

4°75'E) during May–June 2010. All larvae were euthanized as part of an invasive species eradication project and stored at –20°C until further use. At necropsy, liver tissues were collected, and DNA was extracted by using the Genomic DNA Mini Kit (BIOLINE, London, UK). PCR to detect ranavirus was performed as described by Mao et al. (10).

Three samples showed positive results with this PCR. These samples were sequenced by using primers M4 and M5 described by Mao et al. (10) and blasted in GenBank. A 100% homology with the common midwife toad (*A. obstetricans*) ranavirus partial major capsid protein gene (GenBank accession no. FM213466.1) was found (5). Despite the low prevalence of *Ranavirus* infection (0.75%) in the bullfrog tadpoles examined, this study shows that invasive bullfrogs, a known reservoir of chytridiomycosis, are also a likely carrier of ranaviral disease in Europe.

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## Rift Valley and West Nile Virus Antibodies in Camels, North Africa

**To the Editor:** Different arboviral diseases have expanded their geographic range in recent times. Of them, Rift Valley fever, West Nile fever, and African horse sickness are of particular concern. They are endemic to sub-Saharan Africa but occasionally spread beyond this area. Trade and transport of animals and animal products, along with wildlife movements, are considered the driving factors in the spread of these pathogens.

In wide regions of Africa, 1-humped camels (*Camelus dromedarius*) are valuable livestock appreciated as a meat source and as a means for transportation of goods. Camels are susceptible to infection by Rift Valley fever virus (RVFV), West Nile virus (WNV), and African horse sickness virus (AHSV), although their epidemiologic role in these diseases is uncertain (1–3). Movements of camels across the Sahara Desert could carry these pathogens to northern Africa. To test this hypothesis, we conducted a serologic survey in 1-humped camels intercepted at different points by the Moroccan Veterinary Services in 2009. The camels were coming from the southeastern part of the Sahara Desert going to the northwest.

Serum samples were obtained in Smara-Laayoune, Dakhla, and Tata (Table). Most samples (71 of 100 total samples) were from male camels. Samples were also grouped by age of the camels (Table). RVFV antibodies were detected by using a competitive ELISA (4), and samples yielding positive ELISA results were confirmed by virus-neutralization test. WNV-specific antibodies were detected by ELISA (5), and positive results were confirmed by virus-

neutralization test. AHSV-specific antibodies were detected by using the ELISA prescribed by the World Organisation for Animal Health.

Fifteen of 100 samples were positive for RVFV-specific antibodies by competitive ELISA, all of which were confirmed by virus-neutralization test, with neutralization titers ranging from 40 to 1,280 (geometric mean titer = 229). With regard to WNV antibodies, the ELISA detected 44 positive samples and 1 doubtful sample, of which 29 were confirmed as positive by virus-neutralization test (virus-neutralization test titers ranging from 10 to 640; geometric mean titer = 20). As for AHSV antibodies, none of the samples was positive by ELISA. Prevalence data were analyzed by generalized linear model with locality (Dakhla or Smara), sex, and age as fixed factors. No differences by origin or sex were found in prevalence for WNV ( $p > 0.14$ ) but antibodies were more prevalent in camels  $> 3$  years of age ( $\chi^2 = 14.04$ , 3 df,  $p = 0.003$ ). No differences in prevalence of RVFV antibodies were found by sex ( $p = 0.29$ ), but prevalence was higher in Smara ( $\chi^2 = 3.74$ ,  $p = 0.05$ ) and among camels  $\geq 6$  years of age ( $\chi^2 = 8.37$ , df = 3,  $p = 0.04$ ) (Table). We also examined the co-occurrence of antibodies to RVFV and WNV. Of

15 RVFV-positive samples, 12 were also positive for WNV antibodies, and 12 of 29 WNV-positive samples were also positive for RVFV ( $\chi^2 = 8.37$ , df = 1,  $p < 0.05$ ).

Antibodies to 2 zoonotic arboviruses, i.e., RVFV and WNV, were present in camels moving to the northwestern part of the Sahara Desert, and antibodies to AHSV were absent in the populations examined. Despite the higher percentage of seropositivity for WNV than for RVFV, the epidemiologic consequence of RVFV-specific antibodies in this population could be higher than that for WNV antibodies. Camels can act as reservoir hosts for RVFV (6) but are unlikely to do so for WNV, which cycles between mosquitoes and wild birds with mammals usually being dead-end hosts. High prevalence of antibodies to RVFV in camels has been described in different sub-Saharan and Sahelian countries (7–9). Camels have been involved in the spread of disease in some instances (10). Immunity to RVFV indicates previous infection. Our results showed that seroprevalence of RVFV was higher among older than younger camels, indicating that contact could have occurred some years ago. Nevertheless, these populations should be monitored for RVFV and other arboviruses because

these are known to reemerge under certain circumstances in locations where they have occurred in the past.

The results of this study support that camels moving across the Sahara have contact with RVFV and WNV, and frequently the same animals have been infected by both agents. In a particularly dry environment such as the desert, particular attention should be paid to singular wet areas such as oases. The presence of water in these areas results in an abundance of competent mosquitoes and hosts, which in turn makes these viruses likely to cycle and infect domestic animals such as camels coming to drink and rest.

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Table. Results of testing of camels for virus antibodies, by location, age group, and sex of camels examined, North Africa, 2009\*

Camel characteristic	No. samples	No. positive for antibody			
		RVFV	WNV	AHSV	RVFV and WNV
<b>Origin</b>					
Tata	2	0	0	0	0
Smara-Laayoune	58	13	20	0	11
Dakhla	40	2	9	0	1
<b>Age group, y</b>					
<1	18	1	1	0	1
1–2	25	0	1	0	0
3–5	7	1	3	0	1
6–10	27	6	12	0	10
>10	23	7	12	0	0
<b>Sex</b>					
M	71	10	15	0	7
F	29	5	14	0	5
<b>Total</b>	<b>100</b>	<b>15</b>	<b>29</b>	<b>0</b>	<b>12</b>

\*RVFV, Rift Valley fever virus; WNV, West Nile virus; AHSV, African horse sickness virus.

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## Brucellosis, Taiwan, 2011

**To the Editor:** Human brucellosis is the most common zoonosis worldwide (1–4). The disease is transmitted to humans through the consumption of infected meat and raw dairy products from domestic livestock or by direct or indirect contact with infected animals (1–3). The disease is multisystemic and shows wide clinical polymorphism (2–4).

A 54-year-old woman reported high fever, poor appetite, epigastralgia, mild dysuria, generalized myalgia, and mild left side pain for 6 days before she sought care at and was admitted to National Taiwan University Hospital, Taipei, Taiwan. She had a history of ovarian cancer (clear cell, stage Ic), which had been treated with surgery and chemotherapy 7 years earlier at our hospital. She had traveled to many countries, most recently to Algeria and Morocco 2 months before this admission. During her stay in North Africa, she had close contact with camels, ate cheese and yogurt, and drank milk, even in the desert. Fever occurred 1 month after she returned to Taiwan.

On physical examination, her body temperature was 39.9°C, blood pressure was 97/68 mm Hg, and pulse rate was 89 beats/min. There was mild tenderness on palpation in the epigastric area. Laboratory analysis of serum specimens showed elevated levels of alanine aminotransferase (534 U/L), aspartate aminotransferase (841 U/L), and alkaline phosphatase (337 U/L) but a total bilirubin level (0.48 mg/dL) within reference limits. Renal function was within reference ranges (blood urea nitrogen 9.5 mg/dL, creatinine 0.6 mg/dL). C-reactive protein was elevated (4.59 mg/dL), but procalcitonin level was within reference range (0.13 ng/mL). The leukocyte count was 4,710 cells/mm<sup>3</sup>, and hemoglobin was 11.4 g/dL. Serologic tests for viral hepatitis

were negative for hepatitis B virus, hepatitis A virus, cytomegalovirus, and Epstein-Barr virus infections. Abdominal ultrasound indicated mild splenomegaly and no evidence of vegetation. Abdominal and pelvic computed tomography showed focal splenic infarction with splenomegaly.

Empirical ceftriaxone (1 g every 12 h) and doxycycline (100 mg every 12 h) were administered, and fever subsided 5 days later. Two aerobic culture bottles (BacT/ALERT, bioMérieux Inc., La Balme les Grottes, France) from different sets of blood cultures on the day before admission yielded unidentified gram-negative tiny bacilli after 2 days of incubation. The organism was identified as *Brucella melitensis* by the Vitek 2 GN identification system (bioMérieux Inc.) (probability of identity 99%) and was confirmed by analysis of partial 16S rRNA gene sequencing. Two primers were used: 8FPL (5'-AGAGT TTGATCCTGGCTCAG-3') and 1492 RPL (5'-GGTTACCTTGTTACGAC TT-3'). We compared the partial sequences with published sequences in the GenBank database by using the BLASTN algorithm ([www.ncbi.nlm.nih.gov/blast](http://www.ncbi.nlm.nih.gov/blast)). The closest match was *B. melitensis* (GenBank accession no. CP001852.1; maximal identity 100%). MICs were determined by the Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar (BBL, Becton Dickinson, Sparks, MD, USA) supplemented with 5% sheep blood and were interpreted 2 days after incubation. The isolate was susceptible to doxycycline (MIC 0.25 µg/mL; susceptible MICs ≤2 µg/mL) but not susceptible to trimethoprim/sulfamethoxazole (MIC 1/19 µg/mL; susceptible MICs ≤0.5 µg/mL) (5,6). MIC values of tigecycline and gentamicin were 0.125 µg/mL and 2.0 µg/mL, respectively. A serum sample for examination of *Brucella* antibody by Rose Bengal test using *B. abortus* antigen (VLA Scientific,