

A decade of research on Bluetongue virus in Andhra Pradesh, a Southern state of India

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Summary

High sheep population density, congenial climatic conditions for *Culicoides* propagation, and susceptible sheep breeds may be contributing to the higher incidence of Bluetongue (BT) in Southern states of India. Sheep farming in this part of the country is nomadic in nature and BT is one of the major infectious diseases inflicting huge losses. Andhra Pradesh is one of the Southern states with high sheep population in India. Although isolation studies in this region were started in 1993, concerted efforts only began in 2002. More than 50 isolates were obtained in the last decade, and 7 Bluetongue virus (BTV) serotypes (1, 2, 9, 10, 12, 16 and 21) were isolated. Among them, BTV-10, BTV-12, and BTV-21 were reported for the first time from India and the genome analysis of these viruses revealed that BTV-10 and BTV-12 have high sequence identity with the modified live virus (MLV) vaccines used in USA and South Africa, respectively. At the same time, BTV-21 has probably originated from Southeast Asia. Furthermore, some of the BTV isolated from Europe have high sequence identity with viruses isolated from Andhra Pradesh indicating common ancestry. The analysis of different isolates involved in outbreaks revealed that more than 1 BTV serotype is involved and that mixed infections with different serotypes is not uncommon. In a limited study conducted during 2005-2009, it was observed that most of the sheep seroconverted to more than 1 serotype, which further supports circulation of multiple serotypes and mixed infections in Andhra Pradesh. Based on the virus isolation data, in this study it was observed that a few serotypes dominate for 3-4 years followed by domination of others. Continuous monitoring of circulating serotypes is essential to understand the distribution and spread BTV in endemic areas and for devising suitable control measures.

Dieci anni di ricerca sul virus della Bluetongue ad Andhra Pradesh, India meridionale

Parole chiave

Andhra Pradesh (India),
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Sierotipo,
Tipizzazione,
Topotipo,
Virus della Bluetongue.

Riassunto

L'alta incidenza del virus della Bluetongue (BTV) negli stati dell'India meridionale può essere dovuta a diversi fattori, tra cui: elevata densità della popolazione ovina, condizioni favorevoli alla propagazione di *Culicoides* e presenza di razze ovine suscettibili. L'allevamento degli ovini in questa parte del Paese è principalmente di natura nomade e la Bluetongue (BT) è una delle principali malattie infettive che causa gravi perdite economiche. Andhra Pradesh è uno stato dell'India meridionale con una numerosa popolazione ovina, sebbene studi di tipizzazione virale risalgano al 1993, il problema BT è stata affrontato in modo programmato solo a partire dal 2002. Nell'ultima decade sono stati identificati più di 50 ceppi virali appartenenti a 7 sierotipi (1, 2, 9, 10, 12, 16 e 21); tra questi, i sierotipi 10, 12 e 21 sono stati descritti per la prima volta in India. L'analisi genetica ha rivelato che i sierotipi 10 e 12 hanno una sequenza molto simile a quella dei ceppi utilizzati nei vaccini vivi attenuati (MLVs), impiegati

negli Stati Uniti e in Sudafrica, a differenza del sierotipo 21 che sembra provenire dal Sud-Est dell'Asia. Alcuni degli isolati virali europei hanno mostrato una sequenza genica molto simile agli isolati di Andhra Pradesh, ciò indica probabili origini comuni. L'analisi dei diversi ceppi virali responsabili delle epidemie di BT ha rivelato il contemporaneo coinvolgimento di diversi sierotipi di BTV dimostrando come le infezioni miste siano piuttosto frequenti. Anche uno studio effettuato nel periodo 2005-2009, ha evidenziato in molte pecore presenza di anticorpi nei confronti di più sierotipi. Tutto ciò ha supportato ulteriormente la tesi che nello stato di Andhra Pradesh circolino diversi sierotipi e si sviluppino infezioni miste. Sulla base dei risultati degli isolamenti effettuati, è stato possibile osservare come nello scenario infettivo della BT ogni 3-4 anni si alternino sierotipi diversi. È necessario quindi monitorare continuamente i sierotipi circolanti per comprendere meglio la presenza e diffusione di BTV nelle aree endemiche e per definire misure di controllo idonee.

Introduction

Andhra Pradesh¹ is one of the Southern states of India. Agriculture is the major source of income for rural people in this state, with livestock farming playing a subsidiary role. However, sheep farming in Andhra Pradesh is mostly limited to 2 semi-pastoral communities. Most of the sheep in the state belong to either Nellore and Deccani, or non-descript category related to 1 of these breeds. Sheep population is higher in arid areas owing to the availability of more grazing lands in these areas. Approximately half of the sheep flocks are owned by shepherds who migrate seasonally in search of grazing areas and/or water. The Nellore breed is hairy and more suitable for humid coastal areas and reared for mutton; whereas the Deccani sheep are more common in the interior parts of the state and are reared for mutton as well as for coarse wool production. Due to the lack of demand for wool, and the higher and increased demand for mutton, Nellore flocks are increasingly replacing Deccani flocks.

The climate in Southern states of India is tropical in nature and is highly influenced by monsoons. It can be classified into 3 major seasons: Summer (March-June) followed by rainy season (July to September), and Winter (October to February). The Summer season is hot and dry with occasional showers. The South-West monsoon contributes to the rainfall received by major part of the state, whereas the Southern parts of the state has considerable rainfall from North-East monsoon. Significant rainfall is also observed due to tropical cyclonic storms formed in the Bay of Bengal during October to December (Rao *et al.* 2016).

Bluetongue (BT) was first reported in India in exotic sheep from Maharashtra in 1965. Later, outbreaks of the disease were observed in indigenous breeds

(Prasad *et al.* 1992). Due to the congenial climate, insect-borne diseases are common in animals as well as humans during the tropical monsoon season. Bluetongue is an insect-borne disease, which is endemic and notifiable in India. Although serological evidence of Bluetongue virus (BTV) infection has been reported from different parts of the country in different species of animals, clinical disease has been observed only in sheep. There is a marked variation in disease pattern and reporting between Northern and Southern states of India. Overt disease has been reported more from Southern states of India. Since early 1980's, outbreaks of BTV have been recorded in Andhra Pradesh (Babu *et al.* 1988, Khan *et al.* 1983). Owing to a severe outbreak of BT from different parts of the state during 1983, the Animal Husbandry Department of the Government of Andhra Pradesh has instituted a systematic record-keeping of BT outbreaks since 1985 (Sreenivasulu *et al.* 2004).

Bluetongue outbreaks in Andhra Pradesh

Bluetongue has been recorded since the early 1980's from Andhra Pradesh (Babu *et al.* 1988, Khan *et al.* 1983). Although outbreaks have been recorded continuously throughout the year during the period of time going from 1996 to 2014, modal numbers are observed during August to December (Figure 1A). The incidence correlates with climatic factors. It has been noted that more outbreaks occur when daily average temperature, relative humidity, and wind speed were 26°C, 60-65% and 5 kmph, respectively (Johnson *et al.* 2006). This climate is very congenial for *Culicoides* breeding and survival.

A two-year trap study was conducted from July 2012 to June 2014 at Hyderabad, Andhra Pradesh (17.3700° N, 78.4800° E; Elevation: 530 m), during which weekly collections of *Culicoides* were performed. One insect trap was set up at sheep farm of Livestock Research Institute, Hyderabad,

¹ The undivided State of Andhra Pradesh was partitioned into two States – Telangana and Andhra Pradesh – after the submission of this article. The information contained in the articles refers to the undivided State.

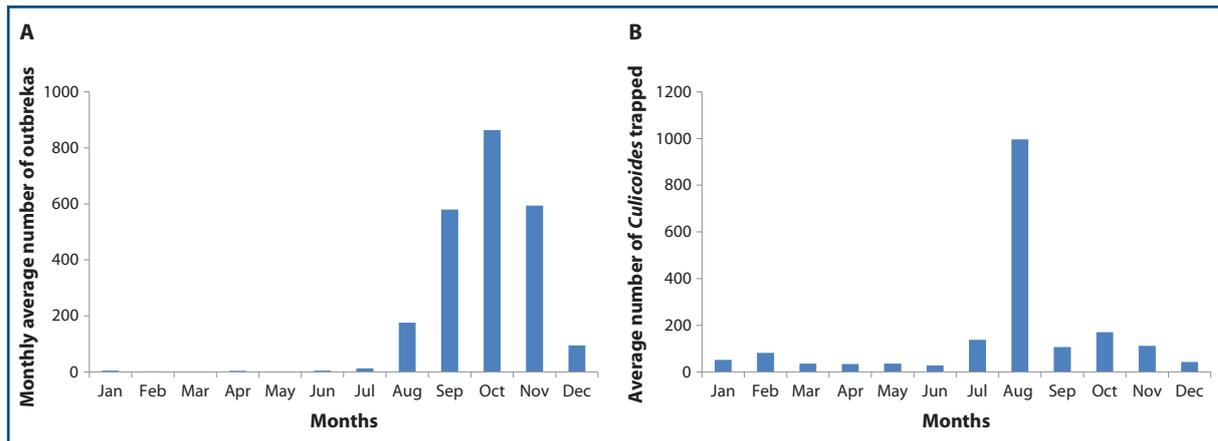


Figure 1. A. Monthly average outbreaks of Bluetongue in Andhra Pradesh during 1996–2014. **B.** Average number of *Culicoides* trapped during different months in the years 2013 and 2014 (total number of catches = 109).

India and insects were collected on a weekly basis for 109 weeks. A total of 1,834 *Culicoides* were collected throughout the study period belonging to the species *Culicoides peregrines*, *Culicoides oxystoma*, *Culicoides imicola*, *Culicoides trithecoides*, and *Culicoides shuffi* with the first 3 species being more abundant. During this 2-year study, it was observed that the population of *Culicoides* insects increased during the monsoon season (Figure 1B), and the disease outbreak either coincided with the midge activity or followed immediately thereafter (Figure 1A).

Similar *Culicoides* population dynamics were observed in the neighbouring state of Maharashtra (Narladkar and Shivpuje 2014). In general, although *Culicoides* population dwindles during the Summer, activity is observed throughout the year, and this may sustain virus circulation in the host population. Apart from these factors, high population density of susceptible sheep breeds may also be contributing to the higher incidence of BT in Southern states of India, especially Andhra Pradesh (Rao *et al.* 2016).

Seroprevalence of BT in domestic ruminants:

In Andhra Pradesh, BT has been clinically observed only in sheep, whereas anti-BTV antibodies have been detected also in other domestic ruminants, indicating inapparent infections. The observed prevalence of anti-BTV antibodies in sheep, goat, cattle, and buffaloes in sera collected between 2007 and 2014 from ruminant populations of Andhra Pradesh was 74.5% ($n = 1,523$), 75.3% ($n = 77$), 83.1% ($n = 138$), and 88.7% ($n = 399$), respectively, as assessed by cELISA (Veterinary Diagnostic Technology Inc, USA). Interestingly, the sero-prevalence in buffaloes was recorded up to 100% in some herds. Another study (Narladkar

and Shivpuje 2014) from the neighbouring state of Maharashtra also reported that higher number of *Culicoides* was trapped near cattle and buffalo herds and not in the proximity of sheep and goat flocks (Narladkar and Shivpuje 2014). Incidentally, the body odour of buffaloes is reported to be more attractive to female *Culicoides* compared to other domestic animals (Mands *et al.* 2004), and this could be a probable reason for the higher observed sero-prevalence in buffaloes. However, more research is necessary to understand the role of cattle and buffaloes in BT epidemiology in India.

Isolation and typing of BTV

Initial attempts to isolate BTV involved intravenous inoculation of lysed blood cells into embryonated chicken eggs (ECE) followed by inoculation of the chicken embryo homogenates into BHK-21 cells (Bommineni *et al.* 2011, Gollapalli *et al.* 2012, Rao *et al.* 2015, Rao *et al.* 2012a, Susmitha *et al.* 2012). However, the success rate of virus isolation was low with this procedure. Recently, *Culicoides* cell line (KC cells) (Wechsler and McHolland 1988) were obtained from The Pirbright Institute and the lysed blood cells were directly inoculated to KC cells followed by passaging in BHK-21 cells. This has enormously improved success of BTV isolation.

The BTV isolates were identified either by amplification of genome segment 5 or immunoperoxidase technique using anti-BTV-VP7 monoclonal antibodies. Isolates belonging to different serotypes were sequenced either following conventional reverse transcription polymerase chain reaction (RT-PCR) or sequence-independent primer amplification technique using primers designed based on segment 2 sequence for BTV-1, BTV-2, BTV-9, BTV-10, BTV-12, BTV-16, BTV-21, and BTV-23 (Rao *et al.* 2015, Rao *et al.* 2013, Rao *et al.*

2012 a, b). Viruses untypeable using this method were considered as either new serotype or a variant of the circulating serotypes. Such viruses were subjected to next generation sequencing (NGS) to identify the type (Rao *et al.* 2013).

Prevalence of different BTV serotypes in Andhra Pradesh

Prevalence of most of the serotypes of BTV in India was reported either by virus isolation or detection of serotype-specific anti-BTV antibodies in domestic ruminants. In Andhra Pradesh, prevalence of antibodies against BTV-4, BTV-6, BTV-12, BTV-13, BTV-14, BTV-17, BTV-18, and BTV-19 was reported during 1993-1995, and BTV-2 was isolated from sheep (Sreenivasulu and Rao 1999, Sreenivasulu *et al.* 1999). Serotype-specific antibodies were demonstrated against BTV-1, BTV-2, BTV-9, BTV-10, and BTV-23 in a study conducted in Andhra Pradesh during 2005-2009 (Sairaju *et al.* 2009). However, it is worth noting that some of the sera, which were positive by cELISA, did not neutralize any of the available viruses, suggesting the existence of serotypes other than BTV-1, BTV-2, BTV-9, BTV-10, and BTV-23. During the same study, it was found that the majority of the sera tested neutralized more than one serotype indicating a mixed serotype infection (Sairaju *et al.* 2013). From some of the outbreaks, BTV-1, BTV-2, BTV-4, BTV-9, BTV-10, BTV-12, BTV-16, BTV-21, and BTV-24 were isolated during 2002-2011 (Table I) (Gollapalli *et al.* 2012, Krishanjyothi *et al.* 2016, Reddy *et al.* 2015, Susmitha *et al.* 2012). Not surprisingly, the involvement of more than one BTV serotype in outbreaks and mixed serotype infections within the same animal was found to be a common phenomenon (Reddy *et al.* 2015). Similar phenomena have been observed earlier from the neighboring state of Maharashtra in India,

and in Israel and South Africa (Dungu *et al.* 2004, Uppal and Vasudevan 1980, Reddy *et al.* 2010a, Shimshony 2004, Verwoerd 2009, Brenner *et al.* 2010). The circulation or multiple serotypes of BTV could be common in tropical regions, where climatic conditions are congenial for *Culicoides* vector propagation for most part of the year.

Several exotic viruses were reported from the Southern states of India during the last decade. Notable among them are BTV-2, BTV-10, and BTV-12 (Gollapalli *et al.* 2012, Maan *et al.* 2012a, Rao *et al.* 2015). All these viruses belong to the Western topotypes and are closely related to the modified live virus (MLV) vaccine strains used in one or the other country. Apart from these viruses, a Western topotype segment 5 related BTV-3 MLV has been circulating in India since 1982 (Maan *et al.* 2012b, Rao *et al.* 2012a). Segment 9 of BTV-12 and BTV-21 isolated from Andhra Pradesh is more related to virus isolates from Southeast Asian countries than to other Indian isolates (Rao *et al.* 2015, Susmitha *et al.* 2012). The congenial climate in Southern India may be contributing to the spread and establishment of foreign viruses once they gain entry into this ecosystem. Some of the European isolates of BTV are related to the viruses circulating in India.

Vaccine development

Initial attempts were made at Sri Venkateswara Veterinary University (SVVU), Hyderabad, for mono and trivalent inactivated vaccine as well as to attenuate BTV-2. Later concerted efforts at SVVU and Veterinary Biological and Research Institute (VBRI), Hyderabad, India focussed on the development of inactivated vaccines using different types of inactivants and adjuvants. Under the All-India Network Project on Bluetongue (AINPBT)², SVVU, along with other partners, was involved in the development of a pentavalent (BTV-1, BTV-2, BTV-10, BTV-16, BTV-23) binary ethylenimine-inactivated vaccine (Reddy *et al.* 2010b). The strains and the technology have been transferred to vaccine manufacturers and the vaccine has been commercialised recently. In view of recent data on the circulation of some of these as well as other serotypes, the dynamics of periodic appearance and/or disappearance of different serotypes is an important area of current investigation by various groups. Prevalence of different BTV serotypes in an area may depend on herd immunity in the host population and continuous monitoring of host immunity and circulating serotypes is an essential

Table I. Isolation of different serotypes of Bluetongue virus from Andhra Pradesh between 2002 and 2011.

Year/ serotype	2002	2003	2005	2006	2007	2008	2009	2010	2011
BTV-1		+		+	+	+	+	+	+
BTV-2					+		+	+	
BTV-4						+			
BTV-9	+	+		+	+				
BTV-10		+							
BTV-12								+	+
BTV-16					+	+		+	+
BTV-21			+					+	
BTV-24								+	

² AINPBT. 2014. All India network programme on bluetongue. AINPBT. 2010. Annual Report, All India Network Project on Bluetongue. New Delhi, India: Indian Council of Agricultural Research.

step towards development of effective vaccine for BT in endemic areas.

Conclusions

Sheep in Southern states of India are frequently affected by BTV. The tropical climate in this part of country is congenial for *Culicoides* propagation, and yearlong activity of the midges has been observed. Due to this congenial climate, BT is endemic here and several serotypes of BTV have been circulating. Over the last decade, 7 serotypes of BTV have been isolated, 3 of which were isolated for first time from India. Continuous monitoring of the circulating serotypes and herd immunity against them is an

essential step towards the development of effective vaccination strategy in endemic countries like India.

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