

Bluetongue Research in Indonesia

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BTV Situation in Indonesia

- No clinical signs in Indonesian ruminants
- In 1981/1982 outbreak in imported Suffolk sheep in Indonesia (West Java)
- 1986: Surveillance in different province in Indonesia, many species of ruminants
- Serum: Serology by AGID → SN
- Blood (heparin): viral isolation in BHK-21
→ no results (depend on viraemia)
- **ESTABLISHED SENTINEL PROGRAM**



Purpose of the study

To describe key aspects of the BTV episystem in Indonesia with respect to:

- The vertebrate hosts
- The distribution of BTV infections
- The seasonality of BTV infections
- The BTV serotypes present
- The Culicoides spp present and their distribution
- The pathogenicity of the BTV serotypes present
- The molecular study





Investigative Approaches



- **Monitoring of sentinel animals (cattle)**
 - > Individually identified seronegative animals
 - > Bled weekly, collections stored in liquid nitrogen (heparin)
 - > Sera in serum Bank freezer (later monthly collection for sera)
 - > Collections transported to IRCVS with cold chain integrity
- **Serological surveys of cattle, buffaloes and sheep in provinces or islands with sentinel herds**
- **Weekly collections of insects by light trap at sentinel herd locations**
 - > Stored in alcohol
 - > Viral isolation



The BTV surveillance program field locations



Laboratory procedures

Virus isolation

- Primary pass by I/V inoculation of embryonated chicken eggs
- 2nd pass in *Aedes albopictus* (C6/36) cells
- 3rd and subsequent passes in BHK cells
- Initial characterization by immuno dot blot,
- Later by Ag-C-ELISA → PCR → sequencing

Serology

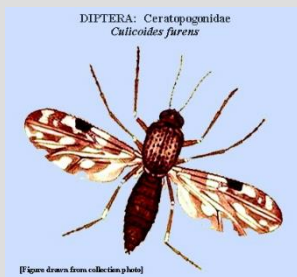
- C-ELISA (replacing the earlier AGID)
- Microtitre VNT for serotype determination



Laboratory procedures

Entomology

- Culicoides spp. were speciated on the basis of morphology and wing patterns using standard procedures



Results – Serology (Representative Data)

% Seroprevalence in the AGID test, by species

Province	Cattle	Buffaloes	Goats	Sheep
West Java	73	77	28	19
Central Java	59	87	NT	NT
West Timor	58	NT	50	26
Sumatera	53	71	NT	NT
Totals	62	75	37	19

- Reactors widely distributed in Indonesia
- Large ruminants have a higher seroprevalence than small ruminants

Sendow et al, 1986, *Veterinary Record* **119**: 603



Results – Virus Isolation

Depok

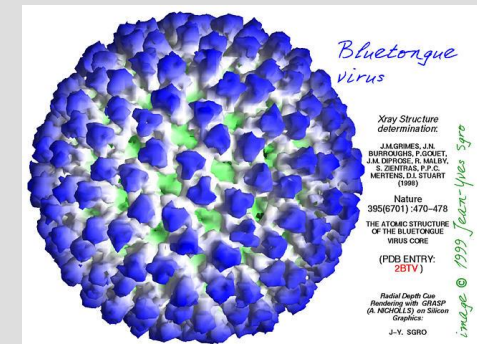
- BTV 1, 3, 7, 9, 12 and 21
- BTV 1, 6 and 21 from pool of *C. Fulvus* and *C. peregrinus*
- EHD, BEF Virus

Kupang

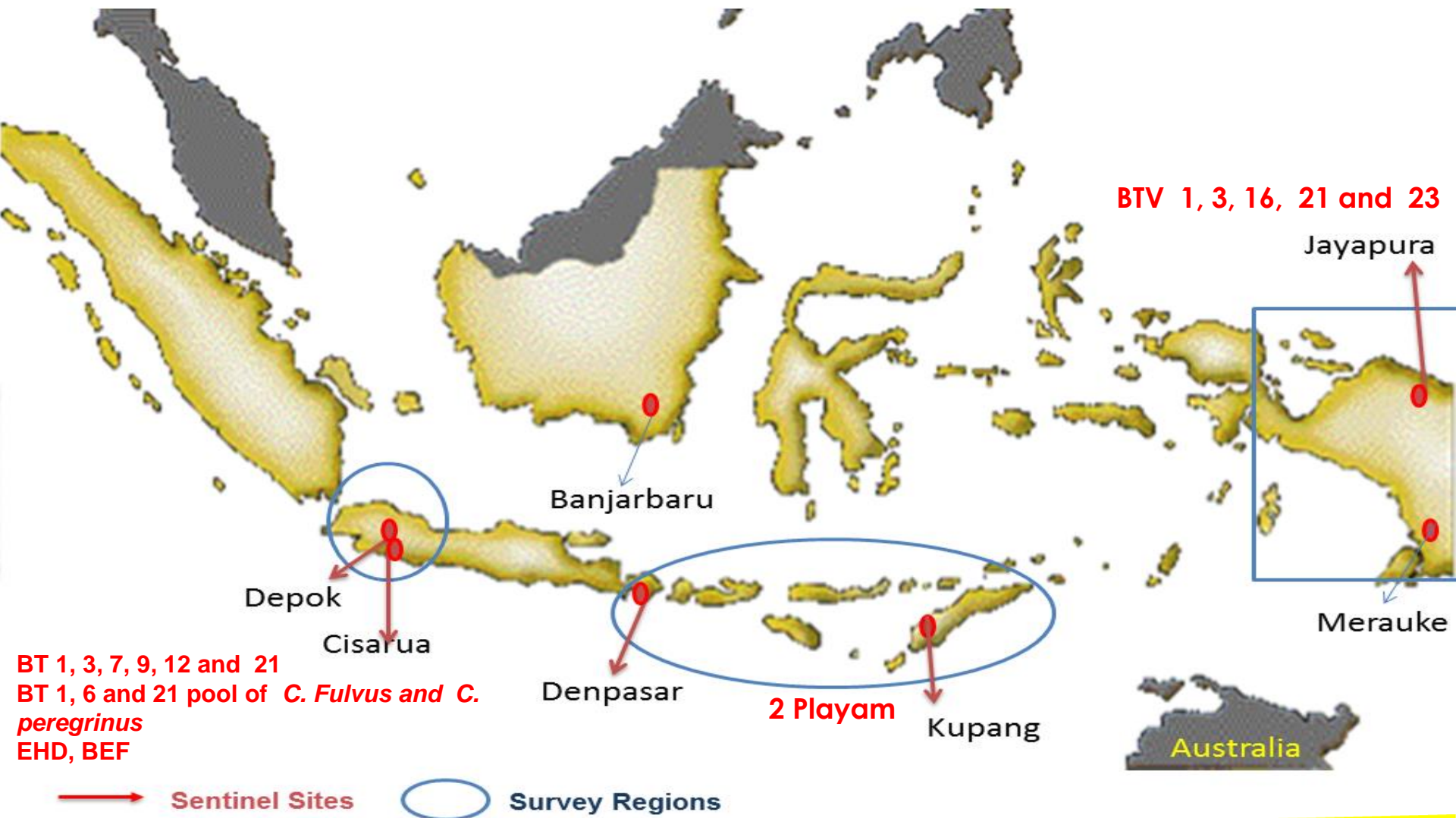
- 2 Palyam group viruses

Jayapura

- BTV 1, 3, 16, 21 and 23



The BTV Surveillance Program Field Locations



Results - Serology

Test results of BTV antibody in sentinel cattle in Depok in 1997 by C-ELISA.

Cattle No.	Sampling Time											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
D01	+	+	+	+	+	-	-	-	-	-	-	-
D02	-	-	-	-	-	-	-	-	-	-	+	+
D03	+	+	+	+	+	+	+	+	+	+	+	+
D04	+	+	+	+	+	+	+	+	+	+	+	+
D05	+	+	+	+	+	+	+	+	+	+	+	+
D06	-	-	-	-	-	-	-	-	-	-	Sold	
D07	-	-	+	+	+	+	+	+	+	+	+	+
D08	+	-	+	+	+	+	+	+	+	+	+	+
D09	-	+	+	+	+	+	+	+	+	+	+	+
D10	-	-	-	-	-	-	-	-	-	-	+	+
D11	-	-	-	-	-	-	-	-	-	-	Sold	
D12	+	+	+	+	+	+	+	+	+	+	Sold	
D13	+	+	+	+	+	+	+	+	+	+	Sold	
D14	-	-	-	-	-	-	-	-	-	-	-	-
D15	-	-	-	-	-	-	-	+	+	+	+	+
D16	-	-	-	-	-	-	-	-	-	+	-	-
D17	-	-	-	-	-	-	-	-	-	-	-	±
D18	NA	+	-	-	-	-	-	-	-	-	Sold	
D19	<i>NA</i>	-	-	-	-	-	-	-	-	-	-	-
D20	<i>NA</i>	-	-	-	-	-	-	-	-	-	-	-

NA = Not available

Culicoides spp collected in Different Areas in Indonesia

	Lampung	Banjarbaru	Depok	Cisarua	Cental Java	Denpasar	NTT			Irian Jaya (Papua)				
							Kupang	Sumba	Rote	Biak	Jayapura	Wamena	Meroke	Timika
Subgenus Avaritia														
<i>Actoni</i>	*	*	*	*	*					*	*		*	
<i>brevipalpis</i>							*							
<i>brevitarsis</i>			*	*		*	*		*	*			*	
<i>Dumduni</i>										*				
<i>flavipunctatus</i>			*	*		*	*			*			*	
<i>Fulvus</i>	*	*	*	*	*	*	*	*	*	*			*	
<i>Jacobsoni</i>			*	*						*			*	
<i>maculatus</i>			*	*				*		*	*		*	
<i>Nudipalpis</i>						*	*		*	*			*	*
<i>Orientalis</i>	*	*	*	*	*	*	*			*	*		*	*
<i>Pungens</i>													*	
<i>Wadai</i>			*	*			*		*	*	*		*	*
Subgenus Haemophorectus														
<i>Gemellus</i>			*	*						*	*		*	
Subgenus Hoffmania														
<i>Effuses</i>						*	*			*				*
<i>insignipennis</i>			*	*										
<i>Liui</i>				*										
<i>parabubalus</i>										*				
<i>peregrinus</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*
<i>Sumatrae</i>	*	*	*	*	*		*		*	*		*	*	*

	Lampung	Banjarbaru	Depok	Cisarua	Cental Java	Denpasar	NTT			Irian Jaya(papua)				
							Kupang	Sumba	Rote	Biak	Jayapura	Wamena	Merauke	Timika
Subgenus <i>Trithecoides</i>														
<i>Albibasis</i>			*				*							
<i>Anopheles</i>			*	*		*								
<i>Barnetti</i>			*	*		*	*		*		*			
<i>flavescens</i>							*							
<i>Gewertzi</i>			*	*			*				*			
<i>Huberti</i>											*			
<i>Palpifer</i>			*	*		*	*		*		*		*	
<i>parabarnetti</i>											*			
<i>parahumeralis</i>	*	*	*	*	*	*	*		*		*		*	
Subgenus <i>Meijerehelea</i>														
<i>Arakawae</i>	*	*	*		*	*	*		*		*			
<i>Guttifer</i>	*	*	*	*	*	*	*		*		*		*	
<i>Histrio</i>							*		*		*		*	
Other:														
<i>Ardleyi</i>											*			
<i>austropalpis</i>												*		
<i>cameronensi</i> (?)			*	*										
<i>clavipalpis</i>						*								
<i>Geminus</i>	*	*	*	*	*	*	*	*	*		*			
<i>Huffi</i>			*			*								
<i>neomelanesia</i>											*			
<i>Ornatus</i>									*			*		
<i>pampangensis</i>											*			
<i>Oxystoma</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*
<i>pangkorensis</i>							*					*		
<i>Papuensis</i>							*					*		
<i>peliliouensis</i>							*		*			*		
<i>Petersi</i>											*			
<i>pseudostigmatus</i>											*	*	*	
<i>pygmaeus</i>											*			
<i>semicircum</i>						*			*			*		
<i>Shortii</i>			*	*					*					

Results – Entomology: 1992 – 1993 Collections

	Jayapura	Merauke	Kupang	Denpasar	Depok
C actoni	**	*	-	-	**
C brevi-tarsis	*	**	*	**	*
C flavi-punctatus	*	*	*	*	*
C fulvus	**	*	**	**	**
C nudi-palpis	*	*	**	*	-
C orientalis	**	*	**	**	**
C wadai	*	*	*	-	*
C oxystoma	**	*	**	**	**

Confirmed vector species

Suspect/potential vector species

Pathogenicity Studies

- To determine the virulence of local BTV strain to local and imported sheep.
- Sero-negative Local sheep and imported Merino sheep
- Inoculated with original heparinised blood positive BTV 1, 9 and 21 (not from passaged cell culture) → Ag C-ELISA.
- Double insect proof pens

Results:

- Inoculated sheep produced seroconversion.
- imported sheep produce mild to moderate clinical signs
- Local sheep produce no to very mild clinical signs.
- No death sheep
- No abortion



Pathogenesis Studies (Imported /local sheep)

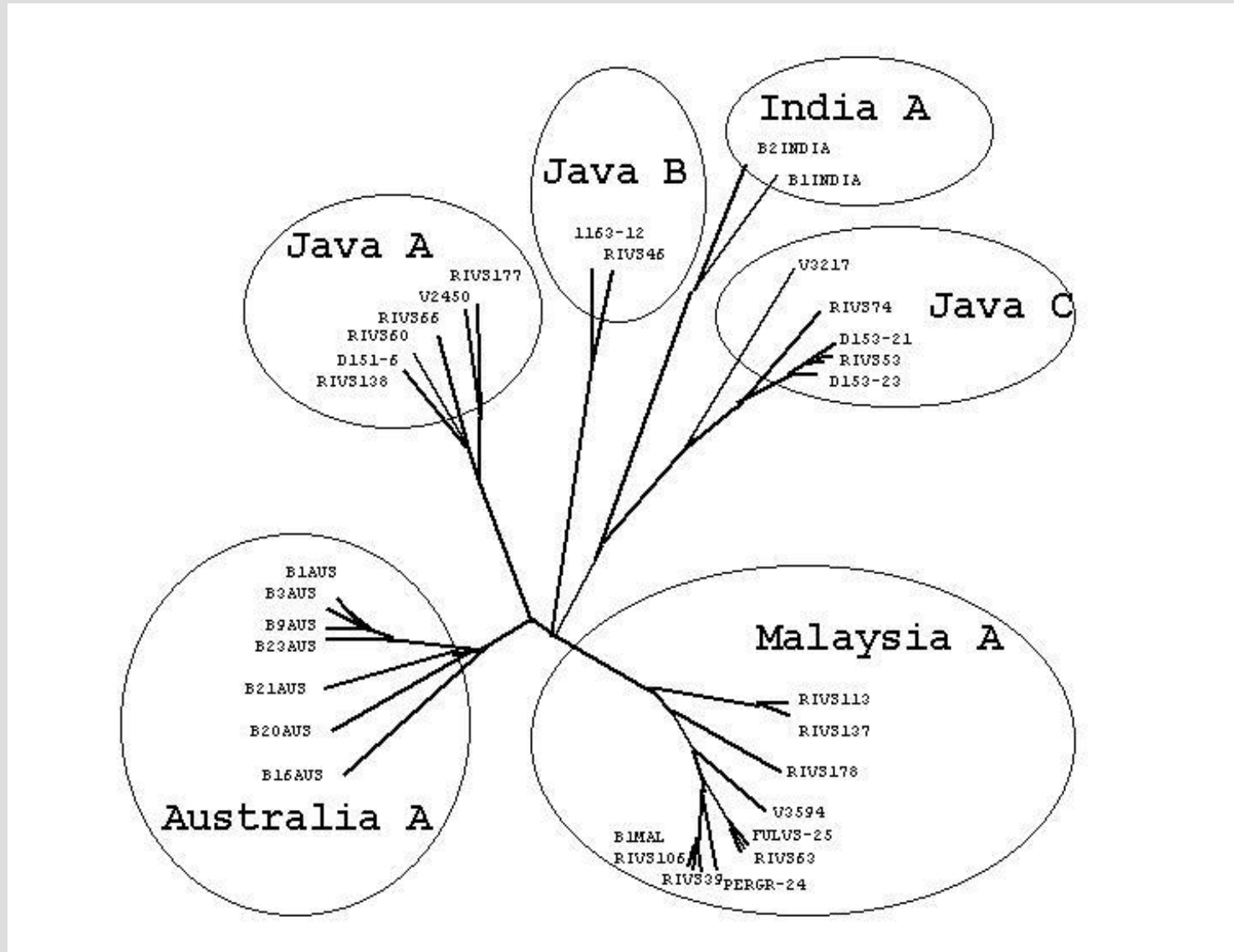
Clinical Signs	BTV 1	BTV 9	BTV 21
Fever > 39.5	+ / +	+ / -	+ / +
Depression	- / -	- / -	- / -
Nasal discharge	+ / +	+ / -	+ / +
Facial oedema	- / -	- / -	+ / -
Coronitis	+ / -	+ - / -	+ / -
Lame	- / -	- / -	- / -
Moribund/ death	- / -	- / -	- / -

Molecular study

- To identify whether each isolates comprised one large homogenous national large population
- Local BTV serotypes sequence
- Analyses to determine the topo-types correlates with geographic of isolation.
- Compare to other BTV from other country
- **Results:**
- Indonesian BTV isolates comprised of 3 group genotypes.
- The molecular analyses led to a new world of understanding about its epidemiology.



Figure 1. Phylogenetic tree of RNA-3 Bluetongue viruses



Conclusions

- A comprehensive BTV surveillance program can be conducted in the Asian setting, using sentinel herds and virus isolation and molecular techniques supported by serology
- BTV infections are widespread without causing disease
- The *Culicoides* spp confirmed as vectors in Australia are present throughout Indonesia
- There are also closely related *Culicoides* spp present whose vector status is unknown
- BTV serotypes investigated experimentally appear to be of low or only moderate virulence



Future Considerations

Predictions from Observations along the Transect

- ⦿ The BTV episystem in Indonesia have changed since the initial studies were done
- ⦿ The vertebrate hosts will be the same
- ⦿ New incursions or reassortants of BTV serotypes and genotypes will possibly be present
- ⦿ These may be of moderate virulence - maybe not
- ⦿ The previously identified vectors will still be present



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THANK YOU

