Orbivirus screening on dried blood spots from captive oryx in United Arab Emirates stresses the importance of pre-import measures

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Introduction

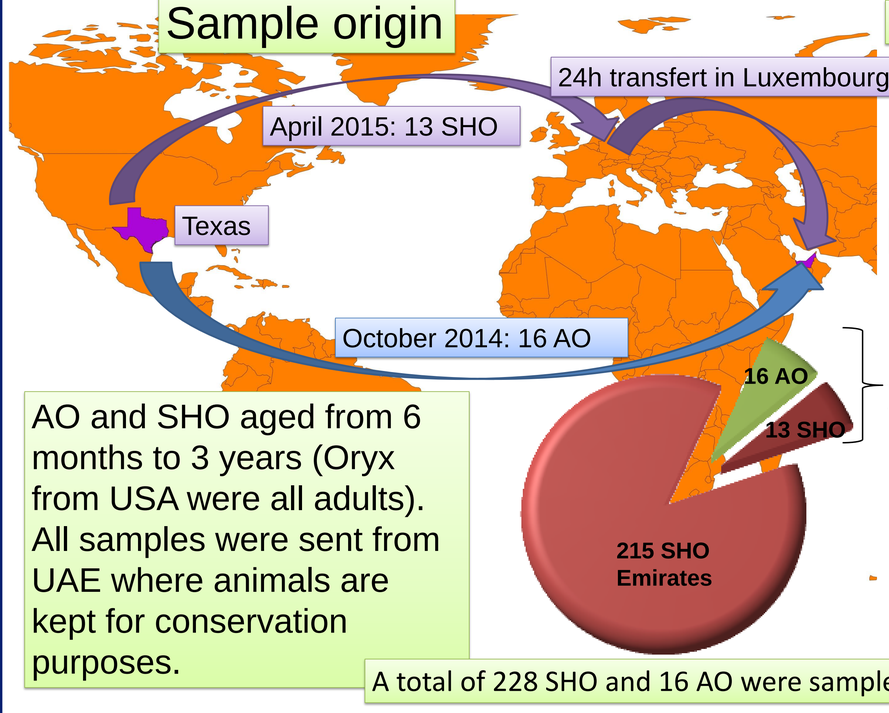
Following reintroduction and conservation programs of the Arabian oryx (AO, *Oryx leucoryx*) and the scimitar horned oryx (SHO, *Oryx dammah*) in the United Arab Emirates (UAE), import of animals from wild game ranches in the United States of America (USA) is not uncommon. Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are orbiviruses that are the causative agents of bluetongue disease (BT) and epizootic hemorrhagic disease (EHD), respectively. BTV and EHDV are endemic in the UAE and the USA. Sheep and some wild ruminant species are usually severely affected by BT whereas EHD mostly affects wild animals and sometimes cattle.
The objective of this study was to estimate the prevalence of these orbiviruses in Arabian Oryx and SHO from captive herds in the UAE using serology and molecular virology. Dry blood spot (DBS) sampling for Orbivirus screening is also discussed.

Arabian oryx (*Oryx leucoryx*)

Scimitar horned oryx (SHO, *Oryx dammah*)

Materials and methods

Sample origin



AO and SHO aged from 6 months to 3 years (Oryx from USA were all adults). All samples were sent from UAE where animals are kept for conservation purposes.

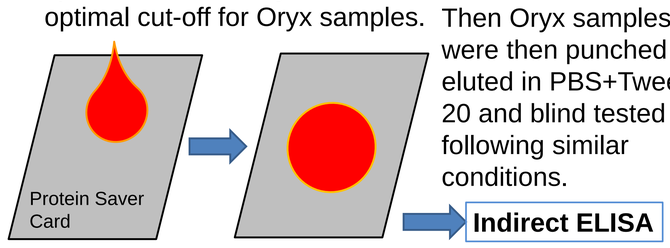
A total of 228 SHO and 16 AO were sampled

Dry blood spots (DBS) for virological and serological testing

Drops of about 80 µl of blood were dispensed on Whatman protein saver cards. Blood spots were punched out in paper discs with a 6 mm diameter punch and diluted in 250 µl PBS and Tween 20 0.05%.

BTV iELISA optimization

Serum samples from cattle used in BTV experimental infection with different BTV serotypes (BTV1, 2, 4, 8, 9, 15 and 16) at different time points were used as references. Corresponding whole blood was used to prepare dry blood spots to be tested by indirect ELISA in order to establish the optimal cut-off for Oryx samples. Then Oryx samples were then punched out, eluted in PBS+Tween 20 and blind tested following similar conditions.



EHDV cELISA

Oryx paper discs were tested to detect antibodies against EHDV by cELISA (LSIVet Ruminant EHDV Serum ELISA Kit).

RTqPCR pan-BTV (S5)

Eluted oryx samples were tested by a pan-BTV RTqPCR targeting a fragment of segment 5, designed to detect all BTV serotypes (Toussaint et al., 2007). Prior to be used on oryx samples, the RTqPCR protocol was validated on cattle dry blood spots of known infectious status. Serial dilutions of *in vitro* constructed plasmid allowed the absolute quantification of the viral cDNA equivalent in samples.

Serotype specific RTqPCR

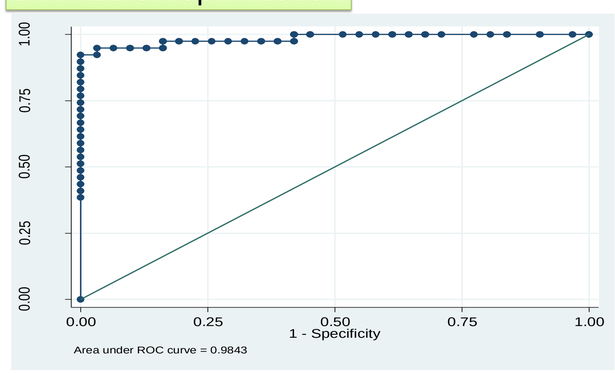
Serotype-specific RTqPCR targeting a fragment of segment 2 were used to test pan-BTV RTqPCR positive Oryx samples. Serotypes 2,10, 11, 13 and 17 were tested.

Seroneutralization (SNT)

Two-fold serial dilutions of the sera (1:10-1:1280) were tested in the presence of 100 $TCID_{50}$ of virus, as previously described (Martinelle et al., 2013). Tested serotypes were BTV2, 10, 11, 13 and 17.

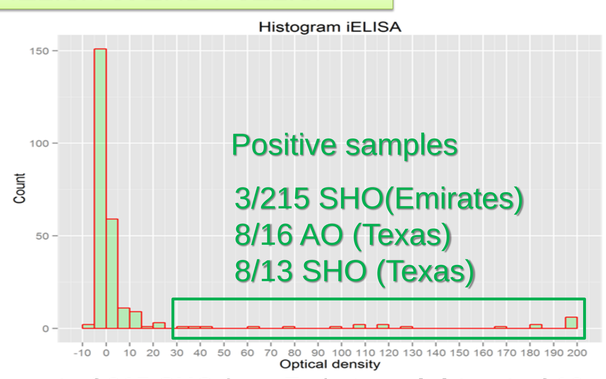
Results

BTV iELISA optimization



The iELISA ROC curve demonstrated an AUC of 0,9843,with an optimal **cut-off of an optical density of 29,4 %;** the sensitivity and the specificity were **94,87%** and **96,77%**, respectively.

BTV iELISA et EHDV cELISA



Three out of 215 SHO from Emirates, **eight out of 16 Arabian Oryx** and **eight out of 13 SHO from Texas** were found BTV seropositive by iELISA. None of the animals could be found seropositive against EHDV.

BTV RTqPCR and RT-PCR

Preliminary tests demonstrated an average higher detection of 0,75 log (+/-0,51) cDNA copy number / ml of blood versus dry blood spots, once corrected for dilutions (P=0,13).
BTV genome was detected in **1/3** seropositive SHO and in **5/16 of the Arabian oryx**, amongst those 2/5 were seronegative. Overall Cq values were high (33-39).

Two Arabian Oryx Samples were found positive for BTV2 by serotype specific RTqPCR. No other positive samples were detected with the other serotype specific RTqPCR.

Seroneutralization

SNT results lacked consistency to identify any particular BTV serotype.

Result summary: positive detection

Animals (origin)	iELISA BTV	cELISA EHDV	pan-BTV RTqPCR	BTV2 RTqPCR
SHO (Emirates)	3/228	0/46	1/46	0/1
SHO (Texas)	8/13	0/13	0/13	not tested
Arabian Oryx	8/16	0/13	5/16	2/5

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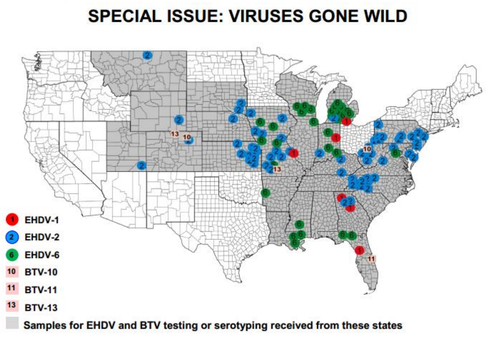
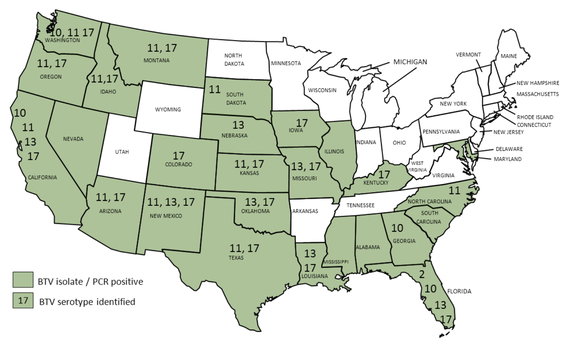
Discussion

BTV seroprevalence and RNA detection in SHO from UAE was very limited. By contrast BTV RNA could be demonstrated in 5/16 imported Arabian Oryx by RTqPCR and 8/16 AO were BTV seropositive. **BTV2 RNA was identified in two out of the five AO samples found positive by pan-BTV RTqPCR**. Moreover, 8/13 SHO imported from Texas were seropositive. **Thus overall 55 % of the animals imported from Texas were BTV seropositive**.



EHDV and BTV cases in USA in 2012
http://wdin.blogspot.be/2013/01/in-spotlight-scwds-briefs-special-issue.ht

The low prevalence in local animals was quite surprising because previous studies showed a higher BTV seroprevalence in domestic and wild ruminants of the Arabian Peninsula (Frölich et al., 2005).
In addition, dried blood spot testing has been demonstrated being a convenient and reliable method of sampling when storage and/or shipment conditions are hazardous. At least 15 different BTV serotypes were reported in the USA and at least 10 in the Middle East. BTV2 was identified in Florida in 1999 and more recently a closely related BTV2 strain was identified in California (MacLachlan et al., 2013).
Additional testing will be performed to further characterize the virus and therefore provide new insights to clarify the origin of the infection of the Oryx. Exporting ungulates from USA requires brucellosis testing based on rose Bengal and bovine tuberculosis based on intradermal tuberculin test, the animals being in the pre-export quarantine.



Bluetongue Virus Isolation and RNA Identification in the United States of America between 1992 and 2011 (Ostlund et al., 2013)

All additional screening is solely based upon importer's requirements. This is of major concern, especially in the cases the animals might transit in a third-party country. These results stress the need for pre-import risk assessment, precaution and implementation of biosecurity measures when considering translocation of wild ruminant species susceptible to BTV and EHDV.

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