The value of camels as sentinels for Bluetongue virus in Morocco

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Bluetongue,
Camels,
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Summary
A serosurvey was conducted to determine the value of camels (*Camelus dromedaries*) as sentinel animals for the detection of bluetongue virus (BTV) in Morocco. Between 2010 and 2013, camels from various localities in Morocco were randomly tested for antibodies against BTV serotypes-1, -4, -6, -8, -11, -14, and -16. Antibodies against 1 or more serotypes were detected in 41.8% of 537 camels tested with a competitive enzyme-linked immunosorbent assay (ELISA) diagnostic test. Of the 7 tested serotypes, only BTV-11 antibodies were not detected with serum neutralisation assays. This study not only confirms the epidemiological presence of BTV-1, -4, and -8 in Morocco, but also presents the first evidence of BTV-6, -14, and -16 in the country. As such, we conclude that camels would be ideal sentinel animals to determine the potential risk of BTV in Morocco.

Il valore dei cammelli come sentinelle per il virus della Bluetongue in Marocco

Parole chiave
Bluetongue,
Cammello,
Marocco,
Rischio.

Riassunto
Tra il 2010 e il 2013 è stata condotta un'indagine sierologica per valutare se i cammelli (*Camelus dromedaries*) possano essere utilizzati come animali sentinella per rilevare la circolazione del virus della Bluetongue (BTV). Campioni di siero di 537 cammelli provenienti da varie località del Marocco sono stati quindi testati mediante ELISA competitiva e nel 41.8 % degli animali sono stati rilevati anticorpi nei confronti di BTV. Mediante il saggio di sieroneutralizzazione, effettuato verso BTV-1, -4, -6, -8, -11, -14 e -16, è stato possibile rilevare la presenza di anticorpi verso tutti i sierotipi testati ad eccezione del BTV-11. Questo studio, dunque, non solo conferma la circolazione in Marocco dei sierotipi BTV-1, -4, -8, ma prova, per la prima volta nel Paese, la circolazione di BTV-6, -14, e -16. Si può quindi concludere che i cammelli potrebbero essere utilizzati come animali sentinella per rilevare l'eventuale circolazione e possibili nuove incursioni di BTV in Marocco.

Bluetongue (BT) is a sub-acute to acute *Culicoides*-borne viral disease of ruminants in temperate and tropical areas throughout the world (Toussaint *et al.* 2006, Mellor *et al.* 2008). The World Organization for Animal Health (OIE) listed BT as an economically important emerging disease, especially in sheep. According to Savini and colleagues (Savini *et al.* 2017), the causative orbivirus, Bluetongue virus (BTV), exists as 28 serotypes (Hofmann *et al.* 2008, Maan *et al.* 2011, Zientara *et al.* 2014), the last

of which was detected in 2014 in China (Sun *et al.* 2016). Further potential novel BTV serotypes have recently been described. Amongst them we cite: BTVXITL2015 detected in Sardinian goats in 2015 (Savini *et al.* 2017), that which was detected in a sheep pox vaccine preparation in Israel (Bumbarov *et al.* 2016) and another strain, for which only partial genome sequences exists, which was isolated from an alpaca in South Africa (Wright 2014).

The spread of at least 5 serotypes of BTV – BTV-1, BTV-2, BTV-4, BTV-9, and BTV-16 – throughout Mediterranean Europe since 1998 suggests changes in the abiotic and biotic factors that first defined Orbivirus distribution, and highlights the importance of understanding these underlying mechanisms (Purse *et al.* 2015). The timeously detection of circulating serotypes in Morocco will contribute to the implementation of effective control programmes.

In Morocco, BT (BTV-10) was identified for the first time in 1956 in the southern area of Laarach and west of Arbua (Placidi 1957). In September 2004, after an epidemiological silence of almost 50 years, 1876 sheep exhibited subclinical disease involving BTV-4 in Morocco (OIE 2004). A serological survey conducted in camels (*Camelus dromedaries*) in 2003 suggested that BTV had actually been subclinically present in camels in Morocco before 2004 (Touil *et al.* 2012). In August 2006, BTV-1 spread into Algeria and reached Tunisia and Sardinia during late 2006. In September 2006, it re-emerged in Morocco, causing widespread and severe clinical disease in sheep (OIE 2006). The disease intensified in 2007 with outbreaks spreading to different areas of Morocco (OIE 2007).

Although it is generally accepted that BTV is almost exclusively transmitted by certain species of *Culicoides*-biting midges (Diptera: Ceratopogonidae) (Purse *et al.* 2015), vector-free transmission has recently been demonstrated. Transmission can either be vertical, from dam to fetus (Rasmussen *et al.* 2013, van der Sluijs *et al.* 2013, Savini *et al.* 2014), or horizontal, via direct contact (Batten *et al.* 2013, Batten *et al.* 2014).

Vector surveys conducted from April 2009 to March 2010 at 14 sites in Morocco indicated that the 2 most abundant livestock associated with *Culicoides* species were *Culicoides imicola* Kieffer (94.2% to 95.9%) and *Culicoides newsteadi* Austen (2.2% to 2.7%) (Lhor *et al.* 2015). Earlier surveys conducted between 1989 and 1991 indicated that *C. imicola* was widely distributed in Morocco (Baylis *et al.* 1997). Indeed, *C. imicola* was absent at only 1 (near Settat) of 22 sites sampled across most of Morocco. *Culicoides* were most abundant in the low-lying northwestern areas (between Tangier and Rabat) and at Marrakech, where at least one adult *C. imicola* per night was collected with a light trap over one year, thereby providing a possible mechanism for viral overwintering in Morocco (Baylis *et al.* 1997). Other species encountered by Baylis and colleagues (Baylis *et al.* 1997) include *Culicoides circumscriptus* Kieffer, *C. newsteadi, Culicoides puncticollis* (Becker), *Culicoides obsoletus* Meigen, and *Culicoides pulicaris* (L.). While *C. imicola* and *C. obsoletus* are considered proven vectors of BTV (Purse *et al.* 2015), the detection of BTV in field collected *C. newsteadi* in the 2012 to 2014 BT epidemics in Italy implicated this species as a potential vector (Goffredo *et al.* 2015).

Bluetongue Virus infects all known species of ruminants, but severe disease is usually restricted to certain breeds of sheep, especially European breeds, e.g., Dorset Horn and some species of deer, e.g., white-tailed deer (*Odocoileus virginianus*) (Taylor 1986, Barnard 1997). Experimental infection has shown that although camels do not display clinical signs of BT, they can seroconvert and develop BTV specific neutralising antibodies (Batten *et al.* 2011). When infected with the virus, camels developed viremia from 7 days post infection, albeit at lower levels than in experimentally infected sheep and cattle (Batten *et al.* 2011). The isolation of viable virus from the blood of experimentally infected camels suggests that they might act as reservoir hosts, and as such play a role in the epidemiology of BT.

According to the Food and Agriculture Organization (FAO) of the United Nations, the number of camels in Morocco increased from 52,000 in 2010 to 59,000 in 2017. In 2017, about 19,863,000 sheep were estimated to be at risk of BTV (FAOSTAT 2017). In the same year, the number of cows, which are also considered as a reservoir host for BTV, was estimated at 3,364,000 (FAO STAT2017)[1].

This preliminary study focuses on the extent to which camels in the South of Morocco were exposed to the BTV serotypes present in and around the Mediterranean Basin. Information on the role of camels in the epidemiology of BTV, and the extent to which they can be used to monitor circulating serotypes and therefore help determine the risk of this disease occurring in Morocco will contribute to the implementation of timeously and effective control programmes.

From May 2010 to February 2013, sera were randomly collected from 538 camels at abattoirs and from animals intercepted by veterinary services at border posts in southern and southwestern Morocco (Table I). Intercepted camels came from neighboring countries and crossed the border illegally. When seized, the animals were kept in quarantine until tested for a number of infectious and contagious

[1] FAOSTAT. 2017. http://www.fao.org/faostat/fr/#data/QA accessed on 20 December 2018.

diseases, including BT. While quarantined, all contact with local animals was prohibited. The facilities were, however, not insect-proof. In Morocco, the results of the entomological investigations showed a great abundance of *Culicoides* populations, particularly *C. imicola* and *C. newsteadi*, for two periods: spring (April-June) for both species, and autumn (October-November) for *C. imicola* (Lhor 2017). Although camels bled at abattoirs usually represent camels from the immediate vicinity, their origin and age are not always known.

Blood was collected from the jugular vein of adult animals (older than 5 years) using 10-ml vacutainer tubes without anticoagulant. After collection, the blood was left to clot overnight at 4°C. The following morning, after centrifugation at 2,500 rpm, the serum was transferred to 2.5-ml Eppendorf tubes and stored at -20°C. BTV antibodies were detected using a commercial competitive ELISA (cELISA) kit from VMDR, Inc. (Pullman, WA, USA) following the manufactures instructions. The cELISA micro-plates were read using a Biokit spectrophotometer (Bio TekTM ELx 808-Fisher Scientific-USA) with an interference filter 620-650 nm. Sera displaying a mean optical density (OD) of < 50% of the mean of the negative controls obtained from the reference sera bank of Biopharma, were considered positive.

A total of 225 cELISA-positive serum samples were serotyped for antibodies against BTV serotypes that had previously encountered in the Mediterranean region i.e., BTV-1, BTV-4, BTV-6, BTV-8, BTV-11, BTV-14, and BTV-16 (Toussaint *et al.* 2006). Neutralisation titers were determined by serum neutralisation testing (SNT) according to Reed and Muench (Reed and Muench 1938) as the reciprocal of the highest dilution of serum that resulted in a 50% neutralisation endpoint titration. The SNT samples were heat inactivated at 56°C for 30 minutes. Neutralising antibody titers were determined by the micro-method of SNT in 96-well plates according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2009). Back-titrations of each virus were included to confirm the 100 $TCID_{50}$ dose.

The number of camels tested annually, which ranged from 87 in 2010 to 273 in 2012, as well as the proportion of antibodies that were detected at the various collection sites, are shown in Table I. The presence of antibodies against one or more serotypes of BTV in 225 of 538 camels tested (41.8%), indicates that BTV is highly prevalent in camels in Morocco (Table I). This result is consistent with those obtained by Touil and colleagues *et al.* (Touil *et al.* 2012), who reported a 42% seroprevalence in camels in Guelmim, Morocco, in 2003. The proportion of positive samples ranged from 13.3% in 2013 to as high as 60.4% in 2012. The highest number (68.3%) of camels showing high antibody titers against BTV was registered at Errachidia in March 2012 (Table I). As sampling was not conducted at the same time of the year, direct comparison of prevalence between the various sites is not possible.

Overall, the highest seroprevalence rate (66.6%) was obtained in camels bled in March 2012. Relatively high seroprevalence rates (53.6%) were also obtained in May 2010 and July 2012 (Table I). This period coincides with the end of the rainfall season and a gradual increase in temperature. It also coincides with the potential abundance of *Culicoides*

Table I. *Number of camels bled yearly at various abattoirs and border posts in southern Morocco to determine BTV seroprevalence and the number of sera that tested positive for the presence antibodies against BTV.*

Year	Month	Sampling Locality	No of sera collected	No of cELISA pos sera (%)
2010	May	Intercepted at Zagora/ Ouerzazate	35	22 (62.9)
	Oct	Imported from Mauritania (Dakhla)	19	3 (15.8)
	Nov	Intercepted at Ouerzazate	33	4 (12.1)
Total			**87**	**29 (33.3)**
2011	Aug	Intercepted at Errachidia	10	0 (0)
	Aug	Abattoir Laâyoune Province	48	15 (31.3)
Total			**58**	**15 (25.9)**
2012	March	Intercepted at Errachidia	41	28 (68.3)
	March	Abattoir Laâyoune Province	97	63 (65.0)
	July	Abattoir Guelmim-Es Semara	79	48 (60.8)
	July	Abattoir Laâyoune Province	56	26 (46.4)
Total			**273**	**165 (60.4)**
2013	Feb	Laâyoune Province	120	16 (13.3)
Total			**120**	**16 (13.3)**
Grand Total			**538**	**225 (41.8)**

Table II. *Viral neutralisation results of 225 cELISA positive camel sera collected between 2010 and 2013 in Morocco and tested against seven BTV serotypes.*

Year	No of pos camels	BTV Serotype							
		1 (%)	4 (%)	6 (%)	8 (%)	11 (%)	14 (%)	16 (%)	Total
2010	29	8 (27.6)	2 (6.9)	0	6 (20.7)	0	7 (24.1)	6 (20.7)	29
2011	15	2 (13.3)	0	1 (6.7)	5 (33.3)	0	3 (20.0)	4 (26.7)	15
2012	165	108 (24.7)	43 (9.7)	10 (2.2)	113 (25.4)	0	71 (16.0)	100 (22.5)	445
2013	16	7 (28.0)	7 (28.0)	1 (4.0)	3 (12.0)	0	2 (8.0)	5 (20.0)	25
Total	225	125 (24.3)	52 (10.1)	12 (2.3)	127 (24.7)	0	83 (16.1)	115 (22.4)	514

in Morocco (Baylis *et al.* 1997, Baylis and Rawlings 1998). The lowest percentages were recorded in February (13.3%) and November (12.1%) (Table I). Although the samples were not all taken in the same year and/or location, our findings seem to indicate significant differences in seasonal frequencies of BTV-positive animals.

Results of the serum-neutralisation test obtained with samples taken between 2010 and 2013 indicated that antibodies to at least 5 serotypes, BTV-1, BTV-6, BTV-8, BTV-14, and BTV-16, were present in camels. The most abundant serotypes were BTV-8 (24.7%) and BTV-1 (24.3%). Representing 22.4% of all tested sera, BTV-16 serotype was found in 115 of 225 camels (51.1%) that were tested (Table II). Although the 5 serotypes were identified every year, their seroprevalence varies from year to year.

We did not research the co-circulation of antibodies to more than 1 BTV serotype in the 44 camels tested in 2010 and 2011. During 2012 and 2013, 55 sera (40 of 2012 and 15 of 2013) were tested for co-infections with 10 serotypes (1, 2, 4, 6, 8, 9, 11, 14, 16 and 24). Co-infections were detected in 18 of 40 of the sera tested in 2012 and 4 of 15 camels tested in 2013; up to 4 serotypes (BTV-1, BTV-8, BTV-14 and BTV-16) could be detected in a single sample. Co-circulation of BTV-1 and BTV-8 was detected in 7 of the camels tested in 2012. The co-circulation of 2 to 3 serotypes, in no particular frequency, were detected in a further 15 camels. Although the ages of the camels were unknown, it can be expected that the number of circulating serotypes will increase as the camel get older (Figure 1).

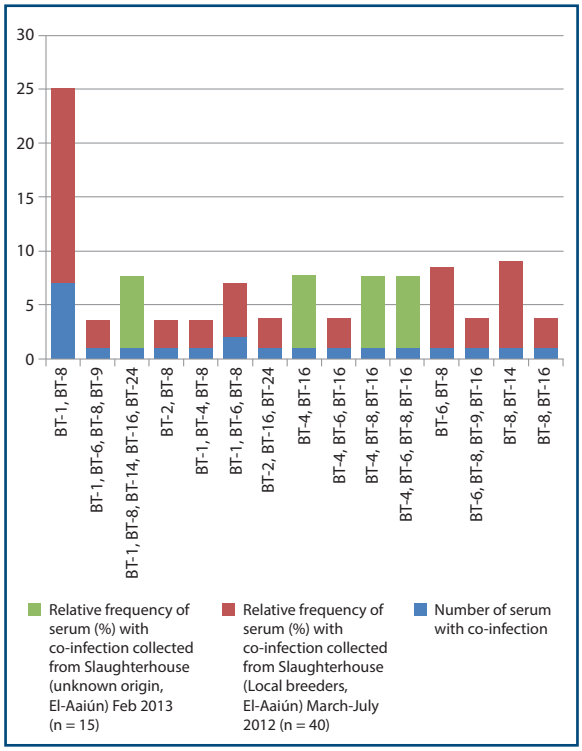

Figure 1. *Relative frequency of Bluetongue virus co-infection detected in camel serum samples collected between 2012 and 2013 in Morocco.* BT = Bluetongue virus serotype.

The serotypes BTV-4 and BTV-1 had been previously detected in Morocco (OIE 2004, OIE 2006). Between 2009 and 2012, BTV-8 was detected for the first time in sheep and cattle and appears now to be endemic (Drif *et al.* 2014). The present study presents the first evidence of BTV-6, BTV-14, and BTV-16. Although only antibodies were detected in this survey, the relative abundance and widespread occurrence of seropositive camels indicates that the virus must have been circulating in Morocco during the past 5 years.

It has previously been demonstrated that BTV-1 can persist asymptomatically for up to 2 months (i.e. low levels of viral RNA was found in the blood) in artificially infected camels (Batten *et al.* 2012). In addition, infected camels were found a year before the onset of the BT epidemic in Morocco; results of a serological survey of 1,392 camel sera showing 42% positive sera in Guelmim in 2003 clearly indicate that BTV was circulating in camels before the first notified outbreak in sheep in 2004 (Touil *et al.* 2012). In a study conducted in 2011 in neighboring Algeria, BTV seroprevalence was 14% in sheep, 21% in goats, and 29% in cattle (Madani *et al.* 2011). In the same study, the seroprevalence was 21% in camels. In 2016, Lorusso and colleagues (Lorusso *et al.* 2016) reported the circulation of BTV-26 in neighbouring Mauritania, with BTV-26 antibodies present in 108 of 159 camels that were tested. Since BTV-26 was not included in this study, it remains to be determined if this serotype is present in Morocco.

Touil and colleagues (Touil *et al.* 2012) indicate a relatively high abundance and geographical distribution of camels in Morocco in addition to the sub-clinical infection of BTV in camels. The fact that the camels move frequently across Morocco increases their potential to transport BTV over long distances. Due to an apparently low viremia, the potential reservoir contribution of camels to BTV epidemiology may be low, but a steady rise in population, along with the sub-clinical nature of their infections could cause the species to contribute to the short-term persistence of BTV. The clandestine movement of animals across borders could be one potential entry route for other serotypes of BTV into Southern Morocco.

The fact that at least seven different BTV serotypes – BTV-1, BTV-2, BTV-4, BTV-8, BTV-9, BTV-14, and BTV-16 – have invaded the west Mediterranean basin since 1999 (Youssef *et al.* 2017) emphasises the need for a reliable early warning sentinel system in Morocco. The present study not only confirms the presence of BTV at epidemic levels in Morocco, but

also supports previous studies (Madani *et al.* 2011, Touil *et al.* 2012, Youssef *et al.* 2017) that demonstrate that camels will be reliable sentinels to monitor the abundance and occurrence of BTV.

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