Monitoring Taiwanese bovine arboviruses and non-arboviruses using a vector-based approach

H.-Y. TZENG¹, H.-H. WU², L.-J. TING³, N.-T. CHANG⁴, Y.-C. CHOU⁵
and W.-C. TU¹

¹Department of Entomology, National Chung Hsing University, Taichung City, Taiwan, ²Bachelor's Degree Programme in Pet Grooming, Tajen University, Yanpu Township, Taiwan, ³Epidemiology Division, Animal Health Research Institute, New Taipei City, Taiwan, ⁴Department of Plant Medicine, National Pingtung University of Science and Technology, Neipu Township, Taiwan and ⁵Tainan City Animal Health Inspection and Protection Office, Tainan City, Taiwan

Abstract. In current sampling approaches, there exists a divergence between the surveillance of arthropod-borne and that of non-arthropod-borne viruses. It is commonly held that the collection of vector specimens applies only to arbovirus surveillance and that the surveillance of non-arboviruses must rely on traditional methods that involve the sampling of blood, faeces or saliva, or other examinations. The vector-based approach is a sampling method that has the ability to survey both arboviruses and non-arboviruses by distinguishing engorged vector specimens from entire vector samples. Accordingly, five arboviruses and three non-arboviruses were detected in a study using a vector-based approach conducted during 2012–2015. Hence, this report provides the first description of the Taiwanese vector species for the bovine arboviruses detected. The present investigations demonstrate that the vector-based approach applies not only to the surveillance of arboviruses, but also has potential as a possible tool for monitoring non-arboviruses on livestock farms in the future.

Key words. *Culicoides* biting midge, cattle, mosquito.

Introduction

Infectious viral diseases pose continual and substantial threats to the dairy industry and have serious implications for animal welfare, food security and even human health. In the interests of farm animal welfare, rapid diagnosis and treatment are required to avoid pain, injury and disease in animals. Furthermore, outbreaks of disease directly reduce the production or quality of dairy products, resulting in huge economic losses and threatening food security. In 2001, an outbreak of foot-and-mouth virus infected around one million cattle in the U.K. and the resulting economic losses were estimated to amount to US\$12.3–13.8 bn (Thompson *et al.*, 2002). However, zoonotic viral diseases of cattle, such as Rift Valley fever and Crimean–Congo haemorrhagic fever, remain constant threats to human populations (Ganter, 2015; Schuster *et al.*, 2016). Therefore, a sustainable system for the surveillance

of infectious disease on livestock farms is essential to the monitoring and control of such diseases.

In Taiwan, several bovine arboviruses are reported, including bovine fever ephemerovirus (BEFV) (Ephemerovirus: Rhabdoviridae), a Simbu serogroup virus, Akabane orthobunyavirus (AKAV) (Orthobunyavirus: Peribunyaviridae), a Palyam subgroup virus, Chuzan virus (CHUV) (Orbivirus: Reoviridae), a strain of epizootic haemorrhagic disease virus serotype 2, Ibaraki virus (IBAV) (Orbivirus: Reoviridae), and blue-tongue virus (Orbivirus: Reoviridae) (Liao *et al.*, 1994, 1996a, 1996b; Lee *et al.*, 2011; Ting *et al.*, 2016). However, entomological investigations into the relationships between vectors and bovine arboviruses are still meagre in Taiwan. In addition, several non-arboviruses have been reported, including bovine leukaemia virus (BLV) (Deltaretrovirus: Retroviridae), foot-and-mouth disease virus (Aphthovirus: Picornaviridae), bovine respiratory syncytial virus (BRSV) (Pneumovirus:

Correspondence: Wu-Chun Tu, Department of Entomology, National Chung Hsing University, No. 145 Xingda Road, Taichung 40225, Taiwan. Tel.: + 886 4 2284 0361 (ext. 507); Fax: + 886 4 2287 5024; E-mail: wctu@dragon.nchu.edu.tw

Paramyxoviridae), bovine coronavirus (BCoV) (Betacoronavirus: Coronaviridae), bovine viral diarrhoea virus (BVDV) (Pestivirus: Flaviviridae), rotavirus A (RVA) (Rotavirus: Reoviridae) and bovine enterovirus (BEV) (Enterovirus: Picornaviridae) (Wang, 1991; Huang *et al.*, 2000; Tsai, 2001; Lee, 2004; Pan, 2013). However, a comprehensive surveillance system that is effective in detecting a broad range of targets and is also affordable is still lacking in Taiwan.

Surveillance of arboviruses through the monitoring of vector insects, mosquitoes and midges is an intuitive concept and is commonly applied on livestock farms. Recent studies have indicated that surveillance of haemoparasites or human viruses has been carried out in engorged mosquitoes, but has not yet been applied to cattle (Boothe *et al.*, 2015; Grubaugh *et al.*, 2015). The present study monitored five bovine arboviruses [BEFV, AKAV, Aino virus (AINOV), CHUV and IBAV] and six bovine non-arboviruses (BLV, BVDV, BEV, RVA, BRSV and BCoV) in vector insects, and examined the feasibility of a vector-based approach as a possible surveillance tool in dairy farms.

Materials and methods

Vector sampling on dairy farms

The six sampling sites were distributed in Yunlin County, Chiayi County, Tainan City, Kaohsiung City and Pingtung County in southern Taiwan (Fig. 1). A modified blacklight trap (wavelength: 365 nm) was set up on each farm, and insect collections were conducted 2 h after sunset, when *Culicoides* (Diptera: Ceratopogonidae) biting midges and mosquitoes are more active on dairy farms. Live mosquitoes and midges were anaesthetized using carbon dioxide in the laboratory and then identified to genus or species level and engorged status under an Ez4D microscope (Leica Microsystems GmbH, Wetzlar, Germany). A maximum of 100 mosquitoes and 200 midges per pool were placed in a 2-mL microcentrifuge tube (Qiagen GmbH, Hilden, Germany) and stored at -80°C for further assays.

Total RNA extraction

Whole insects were homogenized using two 3-mm diameter steel beads in a TissueLyser II (Qiagen GmbH) at 30 grindings/s for 1 min. Total RNA was then extracted with a PureLink RNA Mini Kit (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.). In brief, the vector fragments were mixed with 0.7 mL Lysis solution containing 1% 2-mercaptoethanol in TissueLyser II to make a homogeneous solution and centrifuged at 16 000g for 10 min. Then, 0.6 mL of supernatants was drawn out and well mixed with 0.6 mL 70% ethanol. The solution was transferred to an RNA binding column and briefly centrifuged at 12 000g. The following wash procedures were performed in line with the manufacturer's instructions for the standard wash protocol. For the RNA elution, 50 μL of nuclease-free water from the mixture was added to the RNA for 3 min at room temperature and then centrifuged at 12 000g for 3 min for elution. The total RNA was stored at -80°C for further assays.

Reverse transcription

The reverse transcription (RT) procedure was performed using an iScriptTM cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). The concentrations of total RNA samples were measured with a NanoDropTM 2000 spectrometer (Thermo Fisher Scientific, Inc.). The total RNA was drawn out at between 1 ng and 1 μg for cDNA synthesis, according to the measuring results. Each RNA sample was mixed with 1 μL of reverse transcriptase, 4 μL of 5 $\times$ reaction buffer containing random hexamer and oligod(T) primer, and then increased to 20 μL with nuclease-free water. The synthesis of cDNA was conducted at 25°C for 5 min for primer annealing, at 42°C for 1 h for synthesis and at 85°C for 5 min to stop the activity of reverse transcriptase in an Applied Biosystems[®] 2720 Thermal Cycler (Thermo Fisher Scientific, Inc.). When the RT reaction was completed, cDNA was diluted to 40 μL using nuclease-free water and stored at -80°C .

Detection of arboviruses

Five arboviruses (BEFV, AKAV, AINOV, CHUV and IBAV) were detected by nested RT-polymerase chain reaction (PCR). The primer sets are listed in Table S1 following Shin *et al.* (2009). All viruses were detected using an Ex-Taq DNA Polymerase Kit (Takara Bio, Inc., Shiga, Japan). The PCR mix was composed of 0.2 μL of Ex-Taq DNA polymerase, 1 μL of DNA template (cDNA or first PCR product), 1 μL of each primer at 10 μM , 2 μL of 10 $\times$ reaction buffer and 1 μL of 2.5 mM dNTP mix, and increased to a final volume of 20 μL by the addition of nuclease-free water. The PCR thermal conditions are listed in Table S2. The secondary PCR products were analysed by electrophoresis using 1.5% agarose gel.

Detection of non-arboviruses

Three non-arboviruses (BLV, BVDV and BRSV) were detected by nested RT-PCR, and three other viruses (BEV, BCoV and RVA) were detected by RT-PCR in 192 pools of engorged vector specimens in 2014 and 2015, respectively. The primer sets and PCR thermal conditions are listed in Tables S1 and S2. The PCR mix and electrophoresis analysis were identical to those used in arbovirus detections.

Cloning and sequencing of bovine viruses

The virus amplicon was extracted using a Quick GelTM Extraction Kit (Thermo Fisher Scientific, Inc.). Then, the purified DNA was ligated into a pGEM[®]-T Easy Vector using T4 DNA ligase (Promega Corp., Madison, WI, U.S.A.) at 16°C for 14 h. The ligated plasmids were transformed into competent cells of *Escherichia coli* DH5 α at 42°C for 30 s and then incubated on lysogeny broth (LB) agar plates with 50 μM ampicillin (Sigma-Aldrich Corp., St Louis, MO, U.S.A.), 40 μL of 100 mM IPTG solution (Sigma-Aldrich Corp.) and 40 μL of 1% X-gal solution (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside)

Fig. 1. Localities of sampling farms in Taiwan. YL, Yunlin; CY, Chiayi; TN, Tainan; KH, Kaohsiung; PT, Pingtung.

(Sigma-Aldrich Corp.) at 37 °C for 16 h. The target inserts in colonies were checked by PCR with specific target primers and electrophoresis run in 1% agarose gel. The checked colonies were sequenced by a local company (Genomic Ltd, Taipei, Taiwan). The sequencing results were transferred into VECTORNTI® Version 10 (Thermo Fisher Scientific, Inc.) and trimmed. The sequence identity was verified by BLAST, and each verified sequence was uploaded to GenBank at the National Center for Biotechnology Information (NCBI) (Bethesda, MD, U.S.A.).

Minimum infection rates

Minimum infection rates (MIRs) for five arboviruses detected in the unengorged vector specimens via species identification were calculated with the following equation, with only one infected individual assumed in each positive pool:

$$\frac{\text{number of positive pools}}{\text{total number of unengorged specimens tested}} \times 1000$$

Results

From September 2012 to December 2015, a total of 58 576 *Culicoides* biting midges in 520 pools and 11 741 mosquitoes in 569 pools were collected. Overall, 5855 *Culicoides* biting midges in 123 pools and 8630 mosquitoes in 425 pools were identified to species and the remainder were identified to

genus level. Unengorged pools accounted for 39.8% ($n=450$), engorged pools for 28.1% ($n=317$), and unidentified pools for 32.1% ($n=363$) of the total. Five *Culicoides* species (*Culicoides oxystoma*, *Culicoides nipponensis*, *Culicoides arakawae*, *Culicoides actoni* and *Culicoides bubalus*) were identified. *Culicoides oxystoma* was the dominant species ($n=5484$, 93.7%), followed by *C. nipponensis* ($n=236$, 4.0%), *C. actoni* ($n=108$, 1.8%), *C. arakawae* ($n=16$, 0.3%) and *C. bubalus* ($n=11$, 0.2%). Of the 11 mosquito species identified within the genera *Anopheles*, *Aedes*, *Armigeres* and *Culex* (all: Diptera: Culicidae), six belonged to the genus *Culex*. Among these, *Culex tritaeniorhynchus* was the most abundant ($n=7758$, 89.9%), followed by *Culex quinquefasciatus* ($n=354$, 4.1%) and *Armigeres subalbatus* ($n=252$, 2.9%). Each of the other eight species accounted for < 1% of the mosquitoes identified to species level (Table 1).

In this study, four arboviruses (BEFV, AKAV, AINOV and CHUV) were detected. The finding of *Shamonda orthobunyavirus* [Peaton virus (PEAV)] was unexpected. Samples containing PEAV were distinguished from AINOV samples after sequencing and BLAST analysis. Taiwanese and Japanese AINOV exhibited 98.8–99.7% NP gene homology and PEAV exhibited 98.8–99.1% NP gene homology (Yanase *et al.*, 2010). The detected arboviruses were deposited in GenBank under accession numbers KF676987–KF676999 and MF083618–MF083684. In the detected arboviruses, the structure genes on the M segments of AKAV, AINOV and PEAV and segments 2 and 6 of CHUV were sequenced for further molecular epidemiological studies (data not shown).

In 2012, BEFV, AINOV and PEAV were detected in Yunlin County site A (YL-A), and AKAV was detected in YL-A, Yunlin County site B (YL-B) and Pingtung County (PT) (Fig. 1). In 2013, positive detections of AKAV, AINOV and PEAV were observed from October to December. Akabane orthobunyavirus was detected in Kaohsiung City (KH), AINOV in YL-A, YL-B and PT, and PEAV in YL-A. Chuzan virus was detected from July to September in YL-A and YL-B. In 2014, there were 13 detections of BEFV from April to September and 18 detections of AINOV from January to June. Five viruses, BEFV, AKAV, AINOV, PEAV and CHUV, were detected in 2015; among them, CHUV accounted for 45.5% of overall detections (Table 2).

Two species of *Culicoides* biting midge and six species of mosquito were found to carry target arboviruses that were determined in unengorged specimens. *Culicoides oxystoma* was found to carry AKAV, AINOV, CHUV and BEFV, and *C. arakawae* carried BEFV. *Anopheles sinensis* carried BEFV and AKAV, and the other mosquito species carried various viruses (Table 3).

The MIRs in *C. oxystoma* for infection by the five arboviruses detected ranged from 0.191 to 0.572, whereas MIRs in *Cx. tritaeniorhynchus* were 0.644, 0.805 and 0.161 for infection with BEFV, AINOV and CHUV, respectively. The MIRs in the other six vector species are shown in Table 4.

In surveys of non-arboviruses, BLV, BVDV and RVA were detected in engorged *Culicoides* biting midges and mosquitoes. The results of BLAST analyses indicated that 160 bp of 5'UTR sequences in BVDV belonged to BVDV type 1 and 384 bp of the VP6 gene sequence in RVA belonged to rotavirus A. In 2014, BLV was detected in YL-A, and BLV and RVA were detected in Tainan City (TN). In 2015, BLV was detected in YL-A, YL-B, TN and KH, whereas BVDV was detected in YL-A (Table 5).

Discussion

Investigations into arbovirus vectors and their ecological characteristics are important for controlling arboviruses in the field, but the vector species of bovine arboviruses in Taiwan are still unknown. A comprehensive investigation conducted in dairy farms in Taiwan found 17 species of *Culicoides* biting midge (Chien, 2015). However, the present study identified only five species, although this may reflect the short duration of the period of collection of live specimens for virus detections. *Culicoides oxystoma* is a dominant species on dairy farms, and is also associated with paddy rice fields (Yanase *et al.*, 2013). This species is identified as a vector of BEFV, AKAV, AINOV, PEAV and CHUV in Taiwan and Japan (Yanase *et al.*, 2005). The present findings revealed an ornithophilic species, *C. arakawae*, which may also be a potential vector of BEFV, and its occurrence may be related to the dynamic of wild birds or to distance from a poultry farm (Yanase *et al.*, 2005). Although target bovine arboviruses are considered to be mainly *Culicoides*-borne viruses, mosquitoes may also play an important role in bovine arbovirus transmission. *Culex tritaeniorhynchus* may be a potential vector of BEFV, AINOV and CHUV. In a previous study, AKAV was isolated from *Cx. tritaeniorhynchus* in Japan (Yamakawa *et al.*, 2006), but was not in the present study; however, AKAV was detected in *Cx. quinquefasciatus* in Taiwan. Thus, the susceptibility of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* to Taiwanese AKAV strains requires further examination. *Culex quinquefasciatus* may be a potential vector of AINOV, whereas *Cx. tritaeniorhynchus*, *Culex pipiens molestus* and *Culex fuscocephala* are possible vectors of CHUV in Taiwan. The present authors recorded the first finding of *Ar. subalbatus* as a potential vector of AINOV and CHUV

Table 1. Vector insects collected for the detection of arboviruses and non-arboviruses.

Species	Specimens, <i>n</i>	Pools, <i>n</i>	Engorged pools, <i>n</i>		
			No	Yes	Unidentified
Biting midges	<i>Culicoides</i> spp.	52 721	397		
	<i>C. oxystoma</i>	5484	92		
	<i>C. nipponensis</i>	236	10		
	<i>C. arakawae</i>	16	11		
	<i>C. actoni</i>	108	8		
	<i>C. bubalus</i>	11	2		
Mosquitoes	<i>Anopheles</i> spp.	214	63		
	<i>An. sinensis</i>	77	27		
	<i>Aedes</i> spp.	4	4		
	<i>Ae. albopictus</i>	19	7		
	<i>Ae. vexans</i>	11	2		
	<i>Armigeres</i> spp.	154	22		
	<i>Ar. subalbatus</i>	252	42		
	<i>Ar. baisasi</i>	2	1		
	<i>Culex</i> spp.	2739	109		
	<i>Cx. tritaeniorhynchus</i>	7758	208		
	<i>Cx. quinquefasciatus</i>	354	50		
	<i>Cx. pipiens molestus</i>	73	19		
	<i>Cx. fuscocephala</i>	57	8		
	<i>Cx. annulus</i>	26	6		
	<i>Cx. sinensis</i>	1	1		

Table 2. Polymerase chain reaction (PCR) detection of bovine arboviruses on dairy farms in southern Taiwan.

Date	BEFV						AKAV						AINOV						PEAV						CHUV					
	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT
2012 September	•						•	•					•																	
October–December																														
2013 January–March																														
April–June																														
July–September																														
October–December																														
2014 January–March																														
April–June	•																													
July–September	•																													
October–December																														
2015 January–March																														
April–June																														
July–September																														
October–December																														

•, positive by PCR detection.

BEFV, bovine fever ephemerovirus; AKAV, Akabane orthobunyavirus; AINOV, Aino virus (Shuni orthobunyavirus); PEAV, Peaton virus (Shamonda orthobunyavirus); CHUV, Chuzan virus (Palyam virus). YL-A and YL-B are located in Yunlin County, CY in Chiayi County, TN in Tainan City, KH in Kaohsiung City, and PT in Pingtung County.

and propose that *An. sinensis* is a potential vector of BEFV and AKAV. The habitats of these mosquito vectors are associated with rice paddy fields, human residues and cattle dung (Ohba *et al.*, 2013). Dairy farms in Taiwan are commonly located near other livestock farms or close to rice paddies, which provides these vector species with various ideal habitats.

The present MIR data indicate higher estimations in mosquitoes than in *Culicoides* biting midges (Table 4). There are no previous reports of MIRs of the five detected arboviruses, but the MIRs of Schmallenberg virus (*Orthobunyavirus*) and bluetongue virus (*Orbivirus*) were found to fall in the interval between 0.1 and 100 (Braks *et al.*, 2017). However, MIRs are vulnerable to interference by intrinsic and extrinsic factors, such as the extrinsic incubation period in a vector, sampling population size, and the sensitivity of the detection method (Bustamante & Lord, 2010). Thus, the present group proposes to examine the vector competence of the potential vector species identified for detected arboviruses because most of these can be bred in the laboratory.

A nationwide programme of surveillance of AKAV, CHUV and IBV showed their seroprevalences to be 83.95%, 67.78% and 80.77%, respectively (Ting *et al.*, 2004), which suggests that these arboviruses might have become endemic after the first isolation in the 1990s. The current arbovirus detections demonstrate that both AKAV and CHUV, but not IBV, were epidemic during 2012–2015. The present study used a different primer set designed for the Taiwanese IBV strain (Table S1), but the result was the same as in the previous survey. It is possible that the vectors in the current collections may not carry IBV in the field or that IBV was not epidemic in 2012–2015; however, its occurrence needs further investigation.

Both AINOV and PEAV were first detected in Taiwan, although they have since been isolated or detected in Australia and Japan (St George *et al.*, 1980; Yanase *et al.*, 2010). In the present study, the first detections of AINOV were in YL-A in October 2012, but the main detections were recorded from the end of 2013 to the middle of 2014 in five sampling farms. However, PEAV was detected only in YL-A in November 2012, and in 2013 and 2015, and this temporal and geographic profile of PEAV requires further study. Some reassortment between AINOV and PEAV may occur in nature (Yanase *et al.*, 2010), although this phenomenon has not yet been observed by cloning their structure genes (data not shown). In 2012–2013, BEFV broke out in both the Yaeyama Islands, Japan, and Taiwan; BEFV may have been transferred from the Yaeyama Islands to eastern Taiwan through an airstream (Ting *et al.*, 2014; Hayama *et al.*, 2016). The initial outbreak of BEFV occurred in September 2012 in eastern Taiwan, but the virus soon spread to western Taiwan via Pingtung County; this temporospatial pattern of occurrence is in line with predictions made by airstream simulation (Hayama *et al.*, 2016). Another viewpoint indicated that BEFV may spread in a seasonal direction from southern China to southwestern Taiwan (Walker & Klement, 2015). Therefore, phylogenetic and biogeographic analyses of vectors and pathogens are essential for understanding temporospatial patterns of occurrence of bovine arboviral diseases in terms of the influences of geographic distributions and seasonal atmospheric conditions in countries of eastern and southeastern Asia.

Table 3. Polymerase chain reaction (PCR) detection of bovine arboviruses in unengorged vector specimens.

Species		Positive detections in unengorged specimens				
		BEFV	AKAV	AINOV	PEAV	CHUV
Biting midges	<i>Culicoides</i> spp.	•		•	•	
	<i>C. oxystoma</i>	•	•	•	•	•
	<i>C. nipponensis</i>					
	<i>C. arakawae</i>	•				
	<i>C. actoni</i>					
Mosquitoes	<i>C. bubalus</i>					
	<i>Anopheles</i> spp.		•	•		
	<i>An. sinensis</i>	•	•			
	<i>Aedes</i> spp.					
	<i>Ae. albopictus</i>					
	<i>Ae. vexans</i>					
	<i>Armigeres</i> spp.					
	<i>Ar. subalbatus</i>					
	<i>Ar. baisasi</i>			•		•
	<i>Culex</i> spp.	•		•		
	<i>Cx. tritaeniorhynchus</i>	•		•		•
	<i>Cx. quinquefasciatus</i>		•	•		
	<i>Cx. pipiens molestus</i>					•
	<i>Cx. fuscocephala</i>					•
	<i>Cx. annulus</i>					

•, positive by PCR detection.

BEFV, bovine fever ephemerovirus; AKAV, Akabane orthobunyavirus; AINOV, Aino virus (Shuni orthobunyavirus); PEAV, Peaton virus (Shamonda orthobunyavirus); CHUV, Chuzan virus (Palyam virus).

Table 4. Minimum infection rates (MIRs) in the vector species.

Vector species		MIR				
		BEFV	AKAV	AINOV	PEAV	CHUV
Biting midges	<i>Culicoides oxystoma</i>	0.191	0.191	0.191	0.191	0.572
	<i>Culicoides arakawae</i>	66.667	—	—	—	—
Mosquitoes	<i>Anopheles sinensis</i>	17.241	17.241	—	—	—
	<i>Armigeres subalbatus</i>	—	—	5.291	—	10.582
	<i>Culex tritaeniorhynchus</i>	0.644	—	0.805	—	0.161
	<i>Culex quinquefasciatus</i>	—	2.959	5.917	—	—
	<i>Culex pipiens molestus</i>	—	—	—	—	14.085
	<i>Culex fuscocephala</i>	—	—	—	—	18.519

—, virus not identified in the vector species.

The MIRs represent numbers of infected individuals per 1000 vector individuals.

BEFV, bovine fever ephemerovirus; AKAV, Akabane orthobunyavirus; AINOV, Shuni orthobunyavirus; PEAV, Peaton virus (Shamonda orthobunyavirus); CHUV, Chuzan virus (Palyam virus).

Bovine leukaemia virus is a blood-borne pathogen that is responsible for longterm viraemia in cattle and has been diagnosed earlier in Taiwan (Wang, 1991; Barez *et al.*, 2015). Both characteristics are useful for elucidating the feasibility of vector-based approaches in dairy farms. The present results showed a positive rate of 18.75%, but the conversion of data between regular serum investigations and vector-based approaches requires further study. The vector-based approach may also be applicable for the surveillance of other pathogens in cattle blood, such as *Anaplasma* spp., *Babesia* spp. and *Trypanosoma* spp. The detection of BVDV and RVA demonstrated that a vector-based approach can also be applied to transient viraemia viruses. Rotavirus A is abundant in cattle faeces, and although the contamination by faeces of vector specimens could not be completely excluded in this study, RT-PCR analyses in

three other pools collected at the same time from the same trap showed negative results.

The present study is the first to report the use of engorged vectors (i.e. mosquitoes and biting midges) as tools for the surveillance of non-arboviruses in dairy farms. The vector-based approach provides rapid results by RT-PCR detections within 24 h and allows for a high frequency of sampling. It also reduces the pain and possible distress associated with blood sampling in cattle. Furthermore, users of this approach do not need professional expertise to identify vector species if the targets of analysis are non-arboviruses, and the pooling of different vector species will not affect the detection of non-arboviruses. However, the current vector-based approach requires further improvement if it is to be applied in the field because the efficiency of this approach must be comparable with that of other

Table 5. Polymerase chain reaction (PCR) detections of three positive bovine non-arboviruses.

Date	BLV						BVDV						RVA					
	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT	YL-A	YL-B	CY	TN	KH	PT
2014	January–March	•																
	April–June	•		•												•		
	July–September																	
	October–December																	
2015	January–March			•	•	•												
	April–June	•	•	•														
	July–September	•	•	•	•													
	October–December			•														

•, positive by PCR detection.

BLV, bovine leukaemia virus; BVDV, bovine viral diarrhoea virus; RVA, rotavirus A.

YL-A and YL-B are located in Yunlin County, CY in Chiayi County, TN in Tainan City, KH in Kaohsiung City, and PT in Pingtung County.

sampling methods, such as blood or milk sampling, and other details of this approach, such as the parameters of trap setting, limitation of collection time and the effects of blood digested in the midguts of vectors, also require further investigation. Therefore, the present authors suggest that the vector-based approach has the potential to be used as a surveillance tool to increase understanding of epidemics of both arboviruses and non-arboviruses on livestock farms.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Primer sets used in this study.

Table S2. Polymerase chain reaction conditions in this study.

Acknowledgements

The authors thank the Bureau of Animal and Plant Health Inspection and Quarantine, Taiwan, for financial support (project no. 103AS-10.3.1-BQ-B1; 104AS-10.10.3-BQ-B1). The authors are grateful to Kuei-Min Liao, Yi-Hsuan Chien and Chun-Hsien Lin, Department of Entomology, National Chung Hsing University, for their assistance in insect collections, and to Roger F. Hou, Department of Entomology, National Chung Hsing University, Taichung City, Taiwan, for his critical reading of this manuscript. The authors declare no conflicts of interest.

References

- Barez, P.Y., de Brogniez, A., Carpentier, A. *et al.* (2015) Recent advances in BLV research. *Viruses*, **7**, 6080–6088.
- Boothe, E., Medeiros, M.C., Kitron, U.D. *et al.* (2015) Identification of avian and hemoparasite DNA in blood-engorged abdomens of *Culex pipiens* (Diptera; Culicidae) from a West Nile virus epidemic region in suburban Chicago, Illinois. *Journal of Medical Entomology*, **52**, 461–468.

- Braks, M., Mancini, G., de Swart, M. & Goffredo, M. (2017) Risk of vector-borne diseases for the EU: entomological aspects: Part 2. *EFSA Supporting Publications*, **14**, 1184e.
- Bustamante, D.M. & Lord, C.C. (2010) Sources of error in the estimation of mosquito infection rates used to assess risk of arbovirus transmission. *American Journal of Tropical Medicine and Hygiene*, **82**, 1172–1184.
- Chien, Y.C. (2015) Population surveillance of *Culicoides* spp. (Diptera: Ceratopogonidae) in dairies and vector competence of *Culicoides oxystoma* to bovine ephemeral fever virus. Master's Thesis. National Pingtung University of Science and Technology, Neipu Township.
- Ganter, M. (2015) Zoonotic risks from small ruminants. *Veterinary Microbiology*, **181**, 53–65.
- Grubaugh, N.D., Sharma, S., Krajacich, B.J. *et al.* (2015) Xeno-surveillance: a novel mosquito-based approach for examining the human-pathogen landscape. *PLoS Neglected Tropical Diseases*, **9**, e0003628.
- Hayama, Y., Moriguchi, S., Yanase, T. *et al.* (2016) Epidemiological analysis of bovine ephemeral fever in 2012–2013 in the subtropical islands of Japan. *BMC Veterinary Research*, **12**, 47.
- Huang, C.C., Jong, M.H. & Lin, S.Y. (2000) Characteristics of foot and mouth disease virus in Taiwan. *Journal of Veterinary Medical Science*, **62**, 677–679.
- Lee, Y.F. (2004) Isolation, identification, gene characterization and development of inactivated vaccine of bovine coronavirus in Taiwan. Master's Thesis. National Cheng Kung University, Tainan.
- Lee, F., Ting, L.J., Lee, M.S., Chang, W.M. & Wang, F.I. (2011) Genetic analysis of two Taiwanese bluetongue viruses. *Veterinary Microbiology*, **148**, 140–149.
- Liao, Y.K., Lu, Y.S., Hwang, S.C. & Chang, C.E. (1994) The study on the epidemiology of Akabane virus and Chuzan virus infection in cattle in Taiwan. *Experimental Report of Taiwan Animal Health Research Institute*, **30**, 47–53.
- Liao, Y.K., Lu, Y.S., Goto, Y. & Inaba, Y. (1996a) The isolation of Akabane virus (Iriki strain) from calves in Taiwan. *Journal of Basic Microbiology*, **36**, 33–39.
- Liao, Y.K., Lu, Y.S., Huang, S.T. & Lee, S.H. (1996b) Etiological and epidemiological studies on the Ibaraki disease in Taiwan. *Chinese Journal of Veterinary Science*, **22**, 183–191.
- Ohba, S.Y., Matsuo, T. & Takagi, M. (2013) Mosquitoes and other aquatic insects in fallow field biotopes and rice paddy fields. *Medical and Veterinary Entomology*, **27**, 96–103.

- Pan, Y.C. (2013) Prevalence survey of bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) in Taiwan. Master's Thesis. National Chung Hsing University, Taichung.
- Schuster, I., Mertens, M., Mrenoshki, S. *et al.* (2016) Sheep and goats as indicator animals for the circulation of CCHFV in the environment. *Experimental and Applied Acarology*, **68**, 337–346.
- Shin, Y.K., Oem, J.K., Yoon, S. *et al.* (2009) Monitoring of five bovine arboviral diseases transmitted by arthropod vectors in Korea. *Journal of Bacteriology and Virology*, **39**, 353–362.
- St George, T.D., Standfast, H.A., Cybinski, D.H., Filippich, C. & Carley, J.G. (1980) Peaton virus: a new Simbu group arbovirus isolated from cattle and *Culicoides brevitarsis* in Australia. *Australian Journal of Biological Sciences*, **33**, 235–243.
- Thompson, D., Muriel, P., Russell, D. *et al.* (2002) Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Revue Scientifique et Technique*, **21**, 675–687.
- Ting, L.J., Lee, M.S., Kuo, S.T., Shiau, J.R. & Sung, W.H.T. (2004) Serological survey on arbovirus infections of cattle in Taiwan. *Experimental Report of Taiwan Animal Health Research Institute*, **40**, 65–74.
- Ting, L.J., Lee, M.S., Lee, S.H., Tsai, H.J. & Lee, F. (2014) Relationships of bovine ephemeral fever epizootics to population immunity and virus variation. *Veterinary Microbiology*, **173**, 241–248.
- Ting, L.J., Lee, M.S., Lin, Y.L., Cheng, M.C. & Lee, F. (2016) Invasion of exotic bovine ephemeral fever virus into Taiwan in 2013–2014. *Veterinary Microbiology*, **182**, 15–17.
- Tsai, H.J. (2001) Serological survey on antibodies against infectious bovine rhinotracheitis, bovine respiratory syncytial virus infection, and bovine ephemeral fever in cattle in Taiwan. *Chinese Journal of Veterinary Science*, **27**, 190–197.
- Walker, P.J. & Klement, E. (2015) Epidemiology and control of bovine ephemeral fever. *Veterinary Research*, **46**, 124.
- Wang, C.T. (1991) Bovine leukemia virus infection in Taiwan: epidemiological study. *Journal of Veterinary Medical Science*, **53**, 395–398.
- Yamakawa, M., Yanase, T., Kato, T. & Tsuda, T. (2006) Chronological and geographical variations in the small RNA segment of the teratogenic Akabane virus. *Virus Research*, **121**, 84–92.
- Yanase, T., Kato, T., Kubo, T. *et al.* (2005) Isolation of bovine arboviruses from *Culicoides* biting midges (Diptera: Ceratopogonidae) in southern Japan: 1985–2002. *Journal of Medical Entomology*, **42**, 63–67.
- Yanase, T., Aizawa, M., Kato, T., Yamakawa, M., Shirafuji, H. & Tsuda, T. (2010) Genetic characterization of Aino and Peaton virus field isolates reveals a genetic reassortment between these viruses in nature. *Virus Research*, **153**, 1–7.
- Yanase, T., Matsumoto, Y., Matsumori, Y. *et al.* (2013) Molecular identification of field-collected *Culicoides* larvae in the southern part of Japan. *Journal of Medical Entomology*, **50**, 1105–1110.

Accepted 1 September 2018

First published online 28 November 2018