Rift Valley Fever and Crimean–Congo Hemorrhagic Fever Viruses in Ruminants, Jordan

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The epidemiology of Rift Valley fever virus (RVFV) and Crimean-Congo hemorrhagic fever virus (CCHFV) in Jordan is unknown. Our investigation showed 3% of 989 tested dairy cattle, sheep, and goats were RVFV seropositive and 14% were CCHFV seropositive. Ongoing surveillance is needed to assess risk to humans and protect public health.

Rift Valley fever (RVF) virus (RVFV) and Crimean-Congo hemorrhagic fever (CCHF) virus (CCHFV) are zoonotic arboviruses. RVFV has been causing sporadic outbreaks in East, West, and southern Africa; the Indian Ocean region; and the Arabian Peninsula (Saudi Arabia and Yemen) (1). Although Jordan is considered an at-risk country, the disease has not been reported in Jordan (2). Meanwhile, no seroprevalence studies for CCHFV in human or animals have been conducted in Jordan despite the endemicity of CCHF in neighboring countries (https://www.cdc.gov/vhf/crimean-congo/outbreaks/distribution-map.html), the presence of a necessary tick vector (*Hyalomma* sp.) (http://www.who.int/csr/disease/crimean_congo-HF), and the classification of Jordan as an at-risk country (3). Accordingly, we aimed to determine whether livestock populations across Jordan have been exposed to CCHFV and RVFV (Appendix, https://wwwnc.cdc.gov/EID/article/27/2/20-3713-App1.pdf). Jordan University of Science and Technology Animal Care and Use Committee approved the study.

Using EpiTool (https://epitools.ausvet.com.au), we determined that a minimum of 665 samples were required based on an assumed prevalence of 0.5% and a 95% CI. We tested 989 serum samples from 109 farms (31 dairy cow farms, 44 sheep farms, and 20 goat farms, as well as 14 mixed sheep and goat farms) that were randomly selected from different regions of Jordan during 2015–2016. Serum samples were shipped to the US Centers for Disease Control and Prevention (Atlanta, Georgia USA) for laboratory testing by indirect ELISA (Appendix).

Overall seroprevalence was 14% for CCHFV and 3% for RVFV. The greatest differences in seroprevalence were among sheep, 16.7% (85/509) for CCHFV and 4.5% (23/509) for RVFV, followed by a similar difference for goats, 14.7% (48/327) for CCHFV and 0.6% (2/327) for RVFV (Table). CCHFV and RVFV seroprevalence did not differ in cows at ≈1% (4/152 for CCHF and 2/152 for RVF) (Table).

The provinces that had the highest respective seroprevalence for CCHFV or RVFV did not coincide (Figure). The highest CCHFV seroprevalence was found in the northwest and the highest RVFV seroprevalence in the provinces along the central western border area with Israel (Figure). In total, 29 farms had seropositivity for CCHFV: 19 sheep farms (10 in Irbid, 5 in Tafela, 2 in Jarash, 1 in Ma'an, and 1 in Mafraq), 5 mixed sheep and goat farms (1 in each of Irbid, Jarash, Ajloun,

Table. Seroprevalence of CCHFV and RVFV by location and animal species, Jordan, 2015–2016*

Location	Sheep			Goat			Cow			All animals		
	No. tested	CCHFV	RVFV	No. tested	CCHFV	RVFV	No. tested	CCHFV	RVFV	No. tested	CCHFV	RVFV
Ajloun	36	80.5	5.6	42	85.7	0	0			78	83	2.6
Zarqa (Al-Dulail area)	0	NA	NA	0	NA	0	100	0	2	100	0	2
Amman	0			12	0	0	3	0	0	15	0	0
Irbid and Northern Jordan Valley	206	16.5	1.4	39	2.6	0	26	15.4	0	271	14.4	0.7
Jarash	78	8	0	127	4	0	10	0	0	215	5	0
Karak	8	0	0	24	0	4	1	0	0	33	0	3
Ma'an	12	8	0	13	0	0	0			25	5	0
Mafraq	94	5	13	42	5	0	8	0	0	144	5	8
Balqa	16	0	0	7	43	14	4	0	0	27	11	4
Tafilah	59	17	10	22	0	0	0			81	13	8
Total	509	16.7	4.5	328	14.7	0.6	152	2.6	1.3	989	14	3

*CCHFV, Crimean-Congo hemorrhagic fever virus; RVFV, Rift Valley fever virus.
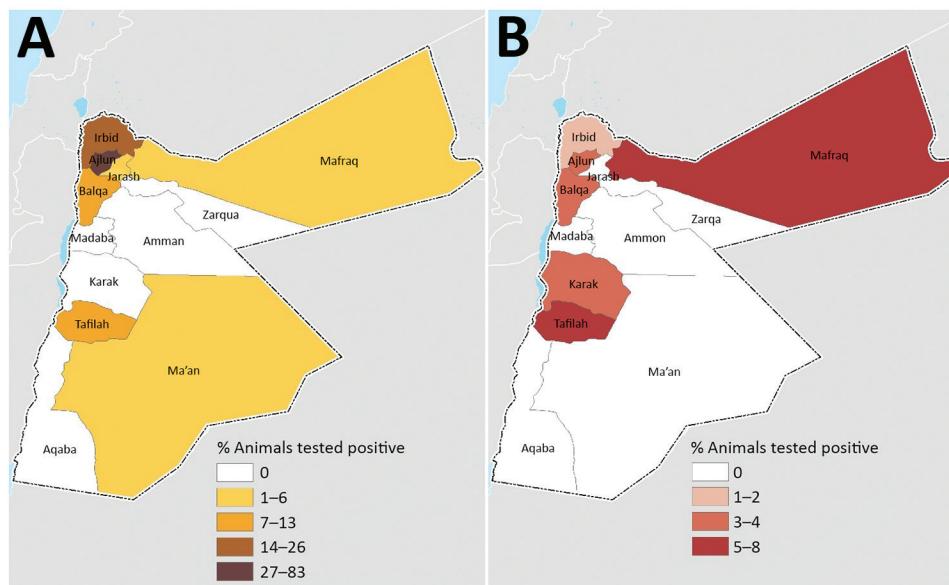
Figure. Seroprevalence of Crimean-Congo hemorrhagic fever (A) and Rift Valley fever (B) in ruminants, by province, Jordan, 2015–2016.

Mafraq, and Balqa), 3 goat farms (all in Jarash), and 2 dairy cow farms in Irbid. Ten farms had animals seropositive for RVFV: 5 sheep farms (2 in Tafelah, 2 in Irbid, and 1 in Mafraq), 3 mixed sheep and goat farms (1 in each of Ajloun, Mafraq, and Balqa), 1 goat farm in Karak, and 1 dairy-cow farm in Zarqa.

This study reports RVFV seropositivity in Jordan's ruminant population without any previously reported animal cases. Observing seropositive animals without disease, however, is not unique; 22% of the small ruminant population in Mayotte were seropositive (4) without any documented human or animal clinical cases. Similarly, South Africa reported high proportion of seropositive ruminants in the absence of a reported outbreak (5). In addition, IgG seroprevalence of 6.5% was detected in sheep and goats in southern Gabon without a reported outbreak (6).

In Jordan, small ruminants are short day breeders; June–September are breeding months. After a ≈5-month gestation period, lambing occurs during November–February, which places gestation and lambing periods during the rainy months in Jordan. The shift of RVF from enzootic to epizootic or epidemic cycle typically follows extended periods of heavy rainfall (7). Because rainy season and gestation periods overlap, RVFV spread poses a potential high risk for abortions and neonatal death in Jordan.

In light of the regional distribution and general expansion of RVFV and CCHFV into newly identified areas, it is not surprising that animals in Jordan tested seropositive to either virus. This finding is consistent with recent studies that reported other mosquitoborne viruses in Jordan, such as West Nile (8) and dengue viruses (9), and tickborne viruses such as *Coxiella burnetii* (10).

The findings of seropositive animals for CCHFV and RVFV in different regions of Jordan call for implementing an early warning contingency plan. Such a plan would include training field veterinary officers, developing strong epidemiologic capabilities, sustaining active disease surveillance, and enhancing laboratory diagnostic capabilities. On the basis of our identification of the subprovinces with the highest seroprevalence, small ruminant sentinel herds should be monitored for IgG and IgM to these viruses in conjunction with seasonal weather, particularly before and during the rainy months. Despite CCHF virulence in humans and the potential public health impact because of severe outbreaks, the virus is not pathogenic for the amplifying hosts (i.e., ruminants). Thus, farmers and veterinarians are at higher risk for infection compared with the general population. Future studies should be conducted to determine the prevalence and potential incident cases of CCHF and RVF in Jordan's human and animal populations. Ongoing surveillance will inform contemporaneous risk assessments and enable development of effective public health messaging for identified risk groups.

About the Author

Dr. Obaidat is associate professor at the faculty of veterinary medicine at Jordan University of Science and Technology. His research interest includes the epidemiology of zoonotic diseases in Jordan.

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Genomic Diversity of *Burkholderia pseudomallei* Isolates, Colombia

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We report an analysis of the genomic diversity of isolates of *Burkholderia pseudomallei*, the cause of melioidosis, recovered in Colombia from routine surveillance during 2016–2017. *B. pseudomallei* appears genetically diverse, suggesting it is well established and has spread across the region.

Melioidosis is caused by the environmental bacterium *Burkholderia pseudomallei*. Infections are acquired by direct contact with the pathogen, most commonly through traumatic inoculation with contaminated soil or water but also by ingestion or inhalation. Symptoms are nonspecific and can include pneumonia, skin lesions, abscess formation, and sepsis (*1*).

In Latin America, melioidosis is believed to be underdiagnosed because of the absence of reliable surveillance and the lack of available diagnostic tools and methods (*2*). Colombia has previously reported cases as sporadic, isolated events in a few geographic areas (*2,3*). The aim of this study was to genetically characterize isolates of *B. pseudomallei* recovered from clinical specimens in different departments of Colombia (*4*). (A department in Colombia is a geographic unit composed of municipalities led by a governor.) The goal was to better understand genetic relationships among the isolates from Colombia, as well as their relationships to isolates from other tropical and subtropical regions of the Americas. The study was internally reviewed at the US Centers for Disease

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Appendix

Dairy Ruminant Population in Jordan

The population of dairy cattle and small ruminants such as sheep and goats in Jordan was discussed in previous publications (*1,2*). Briefly, Jordan relies on dairy ruminants to meet the local demand on milk and dairy products, so ruminants are an important component of the Jordanian animal industry (*3*). Jordan has 2 dairy cattle production systems, both of which raise Holstein-Friesian dairy cows (*3*): a large intensive production system located in Al-Dulial area that uses modern management practices and accounts for ≈50% of the milk in Jordan (*3*), and a small-scale production system that uses traditional management practices (e.g., cows housed in small brick barns) predominately located in the Northern Highlands but scattered in several governorates.

There are also small ruminant farms (i.e., flocks), generally, more scattered throughout Jordan than the dairy cattle farms. They are raised in both the Northern Highlands and the Badia region. The Northern Highlands region receives the highest amounts of rainfall in Jordan and is occupied by small herders who rely on extensive production systems and use rangelands in a constant search for grass and water (*1*). In contrast, Badia is an arid to semiarid region located in southern and eastern Jordan and occupied by nomadic or pastoralist Bedouin (*1*).

Materials and Methods

Setting

There is no animal vaccination program for Rift Valley fever (RVF) in Jordan. This study was designed as a cross-sectional study that covered all governorates of Jordan except Madaba and Aqaba. Several farms across each governorate were randomly selected for the study.

Serum Sample Collection

From each animal, 10 mL of blood was collected and transported to the laboratory on ice in a cooler on the same day of collection. At the laboratory, samples were registered in a logbook and the serum collected by centrifuging the blood samples at 3,000 rpm for 10 min. Samples were stored in sterile 1.5 mL screw-capped tubes at −20°C and later shipped to Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA for laboratory testing.

Serology (Caprine/Bovine/Ovine)- Immunoglobulin G Detection for RVF and CCHF

Indirect enzyme-linked immunosorbent assays (ELISA) were used to measure immunoglobulin G (IgG) levels in collected serum samples at least twice per sample. For RVF, viral antigen (Gamma-irradiated cell lysate of E6 Vero cells infected with RVF) in PBS (1:1,000) was added to the top half of a 96-well plate, and mock antigen (Gamma-irradiated cell lysate of uninfected E6 Vero cells) in PBS (1:1000) was added to the bottom half and incubated overnight at 4°C. After 3 washes with wash buffer (0.01M PBS-0.1% Tween-20), test serum as well as positive and negative control serum were added at a final 1:100 dilution to the first rows of the top and bottom halves of the plate, followed by 4-fold serial dilution down each column. Plates were incubated at 37°C for 1 h and washed 3 times. Anti–sheep IgG conjugated to horseradish peroxidase (HRPO) (KPL Cat. No. 14–23–06, Seracare, https://www.seracare.com) was added to the whole plate and incubated at 37°C for 1 h, followed again by 3 washes. Substrate ABTS was added and incubated at 37°C for 30 min; in the presence of HRPO and hydrogen peroxide, a detectable color transformation occurs that is described by an optical density (OD) value proportional to the amount of IgG present. Positive samples are determined

using an adjusted OD generated by subtracting the negative antigen OD from the positive antigen OD and comparing against established criteria.

CCHF antibodies were detected as described previously (*4,5*). In brief, the 96-well plate was coated with anti-CCHF Hyperimmune Mouse Ascitic Fluids (HMAF) in PBS (1:1,000) and incubated overnight at 4°C. After 3 washes with wash buffer, CCHF viral antigen (Gamma-irradiated E6 Vero cells infected with CCHF) diluted in PBS (1:10) was added to the top half of the plate with mock antigen (Gamma-irradiated uninfected E6 Vero cells) in PBS (1:10) added to the bottom half. The subsequent steps and accompanying reagents and conditions are the same as described above for the RVF IgG ELISA with the exception of the conjugate, for which mouse anti-bovine IgG conjugated to HRPO (Catalog # MA5–16733; ThermoFisher, https://thermofisher.com) was used for cattle samples. All plates were read at 410 nm using a Biotek Powerwave reader with Gen 5 software (Biotek Instruments, https://www.biotek.com).

Data Management and Statistical Analyses

The criteria we used to call a sample positive for IgG were a sum OD >0.95 and IgG titer $\geq$1:400. Data were entered into Microsoft Excel spreadsheet. The overall seroprevalence for each species was calculated as the total number of serologically positive samples by the total number of all tested samples for that species. A herd was considered positive when $\geq$1 animal was seropositive. Herd-level seroprevalence was calculated by dividing the number of seropositive herds for each animal species by the tested herds for that species.

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