to six days. Mucosal hyperaemia and oedema were seen in mouth, pillars of the tongue and nostrils, with erosion in the corners of the mouth and a grey-yellowish pseudo-membrane on the tops of ulcers on the upper gums.

The leucocyte counts fell from 7050–14650/mm^3 to 3400–8900/mm^3 from day 3 post-infection. These recovered to normal (before infection) levels from day 5 post-infection. Lymphocytes started to increase from day 3 post-infection. The latent period, clinical signs and clinical serological changes of the later four experiments were basically similar.

Table 4. Changes of morbidity and mortality rates of bluetongue disease in sheep during the epidemic season.

Epidemic site	Epidemic month	No. of sick sheep	Morbidity %	No. of dead sheep	Mortality %	Total no. dead/ total no. sick (%)
Xiaofakuai village,	May	0	0	0	0	
Wulong commune	June	1	3.0	0	0	
	July	9	26.5	4	44.4	9/34 (26)
	August	23	67.6	5	55.5	
	September	1	2.9	0	0	
	October	0	0	0	0	
Dachang Village,	May	0	0	0	0	
Wulong commune	June	0	0	0	0	
	July	44	59.4	20	66.7	30/74 (41)
	August	28	37.8	9	30.0	
	September	2	2.7	1	3.3	
	October	0	0	0	0	
Tuoluo village,	May	0	0	0	0	
Longqing commune	June	0	0	0	0	
	July	89	55.6	30	60.0	50/160 (31)
	August	60	37.5	18	36.0	
	September	10	6.25	2	4.0	
	October	1	0.6	0	0	
Shizong Farm	May	1	0.6	0	0	
	June	8	4.9	1	2.1	
	July	117	72.2	37	77.1	48/162 (30)
	August	30	19.1	8	16.7	
	September	4	2.5	2	4.2	
	October	1	0.6	0	0	
Totals	May	1	0.2	0	0	
	June	9	2.1	1	0.7	
	July	259	60.2	91	66.4	137/430 (32)
	August	142	33.0	40	29.2	
	September	17	3.9	5	3.6	
	October	2	0.5	0	0	

Embryonated egg inoculation

The procedure involved inoculating 8- to 10-day-old embryos with whole blood from 19 natural or artificially infected sheep, blind passaged in embryos. Specimen no. 33 was used for 5–24 blind passages and no. 40 for four blind passages. The others were discarded after between two and ten passages. Specimens nos. 40 and 57 were inoculated into a superficial vein of 11- to 13-day-old embryos and then blind-passaged.

All eggs were incubated at 33.5–34°C for another 4–5 days. The clinical pathological signs in the embryos were the gelatinous infiltration of the embryo, and swelling of the organs such as heart, stomach, spleen and liver, with necrosis of the liver. Death then occurred.

Seventeen sheep were inoculated with emulsion of embryo amnion at F4, F10, F5, F11, F13 from specimen no. 33, F7 from no. 71 and F4 from no. 15 in the blind passage period. One sheep appeared to have obviously typical bluetongue symptoms, eight had elevated body temperatures and decreased leucocytes, and eight had no symptoms. All animals with clinical or subclinical signs were inoculated with specimen no. 33. Eight sheep were then inoculated with embryo tissues: whole blood, amnion fluid and organs (heart, spleen, liver and kidney). Two animals (sheep 57 and 61) had severe symptoms, one had minor symptoms, another three had slight reactions and the remaining two had no clinical changes. Bluetongue virus was thus isolated from the above experiments with fundamentally similar results.

Cell Infection

The procedure with baby hamster kidney (BHK) cells was similar with each cell type. The same volume of distilled water as in the original sample was added to

the blood of sheep no. 89 to lyse the red cells. These were then centrifuged at 1000 rpm for 10 min. The supernatant was diluted with Eagle's solution at dilutions of 1/10, 2/10, 3/10 and undiluted. The cells were inoculated with the lysed blood in all dilutions, with one bottle of uninoculated cells kept as a control: all were incubated at 33.5–34.5°C. Cytopathic effects (CPE) occurred at 72 hours after infection in the undiluted group, at 96 hours at the 10^{-1} and 10^{-2} dilutions, and at 120 hours at the 10^{-3} dilution. No CPE was seen in the control cells.

Vero cells were infected with 1/10 and 2/10 dilutions of the third passage from sheep no. 64. The CPE involved cell enlargement and rounding up. The cell boundaries were not clear, and granular spots developed in detached cells. After 72 hours of inoculation, some cells enlarged and a typical CPE was seen after 96 hours in the 1/10 and 2/10 dilutions. Control cells became round due to ageing but did not clump. In monolayer cells of goat embryo kidney inoculated with blood from sheep no. 64, CPE was seen 120 hours after infection.

Isolation and Identification of Virus

Isolation from naturally-infected animals

After the first isolation of bluetongue virus from Shizong Farm in 1979, an outbreak of sheep disease was reported from Xiangfan region in Hubei Province where 132 sheep out of 150 (88%) became sick and 38 (29%) animals died. Agar gel immunodiffusion (AGID) tests of sera from recovered sheep showed 14/16 (88%) were positive. A successful procedure for virus isolation and identification was developed by Yunnan Animal Husbandry and Veterinary Institute (YAHVI) and the Hubei General Veterinary Station (Fig. 1).

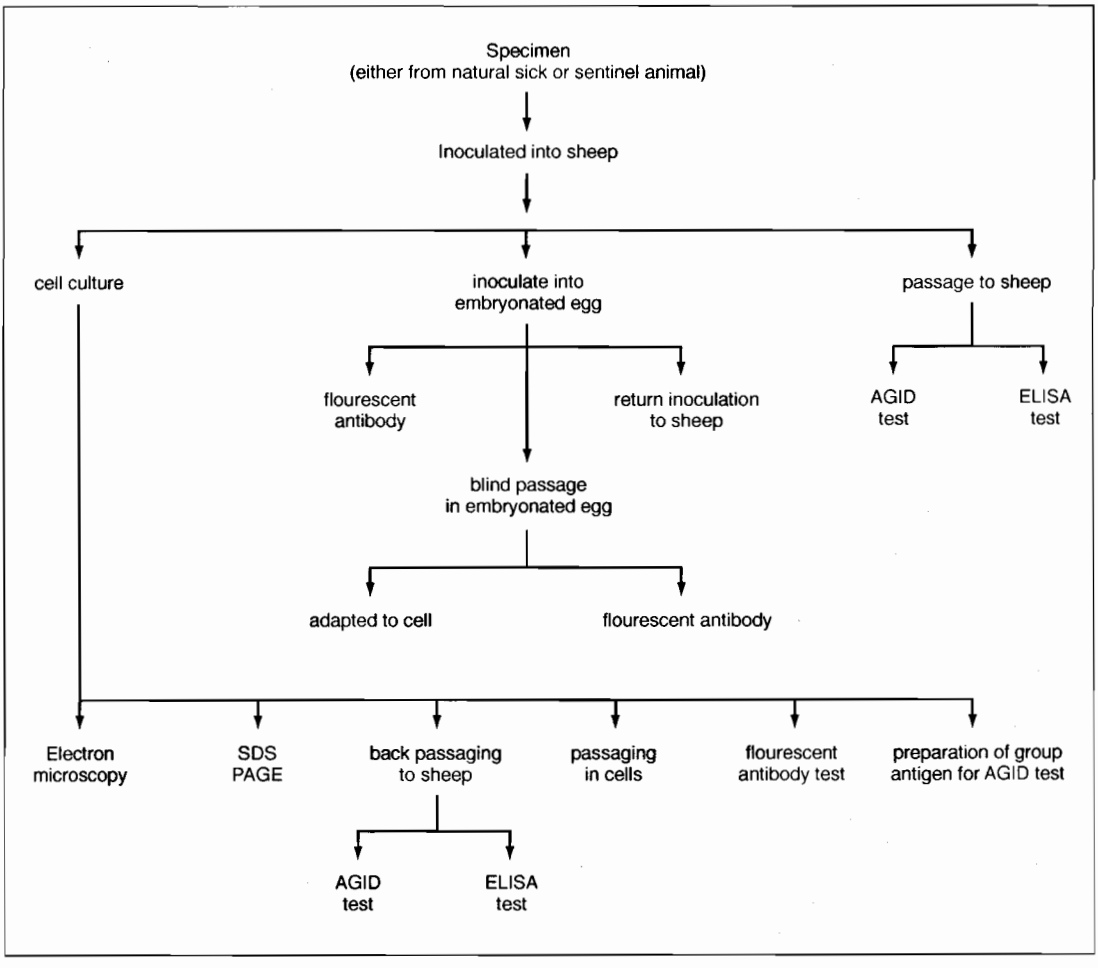

Figure 1. Schema showing procedure used for primary isolation of bluetongue virus in China

Further bluetongue outbreaks occurred from 1987–1993 (Fig. 2). In 1987, some suspected cases were reported by the Sichuan General Veterinary and Prevention Station from a monitoring area in Hongchiba, Wanxian region, where there were no records of bluetongue disease. Hongchiba farm, established in 1958, introduced Ganzi-Tibet, Xinjiang-fine wool and Romney breeds from Jiangsu Province in 1986. No bluetongue disease was found. However, after the introduction of 691 Xinjiang Merinos there was an outbreak of bluetongue, with an incidence rate of 12.4% and mortality rate of 22%. In 1988, YAHVI and the Chengdu and Wanxian Quarantine Stations isolated BLU from the specimens from sick sheep. Viruses were isolated from specimens from Anhui by National Quarantine and Veterinary Institute (NQVI)

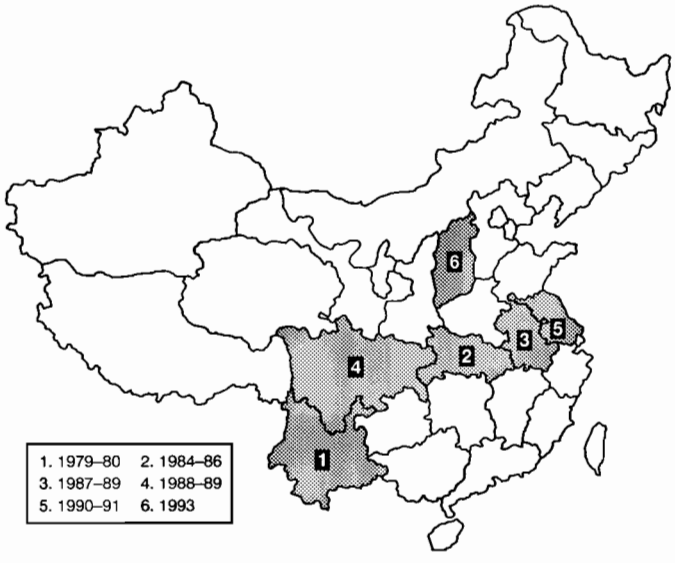

Figure 2. Outbreaks of bluetongue disease in sheep in China.

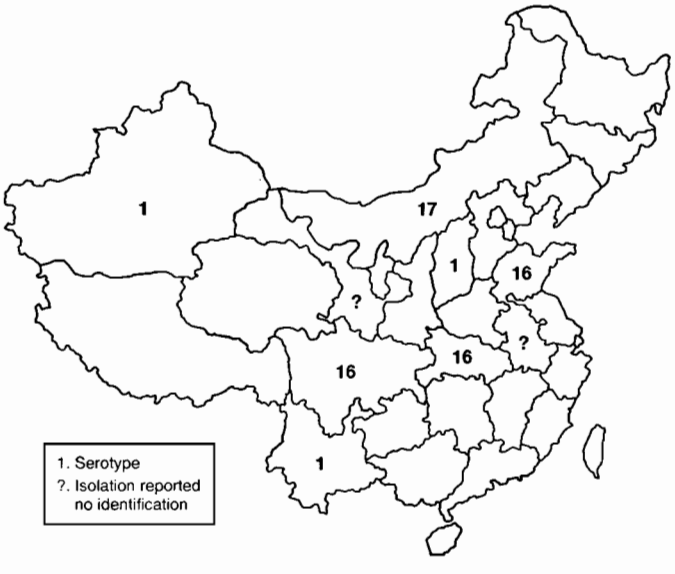

Figure 3. Bluetongue virus (BLU) isolations in provinces in China. (BLU - 1, 16, 17; ? - Isolation reported no identification).

in the same year. In 1993, another outbreak of bluetongue occurred in the Jiaocheng region of Shanxi Province, with 428 (8.2%) of the 5220 sheep affected and 98 (22.8%) dying. Viruses were isolated by Shanxi Provincial General Veterinary Station and Yunnan Tropical and Subtropical Animal Virus Disease Laboratory (Fig. 3).

Isolation of virus from sentinel sheep

From 1987, healthy sheep and goats had been introduced to high prevalence bluetongue areas, such as Xinjiang and the Bayannur region of Inner Mongolia, to establish sentinel stations. These animals were tested regularly in the anticipated bluetongue epidemic season. Viruses were isolated from whole blood from sentinel sheep, the samples having been collected and stored in Inner Mongolia one week before the blood showed AGID-positive. The same results were obtained by NQVI in 1989 from sheep at the Shandong sentinel station and in 1990 from cattle at the Gansu sentinel station.

Serological Study of Bluetongue Virus

General investigation of sheep sera

Serological tests, including AGID, complement fixation, fluorescent antibody, virus neutralisation, enzyme linked immunosorbent assay (ELISA), indirect haemagglutination, haemolysis inhibition and the preparation of monoclonal antibodies (MAb), were modified from published procedures. Antigen for the AGID test was prepared by international procedures. The standard methods of antigen preparation and quarantine were confirmed in China in 1982. A general survey of sera from all over China (Table 5) used the ELISA test, modified by NQVI and YAHVI, and found that:

- the positive rate was higher in the south of China than in the north (i.e. 9.7–35.8% in cattle in the south but 0–0.1% in the north);
- prevalence outside the epidemic area was 0–0.92% in sheep;
- prevalence in goats was higher than in sheep and cattle; and
- infection of suspicious animals all occurred south of 37°N, with none seen further north.

Identification of virus serotypes

The serotypes of viruses from sentinel animals in Inner Mongolia, Xinjiang, Hubei, Sichuan and Yunnan were examined by using sheep cross-protection, neutralisation and indirect agglutination tests. The results showed different strains from these areas.

Serotyping of viruses from blood samples from those Provinces and from Yunnan *Culicoides* by micro-neutralisation tests proved that the major serotypes in China were BLU1 and 16 (Table 6). The study of the epidemiology of bluetongue in China is continuing in Yunnan.

Table 5. Random general survey for bluetongue in China.

Years	No. of provinces and regions	Species	No. of animals tested	No. of seropositive animals (%)
1979–1989	7	sheep	40314	7406 (18.4)
1987–1989	27	sheep	276534	13096 (4.7)
1989	26	cattle	164575	12126 (7.3)

Table 6. Times of isolation, identification and distribution of BLU serotype.

Source of virus strain	Year of isolation	Date identified	BLU serotype
Hubei	1984	September 1994	16
Inner-Mongolia	1988	September 1994	related to 17
Sichuan	1988	September 1994	16
Shandong	1989	1990	16
Shanxi, I	1993	September 1994	1
Shanxi, II	1994	September 1994	1
Xinjiang	1989	September 1994, April 1995	1
Yunnan (*Culicoides*)	1993	August 1994	1
Yunnan, Y-33	1980	August 1994	1

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An Epidemiological Survey of Bluetongue in Yunnan Province, China

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Abstract

A total of 32821 sheep, goats, dairy cows and Chinese Yellow cattle, including five buffalo from Vietnam and 23 Yellow cattle from Myanmar, were surveyed for antibodies to bluetongue virus (BLU) by agar gel immunodiffusion (AGID). This survey gave a clear picture of BLU distribution in 86 counties of Yunnan Province. The 8253 (26.0%) positive animals were distributed in 82 counties, located between 21°30' to 29°N and 97°40' to 106°10'E. Buffalo and Yellow cattle had the highest infection rates (34.1% and 30.6% respectively): these were lower for goats (25.5%), sheep (9.2%) and dairy cows (0.8%). None of the seropositive animals showed clinical signs. The rate of positivity increased from mild climate zones to subtropical and tropical climate zones, and was inversely related to altitude (height above sea level). Disease regulatory authorities were alarmed by the high infection rates found in buffalo and Yellow cattle in the border regions between Yunnan Province and the adjacent countries of Vietnam and Myanmar, as cattle bred in Yunnan Province are often exported to inland provinces of China and could possibly spread bluetongue.

SINCE the discovery in 1979 of bluetongue at and around Shizong County Livestock Farm in Yunnan Province, clinical bluetongue cases have occurred in Wuhan areas, Hubei Province, China. As an emerging disease, bluetongue attracted the attention of relevant authorities because, by infecting sheep and other ruminant animals, and possibly causing high morbidity and mortality, the disease could cause great loss to the local livestock industry.

At that time, the diagnosis of bluetongue relied on clinical observation, virus isolation and bio-assay. It was impossible therefore to define the distribution of epidemic zones and the infection status of other animals in larger areas. By 1982, Yunnan Provincial Institute of Animal Husbandry and Veterinary Science (YPIAHVS) had developed a group-specific soluble agar gel immunodiffusion (AGID) antigen with a Yunnan bluetongue virus (BLU) strain and established diagnostic methods (for which they were awarded second prize for Science and Technology by the Ministry of Agriculture in 1983). Application of the AGID test for the detection of bluetongue provided a specific diagnostic method.

Following instructions from the Ministry of Agriculture, Animal Husbandry and Fishery and the Provincial Bureau of Animal Husbandry to investigate further the distribution and prevalence of bluetongue in Yunnan Province, we conducted a bluetongue survey in areas with the same or similar ecological conditions to Shizong County where the clinical bluetongue had occurred.

Materials and Methods

The diagnostic antigen and positive reference serum were developed and provided by YPIAHVS. Test performance and reading parameters were conducted using the 'Draft protocols of agar gel immunodiffusion test for bluetongue in animals' developed by YPIAHVS. Test sera were separated from bloods randomly collected by the relevant Zhou (district) animal husbandry and veterinary stations and stored at 4°C.

Sera were collected from buffalo, cattle, sheep and goats in 49 counties in 13 Zhous. Cattle imported from Vietnam and cattle, swine and horses from Myanmar were also sampled. The sampling rate was 5–10% in the selected herds.

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Results

Serological surveys

The geographical distribution and prevalence of bluetongue in Yunnan Province, as determined by our results, can be summarised as follows.

- Of the 19083 ruminant animals tested in 49 counties in 13 prefectures or districts in Yunnan Province, 5918 (31.1%) animals were positive to bluetongue with differences among the species(Table 1). Of the non-Chinese animals tested, all five buffalo from Vietnam were positive (100%), and 13 out of 23 cattle from Myanmar were positive (56.5%).
- Positive animals were detected in all 13 districts (Table 2), and in 48 out of 49 counties (Table 3).

Table 1. Prevalence of bluetongue antibodies in various domestic animals in Yunnan Province.

Animals	No. positive/no. tested (%)
Buffalo	1600/3708 (43.1)
Yellow cattle	2644/7307 (36.2)
Sheep	55/990 (5.8)
Goats	1616/6175 (26.1)
Dairy cattle	9/850 (1.1)
Horses	0/13
Pigs	0/40
Total	5918/19083 (31.1)

Table 2. Detection of bluetongue in various districts or regions of Yunnan Province.

District or region	No. positive/no. tested (%)
Lincang District	343/630 (54.6)
Dehong Zhou	615/1292 (47.6)
Simao District	313/719 (43.5)
Honghe Zhou	990/2332 (42.5)
Yuxi District	1346/3273 (41.1)
Xishuang Banna Zhou	28/73 (38.4)
Chuxiong Zhou	161/500 (36.2)
Wenshan Zhou	1391/4078 (34.1)
Zhaotong District	99/363 (27.3)
Lijiang District	53/231 (22.9)
Dali Zhou	116/761 (15.2)
Qujing District	372/3069 (12.1)
Kunming Municipality	66/1757 (3.8)

Geodistribution and epidemiological features

Forty-eight counties had a positive infection rate greater than 50%; 17 counties rates greater than 30%; 12 counties rates greater than 10%, and 7 counties rates less than 10%. Ruili County had the highest positive rate (79.4%) while Songming and Fuyuan Counties had the lowest positive rates (0.9%). The positive areas involving 48 counties were located between longitude 97°40' and 106°10'E and latitude 21°30' and 29°N. The high incidence and wide distribution in many new areas attracted much attention.

Testing of sera of ruminants without clinical signs was done with a group-specific AGID antigen. The infection was limited to ruminants: sera from a few non-ruminant animals were negative to bluetongue. Among ruminants, the highest infection rates were found in buffalo and cattle but no significant clinical signs were seen. Sheep and dairy cattle with low infection rates were also found to be free of clinical cases. The rank order by prevalence in ruminants was buffalo 43.7%, Yellow cattle 36.2%, goats 25.9%, sheep 5.8% and dairy cattle 1.1%. Many of the tested cattle and buffalo also had antibodies to epizootic hemorrhagic disease (EHD) viruses: the reason for the high level of association is unknown.

The distribution of bluetongue infection varied in relation to altitude and climate (Table 4). Positive rates of infection seemed inversely related to the altitudes above sea level ie. low altitude > medium altitude > high altitude, with rates in tropical zones being greater than in subtropical zones, which were in turn greater than in temperate zones. These results suggest that natural ecological conditions vary with altitudes and climate zones, causing different distribution and density of the *Culicoides* insect vectors.

Geographical distribution

The positive rates of infection in border areas were higher than those in inner areas. For example, the prevalence was from 43.1% to 54.5% in the six border districts or prefectures (Lincang, Dehong, Simao, Xishuang Banna, Wenshan and Honghe) and 3.8% to 41.1% in the seven inner districts or regions (Kunming, Dali, Chuxiong, Lijiang, Qujing, Yuxi and Zhaotong), indicating an association with tropical and subtropical climates of the border areas. It seemed the epidemic had been long established and there was a trend for the disease to spread from border areas to inner regions. It was thus possible that the border areas and beyond were the source of the infection.

Table 3. A serological survey of bluetongue by species and district distribution: Yunnan Province, Vietnam and Myanmar.

District or Zhou	County	Total no. animals tested		Buffalo		Yellow cattle		Dairy cattle		Goats		Sheep		Pigs or horses	
		positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%
Lincang	Shuangjiang	165/290	56.9	149/262	56.8	16/28	57.1								
	Genma	133/242	54.9	17/38	44.7	116/204	56.7								
	Cangyuan	42/92	45.6			2/7	28.5			40/85	47.0				
	Zhenkang	3/6	50.0			3/6	50.0								
Dehong	Luxi	337/639	52.7	84/137	61.3	29/63	46.0			219/334	55.0	5/105	4.7		
	Ruili	81/102	79.4	40/50	82.0	40/52	76.9								
	Lianghe	59/174	33.9	5/74	6.3					54/100	54.0				
	Yanjiang	41/102	40.2	2/3	66.6					39/99	39.4				
	Longchuan	84/252	33.3	12/83	14.5	12/50	24			31/46	67.4	29/73	39.7		
Simao	Zhenyuan	66/182	36.3	43/86	50.0	19/56	33.9			4/40	10.0				
	Simao	98/203	48.2			98/203	48.2								
	Manglian	106/206	51.5	79/164	48.2	27/42	63.3								
	Mojiang	43/128	33.6	18/43	41.8	4/23	17.4			21/62	33.8				
Banna	Jinghong	28/73	38.4	9/18	50.0	19/48	39.6			0/7					
Chuxiong	Yuanmao	159/235	67.6	99/178	76.1	32/50	64.0			28/29	95.5	0/26			
	Shuangbo	22/265	8.5	3/78	3.8	9/88	10.2				4.1				
Wenshan	Masupo	252/492	51.2	516/394	54.8	36/98	36.7								
	Xishou	232/517	44.9	69/138	50.0	154/359	42.2			9/20	45.0				
	Funing	233/554	42.1	121/270	44.8	112/284	39.4								
	Yanshan	159/500	31.8	40/125	32.0	48/199	24.1			71/176	40.3				
	Wenshan	200/733	27.2	102/269	37.9	70/246	28.4			28/218	12.8				
	Guangnan	177/478	37.0	51/142	35.9	126/336	37.5								
	Maguang	105/599	17.5	34/137	24.8	55/408	13.5			16/54	29.6				
	Quibei	33/205	16.1			8/98	8.1			25/107	23.3				
Yuxi	Yuanjiang	839/1548	54.2	80/159	50.3	503/1036	48.6			256/353	72.5				
	Eshan	184/425	43.2	153/202	75.7	7/19	36.8			24/199	12.1			0/5	0
	Yimen	192/2644	14.2			0/3				92/641	14.3				
	Xinping	231/3656	35.2	41/101	40.6	2/4	50.0			188/511	34.1				
Dali	Weishan	49/264	18.6			49/213	23.0			0/51					
	Wiangyun	29/272	10.7			25/173	14.5			4/99	4.0				

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		positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%	positive/ tested	%
	Nanjian	38/205	18.5	25/51	49.0	8/51	15.7			5/50	10.0	0/53			
	Yangbi	0/20				0/10				0/10					
Honghe	Jinping	249/548	45.3			240/522	45.9			9/26	34.6				
	Henkou	74/120	61.7			74/120	61.7								
	Honghe	259/483	53.6	23/42	54.7	198/387	51.2			38/54	70.3				
	Luchun	63/177	35.6			37/105	35.2			26/72	36.1				
	Yuanyang	319/901	35.4			269/685	39.3			50/216	23.1				
	Jiashui	26/103	25.2			6/25	24.0			20/30	66.6			0/*40	
														0/†8	
Qujing	Huize	54/966	5.6			3/11	27.3			48/382	8.2	3/372	0.8		
	Luoping	184/366	50.3			59/94	62.7			125/272	45.9				
	Luliang	103/431	23.8	28/99	28.3					68/294	30.4	7/108	6.4		
	Qujing	10/263	3.8			5/143	3.5	4/70	5.7	1/50	2.0				
	Xundian	17/613	2.8			11/307	3.6			6/306	1.9				
	Fuyuan	4/430	0.9			4/189	2.1			0/241					
Zhaotong	Qiasjia	99/363	27.3	29/93	31.3	57/90	63.3			13/130	10.0				
Lijiang	Yongsheng	53/231	22.9	8/44	18.2	13/32	40.6			22/80	27.5	10/75	13.3		
Kunming	Lunan	51/306	16.6	11/73	15.1	26/109	23.8			13/63	20.1	1/61	1.6		
	Yilian	7/330	2.1	0/16		0/8				7/306	2.3				
	Songming	3/341	0.9	3/182	1.6					0/93		0/66			
	Livestock Breeding Farm	0/409						0/409							
	Xiaoshao	5/140	3.5					5/140	3.5						
	Farm	0/31						0/31							
	No. 2 Farm	0/200						0/200							
Vietnam		5/5	100	5/5	100										
Myanmar		13/23	56.5			13/23	56.5								
	TOTAL	5918/19083	31.0	1600/3708	43.1	2604/7307	36.2	9/80	1.1	1610/6175	26.1	55/990	4.8	0/*40	
														0/†13	

*Pigs, †horses

Table 4. Altitude above sea level and climatic zone in relation to prevalence of bluetongue antibodies.

Items	Altitude (metres above sea level)			Climatic Zones		
	Below 1000 m (Low)	Above 1000 m (Medium)	Above 1800 m (High)	Above 20°C	Above 15°C	Above 10°C
No. counties tested	11	28	9	7	33	8
No. of animals tested	4444	9980	4578	2921	11777	4304
No. of positives	2181	3439	280	1539	4110	251
% of positives	49.2	34.5	6.1	52.7	34.9	5.8
% Range	27.3–79.4	2.1–67.6	0.9–22.9	27.3–79.4	2.1–56.9	0.9–22.9

Discussion

Yunnan Province borders Myanmar, Vietnam and Laos, and has some natural ecological conditions in common with these border countries. The survey showed that Yellow cattle from Myanmar yielded a positive rate of 56.6% and all five buffalo from Vietnam were positive. In border areas, trade fairs are frequent and busy. Yellow cattle and buffalo from adjacent countries are often on sale in the markets of the border counties in Yunnan Province. Border crossing or mixed pasturing is a common practice. In addition, as BLU is transmitted by blood-sucking insects, buffalo and Yellow cattle were heavily infected through insect bites and infestation. For example, in Ruili county which borders Myanmar, the positive rate was 79.4%; in Luxi County 52.7%; in Yanjiang County 40.2%; in Minlian County 51.5%; in Genma County 54.9%, in Jinghong County 38.4%; in Hekou County 61.7%; in Jingping County 45.3%; and in Mapo County 51.2% . These counties border Vietnam. The border counties are the main breeding areas for buffalo and Yellow cattle, and their frequent movement inland was a main cause of bluetongue spreading to inner counties in the Province. The results of the survey suggest that bluetongue was introduced into Yunnan Province from the neighbouring countries long ago.

The geographical and climatic conditions are very complex. Due to a great variation in altitude in Yunnan Province, tropical, subtropical and temperate climates exist in the same region or county, particularly in border areas, resulting in higher annual mean temperatures. Among 49 counties investigated, 40 counties have an annual mean temperature greater than 15°C and relative humidity greater than 70%. This suits the activity and survival of blood-sucking insects. There are 34 species of blood-sucking *Culicoides* in this Province: the main BLU vectors, *C. schultzei, C. gemellus, C. peregrinus, C. arakawae* and *C. circumscriptus,* exist in most parts of the Province. Hence, bluetongue has probably existed for a long time with a wide distribution in Yunnan Province.

Because the infected cattle do not show significant clinical signs, people are often unaware of the infection and the animals may carry virus for a long time. According to published accounts from the USA, viraemia may persist for more than one year in cattle after infection with bluetongue. Some experiments showed that bluetongue virus was isolated five years later from erythrocytes of inapparently infected cattle. The viraemia in sheep lasts more than three months, during which time the animals are infectious. Therefore, cattle carrying bluetongue virus would be the main source of infection for bluetongue in Yunnan Province. Furthermore, this survey found no clinical signs in the infected sheep, even though the infection rate in cattle was very high in the same area. Whether the antibodies were produced by cross infection with other related viruses has not been confirmed. Further study and classification of the virus serotypes remained to be done.

In summary, Yunnan Province has the conditions necessary for bluetongue disease to occur and spread: vectors (blood-sucking *Culicoides*); virus-carrying cattle without clinical signs; susceptible animals such as sheep, goats and cattle; and the appropriate natural ecological environment. Since the disease was introduced, it has persisted, spread and been transmitted, with the border region of Yunnan Province as the natural focus area. The 1979 outbreak of bluetongue in sheep in Shizong County can be assumed to have been an example of the disease spreading from border areas to the inner parts of the Province. The survey further indicated that Shizong County was not a single isolated focus of bluetongue infection, but merely the first place where clinical signs were found.

Bluetongue in Yunnan Province has a wide distribution and high morbidity. Any proper and active control measures should take account of the natural and economic conditions of the Province. It is not

recommended that the positively reacting animals be massively slaughtered: rather they should be fed and used as draught animals in locally confined areas and prohibited for use as breeders. In North America and Australia, movement of positive animals has been restricted and this precedent may be followed in China.

The serological survey also revealed that 13/23 Yellow cattle from Myanmar and 5/5 buffalo from Vietnam were positive. There were no reports of bluetongue in these two countries before 1985, according to the FAO Veterinary Year Book. Since this study is the first time that bluetongue antibodies were detected in animals from these two countries, this provides some information for bluetongue research and control there.

The diversity of BLU serotypes complicates control of the disease. To prevent new introductions into the Province, based on the identity of BLU serotypes and their distribution in Yunnan Province, animal quarantine should be strengthened and all seropositive animals prohibited from entry.

Epidemiological Investigations and Control of Bluetongue Disease in Jiangsu Province, China

Zhu Changgui*, Li Yamin*, Shao Cuili*, Xu Shibai† and Zheng Tianran§

Abstract

In 1986 the first bluetongue infections were detected in dairy cows imported from Denmark, using agar gel immunodiffusion (AGID) and ELISA serological tests. In 1988 an epidemiological investigation was carried out throughout Jiangsu Province. Bluetongue antibodies were found in 60/3853 (1.5%) cattle sera and in 63/2426 (2.2%) sheep and goat sera. Serological and clinical observations showed the existence of bluetongue infection in some areas of Jiangsu Province. All seropositive animals were slaughtered, and quarantine and vaccination programs were enforced. The disease was effectively controlled in the Province.

IN 1986, among 32 cows imported from Denmark to Lianyungan City, Jiangsu Province, four animals tested positive for bluetongue antibodies by agar gel immunodiffusion test (AGID) and enzyme linked immunosorbent assay (ELISA) serological tests. Until then, bluetongue positives had never been reported. This result caused special attention to be directed towards the control of bluetongue.

In 1988, a bluetongue epidemiological survey and eradication program were initiated. Some cows, goats and sheep were seropositive although no clinical signs were observed. Most of the seropositive animals were slaughtered but some were kept in isolation without clinical signs being observed.

In the flood season of 1991, clinical bluetongue occurred in Tongshang County, Jiangsu Province, with sera positive by AGID. An epidemiological investigation began soon after the disease outbreak. All animals that were seropositive or had clinical signs of bluetongue were slaughtered. From spring 1994, a vaccination program was enforced for cattle, sheep and goats in the infected area. Bluetongue has been effectively controlled. This paper reports the results of the epidemiological investigations and the vaccination and eradication programs.

Animal Investigations

In Jiangsu Province 76 cattle and 14 sheep or goats in herds or flocks were studied. Some individual animals were also tested. Full clinical examinations were carried out and blood samples collected from all males and 20% of females, breeders and other animals. In total, 3798 dairy cattle, 511 goats and sheep were tested, as well as 229 individually owned buffalo. The AGID test was carried out using the standard protocols and reagents of the Ministry of Agriculture.

No clinical signs of bluetongue were observed. Four dairy farms and one sheep farm proved positive, while a further two dairy farms and one sheep farm were suspect. Forty-two cows and one sheep, plus 55 individual buffalo, tested positive (Table 1).

Epidemiology During Outbreak in Tongshang County

In August 1991, bluetongue broke out in sheep flocks near a border in Tongshang County, Jiangsu Province. The sheep had persistent high fever (41–42°C), swollen faces and muzzles, buccal erosions, excess

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salivation and swollen blue tongues. Lameness and paralysis were observed in some sheep. The clinical course lasted 7 to 14 days. Of the more than 100 clinically ill sheep, 30 died. All remaining clinical cases were slaughtered.

Two serum samples collected two and four days after recovery tested negative by AGID, although three samples collected 15 days after recovery tested AGID positive at the Qingdao Institute for Animal Inspection. After the laboratory confirmation, serum samples from 12 valleys and farms showed 14/34 sheep, 4/58 goats and 4/13 Chinese Yellow cattle were AGID positive.

Prevention and Control

As bluetongue had never before been reported in Jiangsu Province, a restriction and control policy was enforced to prevent further spread of the disease. Four seropositive cows in an imported dairy herd, and all animals demonstrated or suspected as being seropositive in the AGID test, were slaughtered. Some seropositive buffalo in Yizhen and seropositive cows in the Zaicheng dairy farm in Lishiu County were kept under isolation, rather than being slaughtered, as there were too many seropositives. This slaughter out policy for seropositive sheep in Tongshang County continued in 1993 and 1994. However, because of the difficulty of slaughtering all seropositives in such a large area, a vaccination program was introduced in the risk area after spring 1994. Cows, sheep and goats were inoculated with the vaccines, which contained two serotypes of attenuated bluetongue virus produced by the Yunnan Animal Husbandry and Veterinary Medicine Institute. From 63% to 93% of animals in the infected area were vaccinated.

Effects of Preventative Measures

The combination of slaughter or isolation of seropositive animals and vaccination of the remainder reduced morbidity remarkably. On some farms, no seropositive animals have been detected since the original seropositive animals were slaughtered in 1988. However, the seropositive prevalence has remained high where positive animals were detected in 1988 and not slaughtered.

To examine the effects of prevention and control, serological investigations were again conducted, in March 1993, in some of the areas where positive animals were detected in the earlier epidemiological surveys. If the original farm with seropositives had closed down, blood samples were collected from surrounding areas or farms (Table 2).

The slaughter of seropositive animals, especially when these comprised whole flocks, removed the threat of bluetongue infection from flocks and farms (Table 2). In the Zaicheng dairy farm, where the seropositive cows were not slaughtered but raised in isolation after 1988, the seropositive rate remained high. In the buffalo in Yizhen, where the 1988 seropositives were again not slaughtered, the positive rate in the recent investigation was high, at 46%. In Tongshang County, four sheep showed clinical signs in 1992 but were not slaughtered and no other measures were taken. The morbidity and mortality increased dramatically in 1993 when 34 sheep had bluetongue signs.

In Tongshang County, the slaughter and vaccination programs have meant that bluetongue morbidity has decreased year by year since the 1991 outbreak (Table 3). In 1994, only one sheep, which had missed vaccination, demonstrated bluetongue signs.

Table 1. Bluetongue positive farms and animals detected by AGID during 1988 study in Jiangsu Province.

Farms and areas	Species	No.	Results			
			Positive		Suspected	
			No.	%	No.	%
Zaichen dairy farm, Lishui County	Dairy cow	64	38	59		
Dairy breeder farm, Heian County	Dairy cow	20	2	10		
Dairy farm, Rudong County	Dairy cow	61	1	2		
Dairy farm, Nantong County	Dairy cow	23	1	4		
Dairy farm, Dantu County	Sheep	10	1	10		
Fumazhuan dairy farm, Zhengjiang	Dairy cow	50			1	2
Huangtang dairy farm, Zhengjiang	Dairy cow	31			1	3
Xinkun sheep farm, Qidong County	Sheep	30			5	17
Yizhen City	Buffalo	119	55	46		

Table 2. Comparisons of AGID positive rates in infected farms or areas before and after preventative and control measures.

Location	Species	Results from recent surveys			% positive in 1988	Remarks
		No. tested	No. positive	%		
Zaichen dairy farm Lishui County	Dairy cow	20	5	25	59	Original cows on farm all slaughtered
Heian County	Cows, sheep	23	0	0	10	Original cows on farm all slaughtered
Gongxiao breeder farm, Rudong County	Dairy cow	12	0	0	1.6	Original farm closed
Dairy farm, Nantong County	Dairy cow	12	0	0	0	
Rongbin District, Dantu County	Sheep	9	1	11	10	Original farm closed, samples collected from animals nearby
Fumazhuan dairy cow farm, Zhenjiang	Dairy cow	22	0	0	Suspected	
Huanghai sheep farm, Qidong	Sheep	10	0	0	Suspected	Original farm closed
Dongxin dairy farm, Liangyugan	Dairy cow	18	0	0		
Yizhen County	Buffalo	16	6	37	46	Samples came from 119 animals tested in 1988

Table 3. Morbidity and serological investigation of bluetongue in Tongshang County after preventative measures.

Year	Diseased		AGID test results			Remarks
	Species	No.	Species	No. tested	No. of positives	
1991	Sheep	>100	Sheep, goats, cows	305	22	All diseased and seropositive animals slaughtered
1992	Sheep	4				Not slaughtered
1993			Cows	64	5	Positives slaughtered, remainder vaccinated.
1994 (March)			Cows	64	5	Positives slaughtered, remainder vaccinated.
1994 (Dec)	Sheep	1	Sheep	56	1	Diseased and seropositive sheep, 16 of 56 vaccinated

Discussion and Conclusion

The epidemiological investigations in Jiangsu Province, especially in Tongshang County, clarified the regional bluetongue situation. Before 1991, only seropositive animals were known and no clinical cases were reported. The first outbreak in Jiangsu Province was restricted to a small area of Tongshang County and did not spread through the Province because of the combined preventative measures of slaughter and vaccination. Clinical bluetongue was found mainly in sheep, with rare exceptions of cases in goats and cows.

All the above results emphasised the importance of slaughtering clinically ill and seropositive animals to prevent and control the spread of bluetongue. Slaughter of seropositives, especially where they comprised 10% or more of the animals, freed farms of blue-

tongue. Where no slaughter occurred (on the Zaicheng dairy farm and with the Yizhen buffalo), the infection rate remained high (Table 2)

The vaccination program was very effective (Table 3) as the vaccine had a high efficacy. No clinical cases occurred in 3264 vaccinated sheep. Blood samples from 40 of the vaccinated sheep were negative in AGID testing, showing that the vaccine did not induce AGID antibody. However, some unvaccinated animals were positive in AGID testing, which indicated that the vaccine could prevent infection with virus.

An Epidemiological Study of Bluetongue in Anhui Province, China

Zhou Weihan*

Abstract

Between 1987 and 1991, bluetongue was reported by three counties in Anhui Province, and was recognised by the isolation of bluetongue virus (BLU). Sheep morbidity was 33% and mortality 11.3%. A serological survey of domestic ruminants showed that the seropositive rate in the epidemic region was higher than in a non-epidemic region. Local cattle, buffalo and sheep had higher positive rates than goats and dairy cattle. Merino sheep were more likely to be seropositive than Corriedale and Romney sheep, as were grazing herds compared to sheltered feeding herds, but there was no age-related difference in prevalence. Twelve species of *Culicoides* midges are found in Anhui. The dominant mammal-feeding midges are *C. oxystoma, C. homotomus* and *C. nipponensis*, with *C. homotomus* as a potential BLU vector in this region. Local cattle and buffalo may be the overwintering hosts for BLU.

AN outbreak of bluetongue occurred in Chuzhou City in Anhui Province. The isolation and identification of bluetongue virus (BLU) by the Animal Quarantine Institute, Qingdao, confirmed the diagnosis. From then on, comprehensive investigations and observations were conducted on the epidemiology of bluetongue. This paper reports the preliminary results.

The Occurrence of Bluetongue

Between 1986 and 1992, outbreaks of bluetongue occurred in two locations in Anhui Province, at about 32°30'N, 118°E; one at a sheep breeding farm in Lanya District, Chuzhou City, the other in the border area between Xiaoxian and Suixi counties (Table 1). Intensive sheep production occurs at both locations.

Serological Investigation

Between 1986 and 1992, 15 383 domesticated ruminants, in 40 counties and cities of Anhui Province, were tested for BLU antibodies by agar gel immunodiffusion (AGID), using antigens provided by the Yunnan Provincial Institute of Animal Husbandry and Veterinary Science and the Animal Quarantine Institute, Qingdao. The average rate of seropositives was 21.8% (range from 0–75%), with no positive results detected in 602 animals in eight counties and cities. Statistical analysis showed the prevalence rates among Yellow cattle, buffalo and sheep were not significantly different ($P>0.05$), although rates among these three were significantly greater ($P<0.01$) than in goats and dairy cattle (Table 2).

The overall prevalence was significantly greater in epidemic areas than in non-epidemic areas (Table 3): the prevalences in Yellow cattle, sheep and goats in epidemic areas were significantly higher than in the corresponding domestic species in non-epidemic areas (too few buffalo and dairy cattle were studied for adequate comparisons). In epidemic areas, the seropositive rate among sheep was greater than among Yellow cattle, which in turn was greater than among goats ($P<0.01$). In non-epidemic areas, positive rates among Yellow cattle and buffalo were greater than among sheep, which were greater than among goats, which in turn were greater than among dairy cattle ($P<0.01$). The positive rate was higher in sheep than in Yellow cattle in the epidemic areas, while in the non-epidemic areas the positive rate among Yellow cattle and buffalo was higher than that among sheep.

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Table 1. Annual prevalence of bluetongue in Anhui Province, China, 1986–1991.

Year	Months	Foci	No. of animals	No. of sick animals (%)	No. of deaths (%)
1986*	July–August	2 holdings (Chuzhou)	236	130 (55.1)	34(26.2)
1987	June–August	5 holdings (Chuzhou)	914	507 (55.5)	49 (9.7)
1989	August	5 holdings (Chuzhou)	378	160 (42.3)	8 (5.0)
1989	August–October	6 townships (Xiaoxian)	3530	810 (22.9)	37 (4.6)
1991	September–October	9 townships (Xiaoxian)	12358	2794 (22.6)	946 (33.9)
1991	August–October	Maqiao, Suixi	17000	6635 (39.0)	179 (2.7)
Total			34416	11036 (32.1)	1250 (11.3)

*The annual prevalence in 1986 was derived retrospectively.

Table 2. Prevalence of bluetongue antibodies in livestock in Anhui Province.

Species	No. positive/no. tested (%)
Yellow cattle	1231/4554 (27.0)
Sheep	1209/4724 (25.6)
Buffalo	361/1464 (24.7)
Goats	514/3811 (13.5)
Dairy cattle	37/805 (4.6)
Total	3352/15358 (21.8)

Within a single test unit, tests done at the same time showed positive rates among Merino sheep to be greater ($P<0.01$) than among Corriedales, which in turn was greater ($P<0.01$) than among Romneys (Table 4).

A comparison was made between different feeding patterns among goats and sheep in epidemic areas in Xiaoxian County: the positive rates among grazing herds were higher than those among sheltered feeding herds (Table 5). (Cattle have the same feeding patterns so no comparisons were appropriate.) The relationship between age and prevalence was also investigated, in Yellow cattle and sheep of different ages in Fengtai County, a non-epidemic area (Table 6). Positive rates among animals of different ages were not significantly different ($P>0.05$).

Investigation of *Culicoides* Species as Possible Bluetongue Vectors

Culicoides midges were captured by nets and light traps from different animal houses and their surroundings, and also by suction from the skin of bait animals. In this way 22 096 *Culicoides*, identified to 12 distinct species (Table 7), were caught in 33 counties and cities. *Culicoides oxystoma, C. homotomus, C. nipponensis* and *C. arakawae* were the dominant species in the Province, with *C. mihensis* and *C. actoni* less prevalent.

Electrophoresis of *Culicoides* meals was used to demonstrate that *C. oxystoma, C. homotomus, C. nipponensis, C. mihensis* and *C. actoni* feed on the blood of various animals (buffalo, Yellow cattle, dairy cattle, donkeys, goats, sheep and pigs) while *C. arakawae* feeds on the blood of chickens.

Analysis of *Culicoides* species as possible bluetongue vectors

Culicoides actoni was captured in 10 counties in Anhui Province, three of which (Chuzhou, Xiaoxian and Suixi) were in the bluetongue epidemic areas. *Culicoides actoni* has been confirmed as being able to transmit BLU in China. However, because of the small number of *C. actoni* captured, representing only 0.2% (17/7732)of the total captured in Chuzhou, 0.8% (14/1818) in Xiaoxian and 1% (2/194) in Suixi, the seasonal occurrence of the species could not be determined. Further work remains to be done.

Table 3. Seropositive rates in epidemic and non-epidemic areas in Anhui Province.

	Yellow cattle (no. positive/ no. tested)	Buffalo (no. positive/ no. tested)	Sheep (no. positive/ no. tested)	Goats (no. positive/ no. tested)	Dairy cattle (no. positive/ no. tested)	Total (no. positive/no. tested)
Epidemic	716/2446	3/12	814/2862	382/2029	1/1	2016/7350 (27.4%)
Non-epidemic	515/2108	358/1452	295/1862	132/1862	36/804	1336/8008 (16.7%)

Table 4. Prevalence of bluetongue antibodies in different breeds of sheep.

Breed of sheep	No. positive/no. tested (%)
Merino	14/82 (17.1)
Corriedale	4/138 (2.9)
Romney	0/82 (0)

Table 5. Prevalence of bluetongue antibodies in sheep with different feeding patterns.

Feeding pattern	Goats (no. positive/ no. tested)	Sheep (no. positive/ no. tested)
Sheltered feeding	41/720	173/1127
Grazing	120/400	540/1073
Significance	P< 0.01	P< 0.01

Culicoides homotomus is the dominant species feeding on domestic mammals in Anhui Province. It is also the dominant species in the two epidemic counties, comprising 31.3% (2418/7732) of the total captured in Chuzhou and 23.9% (434/1818) in Xiaoxian county. In Suixi County the number of *C. homotomus* comprised 54.1% of the total captured, which was greater than the number of *C. oxystoma.*

Bluetongue outbreaks were associated with the activity peaks of *C. homotomus.* In Chuzhou, the peak activity period of *C. homotomus* falls between 15 May and 13 July. Bluetongue prevalence among sheep occurred from June to August. In Xiaoxian, peaks in *C. homotomus* populations occur between 10 July and 25 August, and bluetongue occurred in sheep from August to October. Taking intrinsic and extrinsic incubation periods into account, the activity peak of *C. homotomus* was considered to be associated seasonally with bluetongue.

Culicoides homotomus is found exclusively in China and Japan, so its vector function may not have been studied in other countries. As both *C. homotomus* and *C. variipennis* (a *Culicoides* BLU vector in USA) are in the same subgenus, *C. homotomus* is a possible suspect as a BLU vector in Anhui Province. In Chuzhou County, *C. nipponensis* comprised 18.1% (1401/7732) of the total captured, although only 6.2% (12/19) in Suixi and 0.8% (15/1818) in Xiaoxian. The difference in the numbers captured in the two epidemic areas was highly significant (P<0.01). In the counties in Anhui Province north of the Huaihe River, *C. nipponensis* was captured in significant numbers. However, the possibility of *C. nipponensis* being a bluetongue vector is quite low.

Table 6. Prevalence of bluetongue antibodies in animals of different ages (no. positive/no. tested).

Age in years	1	2	3	4	5	6	7	8	9	10	Total
Yellow cattle	8/38	19/22	15/48	28/58	14/34	6/23	/20	2/4	1/1	0/1	98/298 (32.8%)
Sheep	1/12	0/8	0/4	1/2	1/5	–	–	–	–	–	3/31 (9.7%)

Table 7. *Culicoides* species identified in 33 counties and cities of Anhui Province.

Culicoides species	No. captured	% of total	No. of counties in which collected
C. oxystoma	10719	48.5	32
C. homotomus	6587	29.8	30
C. nipponensis	3153	14.3	26
C. arakawae	1423	6.4	25
C. mihensis	120	0.5	7
C. actoni	77	0.3	10
C. maculatus	5	0	2
C. sigaensis	4	0	3
C. pulcaris	3	0	3
m (unidentified)	3	0	1
C. matsuzawai	1	0	1
b (unidentified)	1	0	1
12 species identified	22096	100	33 counties investigated

Culicoides oxystoma, the largest population overall, comprised 48.2% (3727/7732) of the total number of *Culicoides* captured in Chuzhou, 66.1% (1202/1818) of those in Xiaoxian, and 28.4% (55/194) of those in Xuixi. However, as there was no apparent association of its seasonality with bluetongue, more data would be needed to consider *C. oxystoma* as a BLU vector.

The other *Culicoides* species are less suspect as BLU vectors, either because they do not feed on mammals, because their distribution is unrelated to the epidemic counties, or because they are less prevalent overall.

Discussion and Conclusion

In 1987, an outbreak of bluetongue occurred in Chuzhou city, Anhui Province and was confirmed by virus isolation. Since then, bluetongue has been reported in three counties, affecting 11 036 sheep with a morbidity of 32.1% and mortality of 11.3%. Other livestock in the epidemic areas were not affected. Bluetongue disease occurred mostly between June and October, with the peak from July to September. In addition to *Culicoides* activity and feeding habits, farming practices must be considered. Farmers have recently begun to postpone shearing by more than a month to encourage greater wool production. Shearing takes place in mid-June in Chuzhou city and in the first ten days of July in Xiaoxian County. As bare sheep are more susceptible to *Culicoides* attack, this practice was probably one of the factors accounting for the seasonal dynamics of the disease.

Serological examination by AGID antigen was conducted on 15 358 ruminants, with 21.8% (3352) testing positive. In descending order of prevalence, seropositives were found among Yellow cattle, buffalo and sheep; goats; and dairy cattle. Prevalence in epidemic areas was greater than in non-epidemic areas. Among sheep, prevalence was greatest in Merinos, followed by Corriedales, followed by Romneys, and greater in grazing flocks than in sheltered flocks. Prevalence in Yellow Cattle and goats showed no significant difference by age or sex.

Culicoides actoni and *C. homotomus* were considered the most likely BLU vectors in Anhui Province. It is therefore recommended that their seasonal occurrence and their relationship with disease prevalence be investigated. There is a need to prepare BLU fluorescent antibody so as to be able to examine the insects for their ability to carry virus. It is important to isolate BLU from *Culicoides* as a first step to ascertaining which species are vectors.

As bluetongue is an arthropod-borne disease, there is a practical question to be answered to aid in research and control, namely, where does the virus overwinter when there is no *Culicoides* activity? These surveys revealed that buffalo and Yellow cattle had the highest seropositive rates and that there were no significant differences in these rates among buffalo, Yellow cattle and sheep in epidemic areas. However, in non-epidemic areas positive rates among buffalo and Yellow cattle were significantly higher than among sheep. Data show that viraemia in sheep lasts 63 days, far less than the 107 days of the non-*Culicoides* activity period in Anhui Province, while viraemia in Yellow cattle may last as long as 42 months. One can thus assume, provisionally, that buffalo and Yellow cattle are the overwintering hosts of BLU in Anhui Province, although the possibility of vertical transmission in *Culicoides* does exist. An attempt is being made to rear *Culicoides* experimentally to study the overwintering dynamics, and to establish an experimental colony of *Culicoides* to elaborate the mechanisms of this aspect.

At the beginning of the investigation, the source of infection was suspected of being linked to the importation into a sheep farm of breeding sheep from other countries. Efforts were directed towards determining whether this notion was correct. As the investigations continued, relatively high prevalences of bluetongue antibodies were detected not only in counties which had no relationships with the farms that had imported sheep but also in areas where no sheep had ever been raised. For example, in Jingde County, which is 200 km away from the farm which imported sheep, the prevalence among Yellow cattle was 75% (15/20). Anecdotally, the author treated a sick sheep in 1955 which, in retrospect, could have been a case of bluetongue. From the investigation of *Culicoides* in the whole Province and other evidence, bluetongue appears to have existed in Anhui Province for many years. There is certainly insufficient evidence to support the hypothesis that bluetongue was introduced to Anhui from outside China.

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Epidemiological Investigations and Isolation of Bluetongue Virus in Gansu Province, China

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Abstract

Serological testing in Gansu Province from 1981 to 1982 showed that 28/6488 (0.4%) cattle and 15/6073 (0.3%) sheep were positive for bluetongue group antibodies as indicated by agar gel immunodiffusion (AGID). A virus isolated from cattle was found to show bluetongue virus (BLU) characteristics by electron microscopy, polyacrylamide gel electrophoresis (PAGE), AGID and sheep inoculation. This was the first isolation of bluetongue virus from cattle in China.

BLUETONGUE virus (BLU) causes disease in sheep but is carried by cattle as a silent infection. Although it is therefore relatively difficult to isolate bluetongue viruses from cattle, this was achieved in this study.

Materials and Methods

An epidemiological survey was carried out in 14 counties of Gansu Province and on Shandan Farm. Sera were collected from cattle and sheep in the same areas and testing for bluetongue antibodies was carried out by standard methods of agar gel immunodiffusion (AGID).

To attempt virus isolation, a heparinised blood sample was collected from a cow which tested positive by AGID on two occasions (Littlejohns 1981). The blood was lysed and used to inoculate BHK21 tissue culture. The cell sheets were examined daily for cytopathic effects (CPE) and blind passaged three or four times.

The virus was purified by ultracentrifugation and RNA-PAGE procedures (Cowley and Gorman 1988) then stained with phosphotungstic acid. The newly-isolated virus was inoculated into 3 one-year-old sheep which had tested negative for bluetongue antibodies. The inoculation dose of 3 mg of the ultracentrifuged virus was administered subcutaneously. The sheep were observed daily and their body temperature monitored. Blood was collected daily for seven days. A nucleic acid probe was used in a Dot-Blot test to assay the blood samples collected seven and eight days post-inoculation. The inoculated sheep were tested for specific bluetongue antibodies at 18 days post-infection .

Results and Discussion

Gansu Province is located in the area between 32°31'–42°57'N and 92°13'–108°46'E. It comprises a high plateau with a complex geological structure. *Culicoides* biting midges are active in the area.

Sheep and cattle in 14 counties and on Shandan Farm were surveyed at random, showing that overall 28/6488 (0.4%) cattle and 15/6073 (0.3%) sheep were positive for bluetongue group antibodies by AGID (Table 1). In the mountainous area (Longnan County), 16/699 (2.3%) cattle and 9/619 (1.5%) sheep were seropositive. In a plains area (Shandan Farm), the prevalence was 8/108 (7.4%) in cattle. Most cattle were local breeds, with only two being crossbreeds.

The CPE in BHK21 cells commenced at 96 hours in the third passage and at 72 hours in the fourth passage. The CPE developed in the same way at the fourth passage level as standard bluetongue virus used as a control. The newly-isolated virus produced a standard positive reaction in a bluetongue AGID test when used as antigen.

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Table 1. Results of serological survey for bluetongue antibodies by agar gel immunodiffusion (AGID) in cattle and sheep in Gansu Province.

Region	Cattle no. positive/no. tested (%)	Sheep no. positive/no. tested (%)	Total no. positive/no. tested (%)
Baiyin	0/413	0/407	0/820
Dingxi	0/410	0/460	0/870
Gannan	0/407	0/432	0/839
Jiayuguan	0/102	0/86	0/188
Jiuquan	1/601 (0.2)	1/581 (0.17)	2/1182 (0.2)
Jinchang	0/124	0/156	0/280
Lanzhou	0/766	0/730	0/1496
Linxia	0/506	0/500	0/1006
Longnan	16/699 (2.3)	9/619 (1.5)	25/1318 (1.9)
Pingliang	0/553	5/502 (0.1)	5/1055 (0.5)
Qingyang	3/591 (0.5)	0/601	3/1192 (0.3)
Shandan Farm	8/108 (7.4)		8/108 (7.4)
Tianshui	0/513	0/203	0/716
Wuwei	0/221	0/224	0/445
Zhangyie	0/474	0/572	0/1046
Total	28/6488 (0.4)	15/6073 (0.3)	43/12561 (0.3)

The virus was spherical, some 60–65 nm in diameter when photographed with an electron microscope (uranium acetate stain), and with a typical orbivirus structure. No other virus particles were seen. An RNA-PAGE preparation showed the 3.3.3.1 pattern typical of bluetongue.

No clinical signs were observed in any of the three sheep inoculated with the ultracentrifuged concentrate, and only one showed a rise in body temperature, to 40°C. This suggested that the virus of cattle origin was of low virulence for sheep. At 18 days post-inoculation, serum from the sheep with the temperature rise proved positive for bluetongue antibodies by AGID. This serum sample was also weakly positive in a Dot-Blot hybridisation with a cDNA probe. These results were consistent with the low virulence of the inoculum.

In summary, a bluetongue virus was isolated from a healthy cow in Gansu Province. This is the first isolation of bluetongue virus from a cow in China. The distribution of animals with bluetongue antibodies in Gansu Province varied with the terrain.

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Bluetongue Epidemiological Survey and Virus Isolation in Xinjiang, China

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Abstract

An epidemiological survey of bluetongue was carried out in 17 counties of Xinjiang. Agar gel immunodiffusion (AGID) tests on 160 671 ruminant sera showed that the proportion of seropositives was highest in goats and declined through sheep and cattle, while all yaks and deer were seronegative. As positives were found as far north as 48°N, this survey has revised the thesis that bluetongue did not exist beyond 45°N. There was also confirmation of a relationship between the distribution of bluetongue and *Culicoides* insects as well as mosquitoes. Males of *Culicoides pseudosarinanus* Kieffer were first reported in Xinjiang. Bluetongue virus (BLU) was isolated, and identified by immunofluorescence antibody, electron microscopy, agar gel immunodiffusion (AGID) and polyacrylamide gel electrophoresis (PAGE) of the viral genome RNA. Neutralisation tests indicated that the virus was serotype 1 (BLU1). The virus strain was inoculated into goats, where it caused pathological changes in target organs of the foetus by crossing the placenta.

IN 1986, a general survey of animal diseases in Xinjiang identified bluetongue-seropositive goats, sheep and cattle in several counties, with a high incidence in some sheep and goat flocks. Seropositive goats, sheep and cattle were also found during veterinary quarantine inspection. To study bluetongue further in Xinjiang, an epidemiological survey and virus isolation and identification procedures were carried out.

Materials and Methods

Serological investigations

In 86 counties, sera samples were taken from ruminants, including 114 568 sheep and goats, 45 664 Yellow cattle, 372 yaks and 67 deer. Overall, 160 671 sera were tested by agar gel immunodiffusion (AGID) for bluetongue antibodies to enable epidemiological estimations. AGID bluetongue antigen and positive reference sera were provided by Yunnan Provincial Institute of Animal Husbandry and Veterinary Science (YPIAHVS), and the Animal Quarantine Institute, Ministry of Agriculture, Qingdao.

As a natural infection experiment, goats and sheep that had proved seronegative after two serological tests two weeks apart were introduced into flocks with high bluetongue infection rates in Qunke Ranch, Weili County, before the mosquito and *Culicoides* activity season. Weekly blood samples were taken into anticoagulant (1 mL 7.6% sodium citrate plus 9 mL blood) and stored at 4°C. Sera were tested by agar gel immunodiffusion (AGID) to detect any seroconversion. Anticoagulant blood samples taken two weeks before seroconversion were used retrospectively for bluetongue virus (BLU) isolation. All introduced animals were examined clinically each week.

Point monitoring of vector *Culicoides*

Culicoides were collected regularly at fixed points at Qunke Ranch, Weili County for species identification and to estimate prevalence.

Virus isolation

Anticoagulant whole blood kept at 4°C was centrifuged at 1000 rpm for 10 minutes and the supernatant discarded. The sediment was reconstituted with minimal essential medium (MEM) to the original blood

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volume, stored, and frozen and thawed twice to release virus particles. Before inoculation, a bacteriological examination was carried out, with penicillin and streptomycin (1000 iu/mL) added at the time of inoculation.

BHK21 cells were grown in a medium of equal volumes of 5% lactalbumin hydrolysate (Earle's) solution and Earle's MEM with 10% calf serum and an appropriate amount of antibiotics. When monolayers formed, they were inoculated with treated anticoagulant blood in the maintenance medium volume and incubated at 37°C for one hour. MEM mixture was then used as a maintenance medium, and the cells were examined for cytopathic effects (CPE) daily.

Seven- to eight-day-old chick embryos were each inoculated with 0.2 mL treated anticoagulant blood, incubated at 33.5°C and examined daily for seven days (Li Zhihua and Peng Kegao 1989). Chick embryos dying within 24 hours were discarded. Chick embryos dying after 24 hours were kept at 4°C and the dead embryos prepared in 1:5 saline emulsions, with penicillin and streptomycin added, and further passaged.

BHK21 cell-adapted virus was used to prepare the AGID antigen. Virus was harvested when cells had been cultured for 72 hours and CPE was evident. When the affected cells comprised more than 70% of the total, the cells were frozen and thawed three times, sonicated for 3 minutes, then centrifuged at 3000 rpm for 30 minutes. The deposit was discarded, and the supernatant was concentrated (100 fold) with polyethylene glycol (PEG) 2000 and inactivated with 0.01% sodium azide. The antigen prepared from isolated virus was tested with positive reference sera by AGID (Lin Lihui et al. 1989).

Cell-adapted virus smears and frozen sections of chicken embryo tissue virus were stained with fluorescein-labelled antiviral antibody (Anon. 1989). Hanging drops were negatively stained. Clarified tissue culture supernatant was absorbed onto carbon-coated copper grids. These were then absorbed with 1:10 rabbit-anti-sheep serum at 37°C for 30 minutes, followed by reaction with bluetongue antiserum at 37°C for 30 minutes, and then with the BLU test at 37°C for 30 minutes (BHK21). Cell-passaged virus culture suspension was frozen and thawed repeatedly, purified by centrifugation and chromatography, stained with 3% phosphotungstic acid, and examined using a DXB-12 transmission electron microscope adjusted to 300 000 total magnification (this work was done in cooperation with the Electron Microscopy Laboratory, YPIAHVS).

The positive cell cultures, as detected by fluorescent antibody and AGID, were purified as described by Wang Zheng (1989) to prepare viral nucleic acid, and run on polyacrylamide gel electrophoresis (PAGE). Microneutralisation tests were conducted to identify the serotype of the isolated virus. Normal and healthy goats and sheep were inoculated with cell culture virus and examined clinically for pathogenic effects.

Results

Geographical factors

Xinjiang, in the centre of the Eurasian continent, is surrounded by high mountains: the Kumlun Mountains to the south, the Altai Mountains to the north and the Tianshan Mountains across the middle. These mountains divide Xinjiang into two greatly differing natural environments. North Xianjian is the semi-enclosed Zinger Basin, in the middle of which is the Gurbantong Youte Desert. South Xinjiang is the wholly-enclosed Tarim Basin, in the middle of which is the Takera Magan Desert. In the three broad valleys are scattered Tulufan, Hami, Tacheng, Yanqi, Baicheng, Greater and Lesser Youdusi and Zhao Sucheng. Xinjiang is in the temperate zone with a continental dry climate, but with great differences between its north and south. The annual rainfall is 150–200 mm in the Zinger Basin (north Xinjiang), about 50 mm in the Tarlim Basin (south Xinjiang), but only 6.3 mm in Takexun County. In winter, north Xinjiang is cold while south Winjiang is relatively warm: in January the mean temperature is –12 to –17°C in north Xinjiang and –5 to –10°C in south Xinjiang. In summer, temperatures differ little, averaging 20–25°C in July in north Xinjiang, 25–27°C in south Xinjiang and up to 33°C in the Tulufan Basin. The frost-free period is short in north Xinjiang (about 150 days) and the mountain areas, and long in south Xinjiang and in the plains(about 200–220 days).

Epidemiological survey

A sentinel herd was established in Weili County, Kuweilun District (844–932 m above sea level), close to the northern margin of the Kelamagan Desert. The average annual temperature is 10.3–10.6°C with extreme highs around 41.8°C. The mean temperature in January is –8.5°C to –10.2°C with extreme lows around –30.9°C. Annual rainfall is 36.6–52 mm, with annual relative humidity of 44%, and an annual evaporation capacity of 2252.3–2921.6 mm. In the Tarim Basin, the annual average temperature is 16°C.

Culicoides and mosquitoes are distributed widely in the north, and as far as the Tianshan Mountains in the south. There are 17 *Culicoides* species in Xinjiang, including *C. alexandrae* Dzhafarov, *C. circumscriptus, C. caucasicus, C. hamiensis, C. homotomus, C. liui* and *C. sinkiangensis*. The mosquito and *Culicoides* activity season occurs between May and September along the Tianshan Mountains in north

Xinjiang; between June and August in the northern part of north Xinjiang; and between April and August in south Xinjiang.

Of the 160 671 ruminants tested (including Yellow cattle, yak, sheep, goats and deer from 17 districts), 4053 (3%) were seropositive (average 2.5%). Among goats in the 42 counties investigated, 17% were seropositive: 1.8% of sheep (85 counties) and 0.9% Yellow cattle (86 counties) were seropositive. No seropositive yak or deer were found.

Sentinel monitoring

The sentinel herd was located at Qunke Ranch, Weili County. This is situated in the valleys of the Peacock and Tarim Rivers, with a warm climate, and plenty of grasses, ponds, lakes and marshes full of reeds where large numbers of mosquitoes and *Culicoides* breed. The dominant species has been found to be *C. pseudosalinus* (82.8%) followed by *C. liui* (18%). *Culicoides pseudosalinus* appears in early May, and reaches peak density in late May or early June, while *C. liui* appears during the first ten days of May, reaches peak density in the second ten days of June, and disappears during the first ten days of August. The mean temperature in the peak density period is 25–28°C and wind is mild to non-existent (Qu Fengyi 1981).

Of the sheep and goats grazing together at Qunke Ranch, 51/98 sheep (52.8%) and 66/125 (52.8%) goats tested positive by AGID. Ten sheep and ten goats, tested negative to bluetongue by AGID, were introduced into the positive flock on 17 May 1989. Sera were collected regularly and tested from May 1989 to January 1990. Goat no. 29 became seropositive on 1 July 1989 and goats 26 and 27 on 8 July 1989 (Table 1). Anticoagulant blood from goats 26 and 27 were used for virus isolation. Although testing of the remaining goats and the sheep continued until the end of January 1990, none became seropositive.

Between May and December 1990, 115 sheep and goats, in mixed grazing in Xiaobaoxiang Township, Hami City, were examined for seroconversion. Tests on sera taken regularly from different animals of different ages and sexes showed that the seroconversion peak in Hami City occurred in June (56.3% of all positives; Table 2). Overall, seroconversion occurred in 27/99 (27.3%) sheep and 5/16 (31.3%) goats. Seroconverting sheep and goats were aged from one to six years old, showing that animals of various ages could be infected.

Clinically suspected bluetongue cases

Six cattle at a breeding livestock farm in Bayinguolun Zhou in 1962 had swollen and bluish tongues, with evident cyanosis and congestion. Some sheep and goats in flocks had lost condition and wool. In January and February 1990, abortions of unknown cause occurred in four flocks comprising 435 goats, which were grazing near Laobagongtai, Hejing Baluntai District. Of the 169 goats (38.8%) that aborted, 15/15 ewes tested negative for *Brucella*, 1/14 tested positive for *Toxoplasma*, and all 15 tested positive for BLU antibody by AGID. The aborted foetuses were abnormal, showing congestion and bleeding in skin, exophthalmos, hydrops abdominis, hepatomegalia and big hind limbs. In two counties of Akesu District, 364 sera from nine aborting goat flocks were tested, with a seropositive rate of 58%.

Table 1. Seroconversion of three sentinel goats tested for bluetongue antibodies by AGID, 1989.

Goat no.	Sex	17 May	1 Jul	8 Jul	17 Jul	25 Jul	2 Aug
26	female	–	–	+	+	+	+
27	male	–	–	+	+	+	+
29	male	–	+	+	+	+	+

+ seropositive
– seronegative

Table 2. Bluetongue seroconversion of sentinel goats and sheep, by month.

Month	No. seroconverting	% of total seroconversion
June	18	56.3
July	2	6.3
August	6	18.8
September	3	9.4
November	3	9.4

Virus isolation

Anticoagulant blood samples, taken one to two weeks before seroconversion of sentinel goats nos. 26 and 27, were inoculated onto BHK21 cells and examined daily. Cells of the first passage began to produce CPE in the third passage, with CPE becoming more extensive with increased passages.

Chick embryos were inoculated, via the yolk sac, with whole blood in anticoagulant of goats nos. 26 and 27, and examined daily. In general, virulence tended to increase with passage (Table 3), but until the eighth passage, pathological changes (congestion of chick embryo with oedema) were not regular.

Virus from blood of goats nos. 26 and 27 were adapted to BHK21 cells. The antigen prepared from the harvested cell-adapted virus 27F5 reacted with hyperimmune serum to Yunnan BLU strain, producing typical precipitating lines.

Table 3. Chicken embryo inoculation death rate.

F	Inoculation group Deaths/Total	Control group Deaths/Total
26F1	1/5	
27F1	1/5	0/5
26F2	2/5	
27F2	4/5	0/5
26F3	1/5	
27F3	3/5	0/5
26F4	2/5	
27F4	3/5	1/5
26F5	2/5	
27F5	0/5	0/5
26F6	6/7	
27F6	5/7	0/5
26F7	1/5	
27F7	1/5	1/5
26F8	3/5	
27F8	0/5	0/5

Smears and sections, made from cell-adapted virus 26F3 and chick embryo passaged virus 27F6, were stained directly and examined by fluorescent microscopy. Typical fluorescent reaction (bright apple-green spots) could be seen in the cytoplasm. Fluorescence was clear in central vein walls and between hepatic cell cords of frozen chicken embryo liver sections.

Electron microscopy showed clusters of spherical particles with uniform size and structure. Capsids were clear, and there were antibody bridges between particles. Two kinds of particles, solid and hollow, could be seen. The morphology and size were identical to those of BLU, with a diameter of 60 nm.

RNA was shown to be separated into ten bands with a 3.3.3.1 distribution pattern, similar to that of BLU17, BTV-Y863 and BLU-W and quite different from that of epizootic hemorrhagic disease virus (EHD). The motility of RNA fragments was slightly different from that of the above bluetongue strains. Bands 4, 5, 6 in the isolate were divided equally, while bands 5 and 6 in the other strains were a little closer.

Normal, healthy goats and sheep were inoculated with cell-adapted virus 27F20. They showed temperature increases; persistent reduction of white blood cells with T and B lymphocytes in peripheral blood; haemorrhage; and lymphocyte and macrophage infiltration of various degrees in lymph node, spleen, tongue, buccal mucosa, pulmonary artery and corresponding organs in the foetus, suggesting the presence of BLU antigen in erythrocytes and target organs. The pathogenicity of bluetongue virus for sheep was higher than for goats, and, as the virus caused pathogenic lesions in the target organs of foetuses by crossing the placenta, the study revealed the association of abortion with BLU infection. There has been no report on the pathology of bluetongue in goats elsewhere in China.

The Xinjiang isolate 27F25 virus was tested against 24 South African BLU serotypes by neutralisation tests. The antiserum to BLU1 neutralised 100 $TCID_{50}$ of the Xinjiang BLU isolate. The control cells did not produce CPE, and the sera to other BLU serotypes did not neutralise the isolate, indicating that the Xinjiang isolate is BLU1.

Discussion

Serological examination of 160 671 goats, sheep, Yellow cattle, yak and deer for bluetongue across Xinjiang showed different prevalences in most parts of Xinjiang, except the Ili, Boertara, and Tulufan Districts, with higher seropositive rates in Changji Zhou and Hami District. The seropositive rate in some goat and sheep flocks in a few counties may be as high as 50%. For example, 52.8% of goats in a flock of Qunke Ranch, Weili County, Bayinguolun District were seropositive, compared to only 0.1% of sheep in the Aletai District. Overall, goats were most likely to be seropositive (1789/10501; 17%), followed by sheep (1845/104067; 8%). The combined positive rate for sheep and goats was 3.2%. Cattle were rarely positive (149/45664; 0.9%), and no yak or deer had bluetongue antibodies. Seropositive goats, sheep and Yellow cattle were distributed mainly in the plains areas and in the margins of the Zinger and the Tarim Basins. This distribution is related to that of *Culicoides* and mosquitoes.

The Xinjiang BLU isolated from a goat has relatively high virulence for sheep and goats. Infected pregnant goat ewes and their foetuses were found to have significant anatomic lesions although they did not abort. The isolated virus passaged in BHK21 produced CPE, suggesting that it had been adapted to BHK21 cells. When it was inoculated into chick embryos, the isolate produced typical lesions and caused deaths, similar to that described by others. Cell cultures revealed evident fluorescent reaction. Clear precipitating lines appeared in AGID using antigen made from cell culture and BLU-positive serum. Immunoelectron microscopy of purified virus showed a morphology and size identical to that of BLU. Passage of the isolate in sheep revealed the characteristic features of bluetongue disease, but with some differences to those induced by Yunnan, Sichuan and Hubei BLU strains. In neutralisation

with sera against 24 South African serotypes, only serum to BLU1 neutralised the isolate. Nucleic acid electrophoresis revealed a band pattern of 3.3.3.1, similar to that of BLU but with minor differences among the strains. All these results showed that the virus isolated in this study was BLU1.

Conclusions

A retrospective study of clinical signs of suspected bluetongue cases and the results of serological investigation have indicated that bluetongue has existed for a long time in the Xinjiang Uigur Autonomous Region. Serological (AGID) bluetongue surveys of 160 571 ruminants in 178 districts in Xinjiang Uigur Autonomous Region showed differing infection rates, with highest seropositive rates occurring among goats, followed by sheep and cattle. The distribution of seropositive animals was apparently influenced by different geographical conditions, climate, and *Culicoides* and mosquito activity. The virus isolated in this study was shown to be BLU1 through immunological assay, morphological observation, animal inoculation experiments, microneutralisation tests, nucleic acid electrophoresis and neutralisation tests.

Acknowledgments

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The Isolation and Characterisation of Bluetongue Virus and Its Epidemiology in Shanxi Province, China

Lei Huaimin, Xu Jianming, He Chongli, Shao Jiangliang and Shi Xiayun*

Abstract

After the first report of bluetongue north of the Yellow River, an epidemiological survey involving 13 858 ruminants in 11 regions/cities and 50 counties was conducted. Bluetongue disease, with typical symptoms, occurred in epidemic sites in the mid-west and south of Shanxi, involving four small towns and 22 villages. At two outbreak sites, morbidity rates were 8.6% and 22.9% respectively and mortality rates 32.6% and 16.0% respectively. The seropositive domestic ruminants, with both active and silent infections, were mainly in the Lu Liang mountains and hills in southern Shanxi. The highest rate of positives was found among goats (35%), followed by sheep (8.2%) and cattle (7.8%). Infection occurred mainly in August and September, the active season for the vector *Culicoides*. Bluetongue serotype 1 (BLU1) was isolated and used to make a vaccine. Sheep from infection-free areas brought into infected areas acquired bluetongue infection. Management of the disease requires limitations on livestock movements, elimination of *Culicoides*, a vaccine and hygiene measures.

THE first outbreak of bluetongue virus (BLU) in China occurred in Yunnan, with BLU subsequently isolated in Guandong, Guangxi, Hubei, Anhui, Sichuan, Inner Mongolia, Hebei, Jiangsu and Liaoning. However, clinical signs of bluetongue were seen only in Yunnan, Sichuan, Anhui and Hubei because bluetongue vectors, *Culicoides* species, are confined to the southern areas, reaching as far north as 40°N. Bluetongue outbreaks occurred close to that zone. Contagious disease in sheep with suspected bluetongue symptoms occurred in the Jiaocheng and Yangcheng regions of Shanxi Province from August to September 1991 and in October 1993 respectively. The virus causing the disease was identified as BLU after a comprehensive investigation, reported here, involving the isolation, identification and epidemiological study of bluetongue viruses in Shanxi Province, China.

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Materials and Methods

Specimens for diagnosis were obtained from whole blood and sera of sick sheep collected on days 4, 5, 7, 10, 15 and 40 after initial symptoms. Samples of heart, lung and blood vessel lesions from critically ill animals were also collected. Specimens for virus isolation were inoculated into cells, blind passaged homogenates of embryonated chick embryos (ECE; provided by General Epidemic Prevention Station of Shanxi Province).

Vero and BHK21 cells were provided by National Quarantine Institute and National Veterinary Medication Institute. Experimental sheep were purchased from bluetongue epidemic-free areas and confirmed as AGID negative. Bluetongue standard antigen plus positive and negative controls were provided by the National Quarantine Institute. Eight- to ten-day-old ECE and experimental mice were supplied by Guandi chicken breeding farm and the Shanxi Medicine College.

Epidemiological surveys involved a general survey and case reports when two outbreaks occurred. The

clinical symptoms of sick sheep, and pathological changes postmortem, were recorded.

Blood cells collected from sick sheep with typical clinical signs were washed three times with saline and inoculated intravenously into 6- to 7-day-old ECE with 0.2 mL blood cells. After incubating for a further three to five days, the whole embryo amnion and the fluid inside were harvested and stored at –20°C until required.

Monolayers of Vero or BHK21 cells were inoculated with either 0.2 mL blood cells or ECE homogenate, then incubated at 35°C and blind passaged. The supernatant from the cell cultures with uniform typical cytopathic effects (CPE) was then harvested.

In attempts to isolate the virus, Vero or BHK21 monolayers were inoculated with either whole blood from sick sheep or the ECE specimen after blind passage. The resultant isolates were sent to Yunnan Tropical and Subtropical Animal Virus Diseases Laboratory (YTSAVDL) for identification.

Ten healthy, one-year-old male sheep, from bluetongue-free areas and individually AGID negative, were inoculated under the skin in the groin area with 0.5–1 mL of diluted blood cells from sick sheep or cell culture. Clinical signs and body temperature were recorded daily.

Results

Epidemiological survey of two epidemic areas of Shanxi site descriptions

Jiaocheng region

Located in a mountain valley at 112°–112°30' E, 37°30'–38° N; 2000 m above sea level; four distinct seasons; average annual temperature 8.5–9.5°C (max 32.5°C, min –20°C), average relative humidity 70% and average rainfall 520.6 mm.

Yangcheng region

Located in a hilly land area between 112°–112°30'E, 35°–35°30'N; 600–1500 m above sea level, similar climate to Jiaocheng, with dry spring and wet autumn; average annual temperature 10–12°C; average rainfall 650–850 mm.

Epidemiological survey outbreaks of bluetongue

Jiaocheng region

In mid-August 1991, a bluetongue-like disease first occurred in Huijiazhuan village, peaking at the beginning of September and declining by the end of the month. The disease was epidemic in an area covering 56 flocks of sheep belonging to 19 villages and three communes. Of the 1125 sheep at risk, 428 (38%) became sick and 98 (22.9%) died. None of the 3825 goats raised in the area became sick. Most sick sheep were more than 1 year old.

Yangcheng region

Three flocks of 133 sheep and 16 goats were in the area. Of these, 56 sheep (32.6%) became sick and 9 (6%) died. No clinical signs were seen in the goats.

Although many blood-sucking insects, such as mosquitoes and *Culicoides*, existed in both epidemic areas, these had nearly disappeared during the bluetongue outbreak period because of cold weather. All sick sheep had an increased body temperature (39.5–40°C) with acute typical clinical signs found in the mucosa of the mouth and in most of the digestive, respiratory and cardiac systems. The course of the disease ran from eight to ten days, with sudden death often occurring after two to three days. A high abortion rate occurred on the Kelan cashmere goat breeding farm (a main flock) and in areas that introduced breeders from Kelan (Table 1).

Serological survey

A general serological survey using the AGID test throughout Shanxi province showed that, in descending order of prevalence, goats (including cashmere goats), sheep and cattle were antibody positive (Table 2).

Animal infection experiment

The number of leucocytes in blood samples and the body temperatures of infected experimental sheep are

Table 1. Abortion rate of ewes in the bluetongue epidemic area

Farm	Year	Total no. of ewes	No. of pregnant ewes	No. of pregnant ewes aborted (%)	No. of pregnant ewes dying
Kelan Breeding Farm*	1991	1100	1000	150 (15.0)	30
	1992		350	88 (25.0)	1
Surrounding farms**	1991–1992		4000	1043 (33.8)	–
Wujiaping Village	1991–1992	149	130	100 (76.9)	–

* breeding goats supplied by Kelan farm
** one of the surrounding farms

shown in Tables 3 and 4. Blood samples from all experimental sheep were AGID positive on 17 January 1994. BLU was isolated from all specimens and was identified by YTSAVDL as BLU1.

Discussion

Epidemiological and serological surveys showed that bluetongue existed in Shanxi Province, with

Table 2. Serological survey by AGID test in Shanxi Province.

Region/survey unit	No. surveyed	Animal	No. of animals tested	No. of positive animals	Positive rate %	Remarks
Jiaocheng region	15	sheep	727	163	22.4	total of 58519 sheep; outbreak site
(farms)		goats	1911	730	40.3	
		cattle	37	6	16.2	
Breeding farms	12	sheep	244	total 103	9.9	animal species mentioned
		goats	260			
		cattle	540			very low positive rate
Neighbouring	11	sheep	total 4814	128	2.3	high positive rates in samples from
counties to		goats		4648	28.3	mountain area
Jiaocheng		cattle	735	39	7.0	
Animal base farm	40	sheep	1518	2	0.13	
		goats	2207	804	36.4	Cashmere base farm – 13, 11 sheep, 16 cattle.
		cattle	500	3	0.6	
Yangcheng region	3 (villages)	sheep	30	11	36	year of outbreak
			20	7	35	1.5 years after outbreak

Table 3. Normal physiological parameters of experimental sheep (measured 24 December 1993).

Sheep no.	Physiological parameter of experimental sheep			
	Respiratory rate (breaths/min)	Body temperature (°C)	Heart pulse (beats/min)	No. leucocytes/mm^3 blood
1	20	39.8	78	8550
3	16	39.5	70	7800
4	18	39.8	80	9300
5	17	39.6	75	8900
7	16	39.5	77	10900
8	14	39.3	73	8800

Table 4. Changes in major physiological parameters after inoculation of specimen.

Date		20 Dec 1993	2 Jan 1994		4 Jan 1994		6 Jan 1994	11 Jan 1994
Inoculum*	Animal no.	Body temperature °C	Body temperature °C	Leucocytes/ mm^3	Body temperature °C	Leucocytes/ mm^3	Body temperature °C	Body temperature °C
Blood cells	1	36.6	41.9	4025	40.2	6550	39.6	39.2
	3	36.6	38.9		38.4		38.6	38.4
ECE	4	38.9	36.4		38.0	7950	38.5	38.2
	5	40.4	39.2		39.0	5000	38.9	38.7
Cell culture	7	39.6	39.4		39.0		38.9	38.0
	8	39.4	399.7	10700	38.7	10700	38.8	38.5

* See text for description of preparation of specimen

most epidemic sites in mountainous and hilly parts where there were active *Culicoides* and other blood-sucking insects. The epidemic season for bluetongue was from mid-August to the beginning of September, although scattered infections occurred up to October (that is in late summer and early autumn). This pattern is slightly different from the reports from elsewhere in the world. There were predictable signs for the acute outbreak of bluetongue disease, with typical clinical symptoms. This suggests that bluetongue could spread north of the Yangtze River.

The source of bluetongue in Shanxi Province was suspected to be within Gaixian County in Liaoning Province, as some breeding cashmere goats had been introduced from there to the Kelan Goat Breeding Farm. The latter, in turn, may have been the source of bluetongue in Shanxi. The most susceptible species in Shanxi was the cashmere goat, unlike other provinces where the highest prevalence of bluetongue was in sheep. The phenomenon of a high abortion rate in ewes may be related to infection with BLU viruses, but this aspect needs further study.

Overall, this serological survey showed that some parts of Shanxi Province remain free of bluetongue, so that prevention and quarantine should be urged immediately.

A Serological Survey of Bluetongue in Cattle in Guangxi Province, China

Chen Libiao, Zhong Peiyi and Zhao Guoming*

Abstract

In 1985, 14 purebred cattle, including four imported from Pakistan, were tested for bluetongue group antibodies using agar gel immunodiffusion (AGID). Four animals tested positive. Subsequently Guangxi Veterinary Epidemic Prevention and Quarantine Station used AGID to test 766 cattle from different areas in Guangxi, including eight regions and three cities. The serum samples were collected from Guangxi Animal Husbandry Research Institute, in cooperation with Kunming Animal and Plant Quarantine Service and the Yunnan Animal Husbandry and Veterinary Research Institute. Of the 766 cattle tested, 88/766 (11.5%) were positive and four suspect. Three regions and one city had no positive results. The highest positive rate (13/18; 72%) was found in the Hechi region. Between 1987 and 1990, a larger scale serological survey was carried out in 30 Guangxi counties. Of the 3712 animals tested, 1328 (35.8%), in 29 counties, were AGID positive. However, extensive investigations through veterinarians failed to find any report of bluetongue disease in cattle or sheep. Bluetongue virus has not yet been isolated. Further research will be carried out to discover why the bluetongue positive rate is so high in cattle in Guangxi: some cross-reaction with epizootic hemorrhagic disease (EHD) virus is considered possible.

THE first report of bluetongue in China came from Yunnan Province in 1979. This paper describes a major survey conducted from 1987 to 1989 in Guangxi Province.

Materials and Methods

In 1985 14 purebred cattle, including four bulls from Pakistan, were tested for bluetongue by agar gel immunodiffusion (AGID). When positive reactions were found, a wider survey was made of 766 cattle and buffalo from eight regions and three cities in Guangxi. Between 1987 and 1990, a larger and more representative survey was carried out on 3712 cattle from 30 counties and 23 special breeding farms in Guangxi province. The sera were stored at 4°C until tested. Control sera and AGID antigen were provided by Yunnan Tropical and Subtropical Animal Virus Diseases Laboratory. Tests were conducted according to the standard protocol. Tests giving suspect results were repeated.

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Results

Of the 14 purebred cattle tested in 1985 by Zhong Peiyi and others, four were positive. In the preliminary survey, 87/766 (11.5%) of cattle were positive, and four suspect. The prevalence in local breeds was slightly higher (15%) than in introduced breeds and crossbreeds (5–10%). Prevalence was highest (44.6–55.7%) in the southeastern and coastal areas including the Yulin, Wuzhou, Nanning and Qinzhou regions, while the lowest prevalence (14.5–16.9%) was in the northern areas, including Guilin and Liuzhou region. The western part of the Province had an intermediate prevalence. The highest prevalence recorded was in the Hechi region at 13/18 (72%). All animals from which blood was collected were healthy.

In the larger survey, 1328/3712 (35.8%) were positive, with AGID-positive animals being found in 29

(96.7%) of the 30 counties tested (Table 1). The survey covered all eight regions of Guangxi Province and the three large cities of Guilin, Liuzhou and Beihai: prevalence in the cities ranged from 5.6% to 77.8%.

Table 1. Results of serological survey using AGID in different regions of Guangxi Province, 1987–1990.

Region	No. of counties tested	No. of blood samples	No. of positive sera (%)
Bose	4	403	137 (34.0)
Breeding farm	23	575	209 (36.3)
Guilin	4	425	72 (16.9)
Hechi	3	308	118 (38.3)
Liuzhou	4	425	62 (14.5)
Nanning	4	409	175 (42.8)
Qinzhou	4	427	196 (45.9)
Wuzhou	4	422	188 (44.6)
Yulin	3	318	171(53.8)
Total	30 (plus 23 breeding farms)	3712	1328 (35.8)

Discussion

The AGID test (Anon 1989), widely used in serological surveys for bluetongue, was used for preliminary tests of 766 cattle and 4 bulls before the large scale survey. The preliminary work showed that the method was suitable for wider ranging sampling. The survey, carried out in all regions of Guangxi, showed that there was bluetongue antibody in most of the Province except for three regions and one large city (Beihai). Thus it was confirmed that bluetongue infection was widespread in the cattle of Guangxi Province. Although no clinical signs were found, cattle could be a large potential source of virus for epidemic outbreaks in this Province.

Guangxi Province is located in the southern part of China, where there is a subtropical monsoon climate with high temperatures, relative humidity and rainfall. This is a suitable habitat for bloodsucking insects such as mosquitoes and *Culicoides*. The natural environment explains the prevalence of bluetongue virus in Guangxi. There is also a lack of proper quarantine, prevention strategies or monitoring of the bluetongue status of introduced breeds from outside the Province. As the survey showed such a high prevalence of bluetongue group antibody in cattle which had no clinical signs, it is highly possible that there may be cross reactions between bluetongue viruses and other species of orbivirus, such as epizootic hemorrhagic disease (EHD) virus. This aspect needs further research.

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Serological Survey of Bluetongue in Sheep and Cattle in Inner Mongolia

Mo Regen, Ao Fulai, Si Qin and Zhao Xinli*

Abstract

The prevalence of infection of bluetongue in sheep and cattle in Inner Mongolia was determined by agar gel immunodiffusion (AGID). In all, 6712 serum samples were tested. The positive rates were 14.8% in 4098 introduced sheep; 74.7% in 463 breeding sheep; and 67.6% in 2004 goats. However, 316 sera from cattle tested negative, as did 103 samples from sheep in an area without introduced sheep. The results indicated that the prevalence of bluetongue antibodies was higher in breeding sheep and goats than in introduced sheep.

SINCE the first discovery of bluetongue in China by Yunnan Provincial Institute of Animal Husbandry and Veterinary Science (YPIAHVS) in 1979, cases of seropositive animals have been detected in other provinces and municipalities, and different strains of bluetongue virus (BLU) have been successfully isolated. In 1986, a quarantine inspection in the Bayannur Meng of Inner Mongolia detected BLU antibody in goat serum, using agar gel immunodiffusion (AGID), and BLU was isolated and identified in collaboration with YPIAHVS. To ascertain further the distribution and prevalence of bluetongue in Inner Mongolia, and to provide a scientific basis for control of the disease, a serological survey of bluetongue was carried out in various districts

Materials and Methods

Serum samples were randomly collected from goats, sheep and cattle in nine districts or cities in Inner Mongolia. Samples of whole blood were collected using routine methods and sera were stored at 4°C until used. Reference antigen and bluetongue positive serum were provided by YPIAHVS. The testing methods and reading followed 'Procedures of agar gel immunodiffusion test for detection of bluetongue' developed by YPIAHVS (Zhang Nianzu et al. 1989a).

Results

Breeding sheep: of the 463 sera samples tested, 346 (74.7%) were positive.

Goats: in areas where breeding animals had been introduced, 1305 (65.1%) of 2004 sera were positive.

Sheep and goats: in areas where breeding animals had been introduced, 609 (14.86%) of 4098 sheep sera were positive. In areas where breeding animals had not been introduced, all 103 sheep and goat sera tested were negative.

Cattle: all 316 cattle sera, collected in different districts, tested negative.

Discussion and Conclusions

Bluetongue in Inner Mongolia has wide distribution and high prevalence. The highest infection rates were in breeding sheep (74.7%) and goats (65.1%). The infection rate was lower in sheep (14.8%). As these seropositive rates were greater than those detected in Yunnan Province in 1983 by Zhang Nianzu et al. (1989b) and in Bayannur Meng, Inner Mongolia in 1986 by Guo Zhaijun et al. (these Proceedings), the disease may be increasing in the region.

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Our results also revealed that seropositive animals appeared to occur mainly in areas where breeding animals had been introduced or where breeding sheep and goats were raised, indicating that the presence and spread of bluetongue were associated with the introduction of breeding animals, mating during the *Culicoides* activity season, and movement of livestock herds.

The emergence and spread of bluetongue has caused great losses in livestock production in Inner Mongolia. We therefore recommend that effective measures be taken, including strict animal quarantine and prohibition of the introduction of seropositive animals. At the same time, seropositive breeding animals should be culled out. In seasons of *Culicoides* activity, measures should be taken to prevent infection of normal breeding animals, and movement of positive livestock should be restricted. Research and application of immunisation measures should be undertaken in a planned way.

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Investigation of Bluetongue Disease in the Bayannur Meng of Inner Mongolia

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Abstract

Serological testing for bluetongue antibodies was carried out by agar gel immunodiffusion (AGID) in seven cities and counties of the Bayannur Meng of Inner Mongolia. Among the samples from sheep, goats, Yellow cattle and camels, 23.2% (650/2799) were seropositive. This proportion was higher among goats at 57.9% (523/903) and lower among sheep at 7.5% (127/1684), with no positive reactions found at all in camels. The seropositive sheep and goats showed no clinical signs. However, some sheep and goats in a sentinel herd showed elevated temperatures and changes in white blood cells. Through the use of sentinel herds, bluetongue disease was found to occur under natural conditions in Bayannur during an epidemic period. Within the sentinel herd, the seropositive rate was 59.2%. After inoculation of embryonated chicken eggs and cell cultures as well as sheep experiments, bluetongue viruses were isolated from sheep. These isolates were shown to be the same group as the Yunnan strain and USA BLU17.

BLUETONGUE disease is well described from other countries (Bowne 1971; Anon. 1980). After the isolation of bluetongue virus (BLU) by Yunnan Provincial Institute of Animal Husbandry and Veterinary Science in 1979 (Zhang Nianzu et al. 1989b), bluetongue viruses were isolated in Hubei in 1984 and Sichuan in 1988 (Lin Lihui and Li Zhihua 1989). In 1986, the Inner Mongolian Animal Quarantine Station, while conducting import and export quarantine inspections in Bayannur Meng, detected bluetongue antibodies in 53.2% (298/549) of goats tested by agar gel immunodiffusion (AGID). To investigate further the presence and prevalence of bluetongue in Bayannur Meng, and to provide a scientific basis for control of the disease, the Bayannur Veterinary Station, in collaboration with Yunnan Provincial Institute of Animal Husbandry and Veterinary Science (YPIAHVS), carried out an epidemiological survey and virus isolation and identification program between 1986 and 1988.

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Materials and Methods

Epidemiological survey

Between April and July 1987, goats, sheep, Yellow cattle and camels were randomly selected in 32 Sumu (townships) and breeding farms in the district for investigation by AGID. Statistical and comparative analyses were made of the test data in terms of range of infection, area, and animals and their ages.

Between April 1987 and January 1988 (before the start of the activity season of the vector *Culicoides*), 50 seronegative goats and 10 seronegative sheep, from agricultural and pastoral areas, were introduced into herds with a 50% bluetongue seropositive rate. All animals grazed together and were observed for natural infection. During the experimental period, the introduced animals were tested weekly by AGID and infection rates were calculated monthly. Clinical signs were recorded. Whole blood samples were taken aseptically with 1/10 (v/v) in

anticoagulant (7.5% sodium citrate) from confirmed infected goats and sheep, and stored at 4°C for later use.

Antigens, positive reference sera and negative sera were provided by the YPIAHVS. Test sera were prepared and stored in a routine manner. Tests were performed and read in accordance with procedures developed by the YPIAHVS (Zhang Nianzu et al. 1989a).

Virus isolation and identification

Passage in embryonated chicken eggs

Sodium citrate anticoagulant blood from infected animals was washed off the red blood cells with sterile normal saline. Sterile distilled water was used to replace the original volume of blood for lysis. The lysed red blood cells were used to inoculate five to ten 7-day-old embryonated chicken eggs (ECE) in the yolk sac (0.2 mL inoculum for each), or to inoculate 0.02 mL inoculum intravenously into 10- to 12-day-old ECE with five uninoculated chicken embryos as control. The inoculated eggs were examined twice daily. The dead ECE were collected, after being confirmed free of bacteria by bacteriological examination. Sterile normal saline was added and the material ground to prepare a 1:5 emulsion, which was then stored at 4°C for 12–24 hours and passaged in ECE as above. Observations of embryo death were made for 48–120 hours. Whether there were reaction or deaths, the embryos were blind passaged five to eight times before final examination. Eggs which died in a regular pattern were kept at 4°C for further virus isolation attempts.

Cell Culture

BHK21 cells were dispersed and grown in Eagle's media according to routine methods. When monolayers were confluent, they were inoculated with 0.1 mL 1:5 emulsion prepared by grinding the infected ECE in normal saline, and were blind passaged until typical cytopathic effects (CPE) appeared.

Experimental artificial infection of goats and sheep

Eight seropositive anticoagulant goat blood samples were taken and mixed in pairs, to give four goat blood inocula. Each blood mixture inoculum was used to inoculate one sheep: eight sheep in all were inoculated with 1 mL each. The inoculated sheep were examined for temperature rises, changes in white blood cell counts etc.

Sheep (goat) virulence test

Each animal was subcutaneously inoculated with 10 mL isolated virus in cell culture suspension, examined daily for clinical signs, and subsequently tested by AGID.

Virus identification

Group specificity test

The virus cell culture was frozen and thawed three times, then concentrated 100-fold to prepare soluble antigen. Positive reference serum was used to carry out the AGID test (Zhang Nianzu et al. 1989a), and control antigens were prepared from Yunnan strain and US BLU17. The virus cell culture sediment was directly stained with fluorescent antibody, as provided and described by YPIAHVS (Hu Yuling and Peng Kegao 1989), and then examined under a fluorescence microscope.

The virus cell cultures were concentrated by treatment with Freon (Hu Yuling and Peng Kegao 1989; Hu Yuling et al. 1989), negatively stained with phosphotungstic acid and examined by electron microscopy for virus morphology. Virus was purified to prepare nucleic acid, analysed with SDS-PAGE (Hu Yuling et al. 1989) and compared with domestic bluetongue isolates and with BLU10 and 17 from the USA.

Results

Epidemiological survey

Bayannur Meng is situated in the western part of the Inner Mongolia Autonomous Region, between 40°13'–42°28'N and 105°21'–109°53'E, and has a total area of 64 413 km^2. The Yinshan mountain range crosses the whole district. North of the mountains is a highland pastoral area (1100–1500 m above sea level), with a medium temperate and highland climate, a frost-free period of 130–160 days and an annual rainfall of 140–250 mm. The activity season of local insects (mosquitoes, flies, gadflies, mites and *Culicoides* midges) falls between April and October. In 1987, the livestock population of Bayannur Meng comprised 4 134 million animals: this included 2013000 sheep, 314 000 goats, 545 000 Yellow cattle and 25000 camels. A relatively rough grazing regime is practiced.

In all, 2799 ruminants were sampled by AGID for the presence of bluetongue antibody. Overall, 23.2% (650) were seropositive: this proportion was higher among goats at 57.9% (523/903) and lower among sheep at 7.5% (127/1684). The situation was basically identical to results from Yunnan Province (Zhang Nianzu et al. 1989b) where no clinical signs of bluetongue were found in either sheep or goats. Follow-up investigations revealed no clinical signs of disease similar to bluetongue. No antibodies were detected in 100 Yellow cattle and 112 camels.

Serological investigations showed that the infection rate was higher in the pastoral areas, at 37.6%, than at the interface of agriculture and pastoral areas,

at 28.1%. No bluetongue antibodies were detected in goats and sheep born in the Hetao (Yellow River Bend) agricultural zone. Follow-up studies showed that the seropositive animals were adult sheep and goats purchased from pastoral areas. The infection rate among goats, mainly purchased animals, was 10.5%.

Statistical analysis of the infection status of 737 goats and sheep of different ages showed that animals above three years old were more likely to be infected than two-year-old animals, and both groups were more likely to be infected than one-year-old animals.

All 60 goats and sheep tested between April and July were seronegative. From August on, however, seropositive animals were detected. Although most seroconversions were detected between August and October, newly positive animals were detected even as late as January in the following year. No clinical signs were observed in the infected animals during the experimental period. A few animals showed transient temperature rises (to 40°C) and reductions in numbers of white blood cells by 25–50%.

Virus isolation and identification

After five passages, regular deaths occurred in the ECE, with significant oedema in corresponding parts of the chicken embryos. Chicken embryo virus from blood sample no. 018 was used to inoculate BHK21 monolayers. After five passages, CPE appeared. Intercellular gaps were slightly broadened after continuous culture for 48 hours. Approximately one third of the cells became rounded and detached in 72 hours. All the cells finally rounded, aggregated and fell off the glass.

Sheep no. M2, inoculated with a mixture of anticoagulant bloods nos. 003 and 017, exhibited seroconversion, with a transient reduction of white blood cells and a temperature rise 22 days post-infection. The sheep inoculated with a mixture of anticoagulant bloods nos. 018 and 019 seroconverted, with mild changes in white blood cells and temperature 22 days post-infection. Seroconversion was not observed in the other infected sheep during one 1 month of observation post-infection. One of the two sheep inoculated with cell culture of the isolated virus seroconverted 15 days post-infection, without significant change in white blood cell count and temperature.

Virus identification

Antigen was prepared using BHK21 cell cultures infected with the virus isolate. An AGID test was conducted with the prepared antigen and reference positive serum. A positive result was obtained, with the control antigen remaining negative.

BHK21 cell cultures with the isolated virus were stained directly with fluorescent antibody. Fluorescent microscopic examination of the preparation demonstrated apple-green fluorescence in stained substances of various sizes in the cytoplasm surrounding the nucleus. The standard viruses (Yunnan strain, Wuhan strain and USA strains) were prepared as reference material. The results were identical. No fluorescence reaction was found in cell cultures not infected with virus.

Viral particles were observed by electron microscopy. These particles were round in form with significant structure at an electronic amplification of 20000 and a five-fold optical amplification. The structure of the preparation was the same as those of the reference virus (US BLU17). The nucleic acid could be divided into 10 RNA fragments, and the band pattern was the same as those of the Yunnan strain, US BLU10 and 17.

Discussion

According to previously published accounts, the approximate distribution of bluetongue is between latitudes 45°N and 45°S (Bowne 1971; Sellers 1981). Bayannur Meng is located between 40–42°N and is thus clearly within the bluetongue epidemiological region. Our serological investigations showed the average prevalence of bluetongue antibodies in Bayannur Meng was 23.2%, with 7.5% in sheep and 57.9% in goats. No bluetongue antibody was detected in the Hetao (Yellow River Bend) agricultural area: whether this is due to a variance of geographical or ecological conditions remains to be investigated.

Among sentinel herds, AGID testing revealed a seroconversion rate of up to 59.2% (28/50 in goats and 4/4 in sheep), indicating that the infection rate was relatively high in an epidemiological cycle. Seroconversion occurred until December of the same year and even in January of the following year. This was probably because infection with trace amounts of virus induced only small amounts of antibodies which could not be detected. The virus then multiplied with prolonged viraemia, resulting in a gradual increase of antibody titre. In our experiments, we observed that antibodies were not detected in goats inoculated with test blood until 116 days later. Furthermore, 8/31 sheep showed seroconversion in December when the extreme cold would suggest there is no *Culicoides* activity. Whether there is another insect vector remains to be determined.

The virus isolated was confirmed as being BLU as it caused CPE in cell cultures, regular deaths and lesion in inoculated chicken embryos, and seroconversion as detected by AGID in experimentally infected animals. Group-specificity determinations, morphological examination and epidemiological data also confirmed the BLU identification.

A virus strain of relatively low virulence was isolated from a goat and from sheep. The infection rate was low in sheep, including pedigree breeds such as Xinjiang fine-wool and Romney sheep. No antibodies were detected in Yellow cattle. It therefore appears that the virus isolated in Bayannur Meng differs from other bluetongue viruses isolated from sheep in China. Whether this is the goat-adapted virus or a hypovirulent sheep strain remains to be investigated. No antibody and no clinical cases were found in cattle where goats were heavily infected. No cross-reaction was found between the positive serum and epizootic hemorrhagic disease (EHD) virus, suggesting that Ibaraki disease in Yellow cattle could be excluded.

Analysis of the isolate's nucleic acid showed that the isolated virus had 10 nucleic acid fragments, with a band pattern similar to that of known bluetongue virus types, indicating that the virus was a bluetongue virus, not a bluetongue-related virus. Study of its type-specificity will be carried out elsewhere.

No previous report has been seen on the isolation of BLU from goats. From our work, it is possible to isolate BLU from goats by natural infection of animals in sentinel herds and the timely collecting of blood samples from infected animals for virus isolation.

Conclusion

Between 1986 and 1988, bluetongue virus was isolated from goats in Bayannur Meng, Inner Mongolia by cell culture, chick embryo inoculation and goat infection experiments. Group-specificity and morphological features of the isolate also confirmed the BLU identification.

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Differential Epidemiology of Bluetongue Antibodies in Ruminants in China

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Abstract

A total of 258 serum samples of ruminants that were positive by AGID and cELISA were tested in neutralisation tests with BLU1, BLU16 and BLU17 viruses. The samples originated from Kunming, Yiliang, Lunan, Nanpeng, Guangnan, Shizong, Luliang, Luqian and Ershan counties of Yunnan Province and Bayannur Meng of Inner Mongolia. BLU1 antibodies were found in 59 serum samples, BLU16 in 24 samples and both BLU1 and BLU16 in nine samples. No antibodies to BLU17 were detected. The results indicated that antibodies to BLU1 and BLU16 exist in Yunnan Province although antibodies to BLU1, BLU16 and BLU17 were not found in sera from Inner Mongolia. The results also indicated that other bluetongue, or cross-reacting, viruses exist in Yunnan Province.

BLUETONGUE disease is an infectious viral disease, transmitted by certain insects, that mostly affects sheep and other ruminants. Systematic study of bluetongue virus (BLU) in China started after the first outbreak in Shizong, Yunnan Province. Several BLU serotypes were isolated from Yunnan, Hubei, Sichuan, Inner Mongolia, Shandong, Shanxi, Anhui and Gansu Provinces, but the identification and distribution of the isolates still remains unknown. Work by Yunnan Tropical and Subtropical Animal Virus Diseases Laboratory on the identification of these viruses has involved cross protection tests of sheep with four BLU isolates. Micro-neutralisation test results have shown that the isolates from Yunnan, Xinjiang, and Shanxi were BLU1, from Sichuan and Hubei BLU16, and from Inner Mongolia BLU17 (Zhang Nianzu et al. 1991, 1993). This paper reports on a survey of serotype distribution in Yunnan and Inner Mongolia by means of testing for neutralising antibodies.

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Materials and Methods

Isolates of BLU1, BLU16, BLU17, tested in previous experiments by this laboratory, were used, passaged three times in Vero cells. The isolates were used at 100 $TCID_{50}$. Positive sera had previously been tested by competitive enzyme linked immunosorbent assay (cELISA) and agar gel immunodiffusion (AGID). The sera for tests were inactivated by being kept at 56°C for 30 minutes. Dilutions were made in minimum essential medium + 10% foetal calf serum. The neutralisation tests were carried out by standard methods (Gard and Kirkland 1993).

Results

Of the total 258 samples, 59 contained BLU1 antibodies, 24 BLU16 antibodies and nine had both BLU1 and BLU16: none had BLU17 antibodies (Table 1).

Discussion

The sera were screened by cELISA using high quality antigen and monoclonal antibodies to avoid cross-reaction with epizootic hemorrhagic disease virus (EHD) and Palyam group viruses. As no BLU17 anti-

bodies were found in any of the 258 samples, the provenance of BLU17 from Inner Mongolia was unconfirmed. Since 82 samples had antibodies to BLU1 or BLU16 or both, this suggests the existence of BLU1 and BLU16 serotypes in Yunnan Province.

Table 1. Distribution of neutralising antibodies to bluetongue in sera positive for group antibodies.

Source of samples	No. of samples	Serotypes of antibodies		
		BLU1	BLU16	BLU17
Lanping, Guangnan	91	26	13	0
Yuxi, Eshan	46	14	4	0
Kunming	34	1	3	0
Shizong	26	4	0	0
Yiliang	20	10	2	0
Lunan	10	4	1	0
Luliang	10	0	0	0
Luquan	7	0	0	0
Bayannur, Inner Mongolia	14	0	0	0

That there were 175 samples with no antibodies to any of these three serotypes suggests that serotypes other than these may be present in Yunnan. The possibility of multiple infections with different serotypes was confirmed, as shown by the nine samples with both BLU1 and BLU16 antibodies.

Further tests, with a wider range of reagents, should be made on the samples from Inner Mongolia. The micro-neutralisation test used in this study is recommended as the standard test for surveying and monitoring the distribution of BLU serotypes.

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Bluetongue Viruses in the Asian and Southeast Asian Region

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Abstract

The first scientific accounts of the presence of bluetongue in the Asian and Southeast Asian region seem to have come from India in the 1960s. Over the next decade, reports of clinical disease in sheep and/or serology from several countries suggested that bluetongue virus activity was widespread in the area. During this period, the inaccessibility of the reference laboratory in South Africa, at Onderstepoort, meant that confirmatory assistance was not available for many countries. The detection of bluetongue virus in northern Australia in 1977 set in train a variety of activities which led to a greatly increased understanding of bluetongue in the region. Knowledge and expertise developed and reagents were prepared and made available. There was also an interest by animal health administrators and scientists in countries where sheep were farmed in unravelling the bluetongue virus and disease status of those countries. In the past ten years, various research groups have isolated and characterised bluetongue viruses in several countries in Asia and Southeast Asia. Significant serological surveys have been undertaken in many countries and entomological investigations have been initiated in a few. Many of the serotypes active are now known and there is increasing information on their biology and natural history. An additional advantage of bluetongue research activity throughout the region is that laboratory technology generally has benefited. Bluetongue has been a vehicle for introducing virological, serological and entomological laboratory and field techniques into numerous laboratories.

BLUETONGUE has been one of the most feared diseases in sheep-producing countries this century (Roberts et al. 1993). For the first half of this century, bluetongue was regarded as another 'African disease'. Then, for 30 years after the Second World War, animal health administrators regarded bluetongue as an emerging disease (Gard 1990). There were explosive outbreaks of bluetongue disease in Cyprus in 1943 and in Portugal and Spain in 1956, as well the more insidious appearance of the disease in USA in the intervening years. Accounts of the disease in other countries soon followed. Subsequent reports from USA that the virus caused chronic infections of cattle, that semen could be a vehicle for virus transmission, and that transplacental passage was a significant feature of virus infection added to the concern about bluetongue. Countries worried about the health of their national sheep flocks imposed strict quarantine to exclude the virus.

A more realistic picture of the international status of bluetongue has been gauged over the last 20 years. As countries have developed their laboratory diagnostic capacities, the presence of the bluetongue virus (BLU) has been established in most countries in tropical, subtropical and adjacent temperate zones wherever substantial populations of ruminants exist. Often the presence of bluetongue was unsuspected because the virus circulated subclinically in cattle, buffalo, goats and native sheep, species susceptible to infection but not to disease.

The recognition that bluetongue virus was present in Australia in 1977 (St. George et al. 1978) led to two decades of broad-ranging research into the virus, its vectors, epidemiology, pathogenesis and diagnosis. Realising that the Australian situation had to be considered in a regional context, Australia and several countries in Asia and Southeast Asia collabo-

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rated in research programs. Thus the bluetongue status of this part of the world is now better understood: the status in Asia and Oceania has been reviewed by Hassan (1992a) and Doyle (1992) respectively.

Current Situation

Australia

The presence of bluetongue was not suspected in Australia until 1977, when a virus isolated from a mixed pool of midges collected during a bovine ephemeral fever vector study was identified at Yale Arbovirus Research Unit as bluetongue (St. George et al. 1978).

This isolation of bluetongue virus initiated a long, productive research program in Australia, a country with a vital sheep industry. As a result, there is now an understanding of the viruses and vectors present in the country, and of their distribution. BLU serotypes 1, 3, 9, 15, 16, 20, 21 and 23 have been isolated in Australia and the main vectors have been shown to be *Culicoides actoni, C. brevitarsis, C. fulvus* and *C. wadai* (Gard and Melville 1989).

Bluetongue disease is the result of a complex interaction between sheep, virus and environment and does not occur naturally in Australia. The Australian vectors of BLU have a strong host preference for cattle, and small ruminants have a low antibody prevalence. Moreover, the BLU serotypes active in sheep-raising areas (strains of BLU 1 and 21) appear to be only mildly pathogenic.

China

Bluetongue disease was first diagnosed in China in Yunnan province in 1979 (Zhang Nianzu et al. 1989). Antibody has been detected in 29 provinces, clinical disease has occurred in four (Zhang Nianzu et al. 1992) and bluetongue viruses have been isolated from sheep, goats and cattle. BLU antibodies have been detected in sheep, cattle, goats and deer (Zhang Nianzu et al. 1992). In Yunnan province more than 30 species of *Culicoides* have been found, of which four are suspected BLU vectors.

India

The disease, ranging from isolated cases to widespread outbreaks, has been reported in India since 1963 (Mehrotra 1992). Mild to severe disease has been recorded in local and exotic breeds of sheep, but disease has not been noted in infected goats, cattle or buffalo. The virus is endemic and widely distributed. In 1973, BLU virus was isolated from experimentally infected sheep inoculated with clinical material (Uppal 1992). BLU1, 3, 4, 9, 16 and 17 have been isolated from sheep, and serological evidence suggests that other serotypes also circulate in India. There is no information on the species of *Culicoides* acting as BLU vectors in India.

Indonesia

More information on bluetongue and its vectors is known for Indonesia than for most other countries in the region. Since 1987 there has been a program to isolate BLU from sentinel cattle located at several widely separated sites in Indonesia, and BLU1, 7, 9, 12, 21 and 23 have been recovered (Sendow et al. 1992). As part of the national bluetongue research program involved the study of vectors, a considerable body of information is available. Sukarsih et al. (1992) listed 49 *Culicoides* spp. collected at six sentinel cattle sites in Indonesia. The four species proven as vectors in Australia are present and widely distributed. Other potential vectors have been trapped and identified.

Japan

Many arboviruses are active in Japan and several have been isolated there for the first time in the world. Orbiviruses are regularly active in summer or autumn, especially in the southern cattle farming districts. The Quarterly Epidemiology Report of the Office International des Epizooties (OIE) often indicates that bluetongue viruses have been active in Japan, as evidenced by the detection of antibody, but diseased sheep have not been seen.

Malaysia

Serological evidence of BLU presence has been recorded in Malaysia since the late 1970s (Hassan 1992b). Antibodies in imported cattle and local ruminants indicated that the virus was endemic. Clinical disease occurred in 1987 when sheep recently imported from a bluetongue-free region of southern Australia suffered dramatic disease in several states in Peninsular Malaysia and BLU 1 was isolated from one of these sheep (Chiang 1989). After the clinical disease episode in 1987, the Australian and Malaysian governments embarked on a collaborative ACIAR-sponsored bluetongue research program. Serological testing showed that multiple serotypes of BLU were endemic in the country and BLU1, 2, 3, 9, 16, and 23 were isolated from sentinel cattle and sheep (Sharifah et al. 1995).

There have been some studies on the *Culicoides* fauna of Malaysia. *Culicoides peregrinus, C. orientalis* and *C. shortii* are considered the most probable vectors on the basis of abundance, distribution and host preference (Cheah and Rajamanickam 1991). However, a program to recover BLU from vectors has not been attempted.

Papua New Guinea

Serological surveys of Papua New Guinea ruminants were conducted in conjunction with testing of Australian animals soon after bluetongue was first recognised in Australia. Doyle (1992) reported that bluetongue virus occurs in Papua New Guinea, with one of the vectors being *C. brevitarsis*.

Taiwan

Serological evidence of EHD virus infection has been reported from Taiwan (Metcalf et al. 1992). The presence of EHD on Taiwan indicates that Taiwanese ruminants could also be at risk from bluetongue.

Thailand

Bluetongue serological surveys have been conducted recently in Thailand. Antibodies have been detected in the absence of clinical disease.

Other countries in the region

The bluetongue status of Myanmar, Cambodia, Korea, Laos, Philippines and Vietnam are unknown, but bluetongue viruses probably circulate subclinically in all these countries.

Discussion

Bluetongue viruses may move between countries by the transportation of viraemic vertebrates or by windblown infected vectors (Sellers 1992; St. George 1992). The latter mechanism is the most likely in the Asia and Southeast Asia region. Because of this regular interchange, the viruses of the region can thus be considered as forming a pool with some commonalities, with the extent of the mixing depending on geography and predominant wind patterns. Conventional quarantine measures cannot prevent infected vectors crossing national borders. There is no evidence that bluetongue has become established through the international trade of live ruminants or their germplasm.

Conversely, bluetongue viruses within the region can be differentiated by topotyping techniques (Gould et al. 1992), reflecting uncommon genetic sequences due to a lack of mixing. Whether there are any correlations between topotype and other biological characteristics is as yet unknown.

An increasing number of countries in the southern and eastern Asian and Pacific region are gathering information on their bluetongue status. One anticipates that, as more data are accumulated, the widespread distribution of bluetongue in all populations of susceptible ruminants will be increasingly accepted by animal health administrators. Decision makers will need to understand the factors contributing to virus virulence and to disease susceptibility of sheep.

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