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**Epizootic haemorrhagic disease virus circulation in Tunisia**

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**Keywords**

Epizootic haemorrhagic disease, Epizootic haemorrhagic disease virus, Tunisia.

**Summary**

Epizootic haemorrhagic disease virus (EHDV) was detected for the first time in Tunisia and in other Northern African countries in 2006. The objective of the present study was to investigate whether EHDV circulated in Tunisian livestock before and after the officially-reported outbreak of 2006. Thus, serum samples from cattle and dromedaries collected in different time periods (before and after 2006) and from different regions of Tunisia were screened for the presence of EHDV antibodies. Serological investigations conducted on cattle and dromedary sera collected in 2000 and 2001 demonstrated no virus circulation on these dates. However, viral circulation was evidenced in 2012 and 2013, although no EHDV cases were officially reported in these years. Serum-neutralization assessed on few ELISA positive samples, confirmed the presence of antibodies against EHDV serotype 6, which was the serotype involved in the EHDV outbreak in the Maghreb region in 2006.

**Indagine sulla presenza e circolazione del virus della Malattia emorragica epizootica in Tunisia**

**Table 1.** The figure shows the results of the Enzyme-linked immunosorbent assay run serum samples from cattle and dromedaries collected in Tunisia arranged according to animal species, date, and geographic region of sampling.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total sampled</th>
<th>Year of sampling</th>
<th>Geographic region</th>
<th>EHDV ELISA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>569</td>
<td>2001</td>
<td>North</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>2007</td>
<td>North</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>577</td>
<td>2012</td>
<td>North</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2012-2013</td>
<td>North, centre and south</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>25*</td>
<td>2013</td>
<td>North</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>25*</td>
<td>2014</td>
<td>North</td>
<td>1</td>
</tr>
<tr>
<td>Camels</td>
<td>84</td>
<td>2000</td>
<td>South</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>South</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Same animals sampled in 2013 and in 2014.
investigated Tunisian strains belonged to EHDV serotype 1, 2, 3, 4, 6 or 7.

Throughout the study, collected samples from sentinel animals were assessed by EHDV RT-PCR. Total RNA was extracted from 100 µl of blood using the Kingfisher 96 robot and the MagVet Universal isolation kit (LSI; reference: MV384) according to the manufacturer’s instructions. Finally, the RNAs were eluted with 80 µl of ultrapure water and used in a commercial EHDV RT-PCR according to the manufacturer’s instructions (LSI, Thermo Fisher, Lissieu, France). This kit allows the detection of all EHDV serotypes (by amplifying the EHDV S9 segment encoding VP6 protein) and does not cross-react with BTV. Five microliter of eluted RNA were denatured by heating to 95°C for 3 minutes in presence of 10% DMSO and added to 20 µl of EHDV mix. Sentinel blood samples were also assessed by RT-PCR that detects BTV according to a previously described protocol (Hoffman et al. 2010). This protocol amplifies the S10 viral segment and is able to detect all BTV-serotypes.

No antibodies against EHDV were detected in sera collected from cattle prior to 2002. All sera collected from dromedaries, before and after 2001, were also negative, EHDV ELISA results are shown in Table I. Thirty-one out of 577 sera collected from cattle in 2012 in Northern Tunisia were seropositive. The mean age of these seropositive animals was 1 and half year. Moreover, 13 out of 150 cattle sera collected from different regions of the country between 2012 and 2013, were also positive. All 25 sentinel animals were ELISA negative at the beginning of the survey (August 2013). In November 2013, seroconversion was detected in 1 of the 25 tested heifers. The same animal tested positive in the following examinations that were conducted in February, March, and April 2014.

All EHDV ELISA positive sera were negative by ELISA detecting anti-BTV.

Serum-neutralization test was performed in the OIE Reference Laboratory for Bluetongue (IZS Teramo, Italy) for 3 ELISA positive sera in order to identify the EHDV serotype. The first 2 of these 3 serum samples were collected from cattle tested in 2012 in Northern Tunisia; whereas the third was the serum of the seroconverted sentinel animal of 2013. Serum-neutralization revealed the presence of specific EHDV-6 antibodies with titres of 20 and 80 for the 2 animals that had been sampled in 2012, while the sentinel animal from 2013 was negative.

Real time PCR was assessed on EDTA blood samples collected from this sentinel animal, before and after seroconversion. However, viral RNA could not be detected.

A limitation of the study is the absence of sera collected in years immediately preceding the 2006 outbreak, so the presence of the virus between 2001 and 2006 cannot be excluded. Moreover, our study does not include samples between 2007 and 2012, so we cannot confirm or exclude any viral circulation in that period. All sera collected from dromedaries, before and after 2006, were negative for EHDV. The same result has been already described in studies in neighbouring countries; for example viral circulation has been detected in cattle and not in dromedaries in Algeria in 2008 (Madani et al. 2011). Also, viral circulation has not been detected in dromedaries in Morocco between 2003 and 2009 (Touil et al. 2012).

The presence of positive sera from 2012 in spite of the absence of officially reported clinical EHDV outbreak in the country was demonstrated. This phenomenon, which is likely due to silent viral circulation, demonstrates the utility of a larger number of sentinel animals placed all over the country for the monitoring of EHDV.

Such survey could be performed using serological methods, especially the ELISA technique that is less expensive. Molecular assays should be, then, performed to investigate samples from seroconverted animals. In the present study, molecular assay did not detect EHDV genome in blood samples collected from seroconverted animal. This could be explained by a low sensitivity of the used RT-PCR technique, by the degradation of viral RNA during transfer to the laboratory, or by a false positive ELISA result. However, this last hypothesis is to be considered with caution, because 3 samples, collected monthly (from February to April 2014) from the same animal, were ELISA positives. Serum-neutralization allowed the detection of antibodies against EHDV-6 in 2 of the 3 investigated samples. This EHDV serotype was the first involved in the 2006 EHDV outbreak (EFSA 2007, OIE 2006 a, b). Surprisingly, serum-neutralization was negative for the sample from the seroconverted animal. However, we can say with caution that it could be due to the infection with an EHDV strain belonging to serotype 5, which was not investigated in the SN test, or with another Orbivirus that cross-reacts with EHDV. The negativity of SN test can also be explained by a false positivity of the ELISA test.

Overall, our data suggest that a strict monitoring and early warning system has to be applied in Tunisia to control the circulation of some vector borne pathogens such as EHDV. For that purpose, strategies against vectors spread have to be developed and associated with the implementation of a surveillance program based on scattered sentinel animals all over the Tunisian territory. Such measures will certainly help to face the emergence of an EHDV outbreak in Tunisia and, by detecting the occurring serotype, it will address prophylaxis measures in the whole Mediterranean area.
References


