Man-threatening viruses isolated from ticks in Saudi Arabia

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ABSTRACT

Objectives: To determine tick viruses transmissible to man in Saudi Arabia.

Methods: We collected tick samples for identification from different region of Saudi Arabia during March 1992 - December 1992. Ticks collected from domestic animals were washed with buffer containing antibiotics, macerated, triturated in minimal essential media (MEM), centrifuged and the supernatant inoculated intracerebrally in suckling mice. Brains of mice showing disease signs were similarly treated and the supernatant inoculated on cell cultures. Antigen spot slides were prepared from material of blind-passed cell cultures showing cytopathic effects. The slides were overlaid by group-specific hyperimmune mouse ascetic fluid (HMAF) to different virus groups and stained with antimouse fluorescein isothiocyanate conjugate. Positive slides were retested with monoclonal antibodies or HMAF to individual viruses within the positive group.

Results: Sindbis virus was isolated from *Hyalomma dromedarii (H. dromedarii)* from Al-Qasim and Jazan regions and *Hyalomma impeltatum* (*H. impeltatum)* from Al-Qasim, Ar-Riyadh and Jazan regions. Chick Ross and Kadam viruses were isolated from *H. dromedarii* from Al-Qasim region. Kadam virus was isolated from *Hyalomma anatolicum (H. anatolicum)* from Ar-Riyadh region. Dhori virus was isolated from *H. impeltatum* and *Hyalomma schulzei* (*H. schulzei)* from the Eastern region. Other alphaviruses were isolated from *H. dromedarii* from Al-Qasim and Tabuk regions, *H. impeltatum* from Jazan region, *H. anatolicum* from the Eastern region and *Rhipicephalus sanguineus* from Ar-Riyadh and Eastern regions.

Conclusion: In the Kingdom, ticks harbor viral infections transmissible from wildlife to man and his livestock. Local physicians should be aware of the symptoms and signs of these infections.

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Ticks transmit a greater variety of infectious agents than any other group of hematophagous arthropods and may cause zoonoses. Many tick-borne agents produce generally benign infections in the wildlife but more or less serious diseases in livestock and humans; the 40-year "modern" history of Crimean Congo hemorrhagic fever (CCHF) virus provided the most dramatic example of a harmless infection of ticks and wildlife flaring into epidemics of great significance in human morbidity and mortality.[1] In the last few decades, great agricultural advancement in the Kingdom of Saudi Arabia has changed large areas of the desert into cultivated lands. Man and his herds of domestic animals in these areas provide the ticks with their blood meals. The ticks thrive in these areas and their harmless infections may be transformed into forms of epidemics and epizootics to man and his domesticated herds.[2,3] A nationwide survey of ticks infesting domestic and wild animals in Saudi Arabia has been carried out during the years 1991 and 1992.[4,5] During that survey, ticks were collected to test the presence of viruses that affect man and his livestock. In the present study, the results of the investigation are reported.

Methods.

Tick collection and identification. During March, April, November 1991 and May, October, November, and December 1992 the samples were collected for their identification from different localities of Saudi Arabia (**Table 1**). Ticks were collected from camels, goats, sheep, cattle, dogs, cats, chicken and pigeons. Identification of tick species was carried out using the keys as described by Diab et al.[5]

Tick sampling and sample size. The ticks were separated into pools (maximum of 30 ticks/pool) by species, gender, stage, collection locality and host. The ticks were sent alive to the Virology Division of the

Naval Medical Research Unit No-3 (NAMRU-3) in Cairo, Egypt, where they were frozen and stored at -70°C.

Tick preparation for virus isolation. To investigate their infection with viruses, frozen ticks were washed using phosphate buffered saline (pH 7.6) containing penicillin and streptomycin and then each pool was macerated and triturated in minimal essential media (MEM) containing 10,000 units of penicillin, 10,000 µg streptomycin and 25 µg fungizone per 100 ml. The triturated suspension was centrifuged for 10 minutes at 3000 rpm.

Virus isolation. The supernatant of the centrifuged tick suspension was inoculated intracerebrally in suckling Swiss Webster mice and on Vero and BHK-21 cell cultures. Inoculated mice and cell cultures were observed for 14 days for signs of disease or cytopathic effects (CPE). The brains of mice showing signs of disease were harvested, triturated in MEM containing antibiotics and centrifuged as described above. The supernatant of the brain suspensions were then inoculated on cell cultures. Ten days post-inoculation, material harvested from cell cultures was blind-passed again in cell cultures and observed for CPE. Cell cultures showing CPE were harvested and antigen spot slides were prepared from the cell suspension.

Virus identification. The antigen spot slides were tested by immunofluorescence; the slides were overlaid by group-specific hyperimmune mouse ascetic fluid (HMAF) to different virus groups and then stained with antimouse fluorescein isothiocyanate conjugate (Kirkegaare and Perry, MD, USA). Slides reacting with a certain virus group were retested with monoclonal antibodies or HMAF to individual viruses within this group.

Results. A total number of 1,727 ticks were collected. The ixodid (hard) ticks included 8 species of *Hyalomma* (140 pools), 2 species of *Rhipicephalus* (24 pools), *Haemaphysalis sulcata* (2 pools) and *Boophilus kohlsi* (one pool), and the argasid (soft) tick *Argas persicus* (11 pools). Twenty viruses were isolated from 20 tick pools (**Table 1**). With the exception of 2 isolates from male *Hyalomma dromedarii (H. dromedarii)* ticks, all of the other 18 viruses were isolated from female ticks. No viruses were isolated from *Argas persicus*, *Haemaphysalis sulcata* or *Boophilus kohlsi*, and no viruses were isolated from ticks collected from sheep, cats, pigeons or chicken. Of the 20 isolated viruses, 16 were of the *Alphavirus* group, 2 were of the *Flavivirus* group and 2 belonged to Orthomyxoviridae. Of the alphaviruses, 6 were identified as Sindbis virus and one as Chick Ross virus. The remaining 9 alphaviruses were not identified beyond the group level owing to a disastrous electricity failure. The flaviviruses were identified as Kadam virus while those belonging to the Orthomyxoviridae were identified as Dhori virus.

Table 1 - Viruses isolated from ticks collected from livestock in certain localities in Saudi Arabia.

Tick species	Region	Animal hosts	No. of pools	Isolated viruses
Hyalomma dromedarii	Al-Qasim	Camel	1	Sindbis
Hyalomma dromedarii	Al-Qasim	Camel	1	Chick Ross
Hyalomma dromedarii	Al-Qasim	Camel	1	Kadam
Hyalomma dromedarii	Jazan	Cattle	1	Sindbis
Hyalomma impeltatum	Al-Qasim	Camel	1	Sindbis
Hyalomma impeltatum	Ar-Riyadh	Camel	2	Sindbis
Hyalomma impeltatum	Eastern	Camel	1	Dhori
Hyalomma impeltatum	Jazan	Cattle	1	Sindbis
Hyalomma anatolicum anatolicum	Ar-Riyadh	Cattle	1	Kadam
Hyalomma Schulzei	Eastern	Camel	1	Dhori
Hyalomma dromedarii	Al-Qasim	Camel	3	*Alphavirus*[*]
Hyalomma dromedarii	Tabuk	Camel	2	*Alphavirus*[*]
Hyalomma impeltatum	Jazan	Cattle	1	*Alphavirus*[*]
Hyalomma anatolicum anatolicum	Eastern	Goat	1	*Alphavirus*[*]
Rhipicephalus sanguineus	Ar-Riyadh	Goat	1	*Alphavirus*[*]
Rhipicephalus sanguineus	Eastern	Dog	1	*Alphavirus*[*]

[*]Identification was not carried beyond the group level.

Discussion. The present study was the first nationwide project that investigated the presence of viruses in ticks commonly infesting livestock in Saudi Arabia. Isolation of Sindbis, Kadam, Dhori and Chick Ross viruses during the present study from different Regions in the Kingdom strongly suggested that these viruses might be circulating in the Kingdom. The tick species collected from different animals in Saudi Arabia during the present work as well as during previous studies[5,6-13] have been recorded to harbor several viruses.[14-16] Kadam virus was previously isolated from *H. dromedarii* parasitizing camels in Saudi Arabia.[17] Moreover, El-Azazy and Serimgeour[18] reported an outbreak of a suspected CCHF infection involving 7 individuals in Makkah. Out of 13 species of ixodid ticks (6 *Hyalomma*, 5 *Rhipicephalus*, one *Amblyomma* and one *Boophilus*) collected from camels, cattle, sheep and goats, 10 have been reported to be capable of transmitting CCHF.[1] However, their attempts to isolate viruses from pools of *H. dromedarii* and *H. anatolicum* were unsuccessful. They suspected that the CCHF virus in Saudi Arabia was introduced through infected ticks from imported sheep arriving in Jeddah seaport. Introduction of ticks infected with viruses to Saudi Arabia on imported animals from neighboring countries was highly possible since several reports of infection with tick-borne viruses in these countries were well documented.[3,19-21] In Iraq, 5 cases of CCHF virus were reported in 1979,[3] and in the United Arab Emirates 7% of the imported and domestic ruminants including cattle, and 5 indigenous camels were positive for CCHF.[19] Hemorrhagic fever viruses were also isolated from 4 persons and from 19 pools of *Hyalomma anatolicum* in the Sultanate of Oman.[20] In addition, *H. impeltatum* and *H. excavatum* collected from Iran and Somalia cattle and *Hyalomma anatolicum* from Somalia goat were positive for CCHF virus antigen.[20] Wood et al,[21] also have isolated viruses of CCHF, Thogoto, Dugbe and Jos viruses from several ixodid tick species in Ethiopia.

Viruses are of the most important groups of disease agents carried by ticks to man and animals. Hoogstraal[22] has listed 110 arboviruses associated with 84 ixodid tick species and subspecies and 32 argasid tick species. Several factors make ticks efficient vectors and reservoirs of pathogens and hence, it account for the potency of ticks in spreading diseases to man and animals. A susceptible tick becomes infected when feeding on a host in which the disease agent is circulating at or above an infective threshold titer.[14,23-26] The agent migrates from the tick digestive tract and invades the body cavity and almost all the tissues where it multiplies and may be maintained horizontally by transstadial survival to the next life stages (from larva to nymph and from nymph to adult). Transstadial survival accounts for a great deal of the tick efficiency in disease transmission. In addition, if a feeding tick is dislodged before engorgement, even if it is a highly host-specific species, it may indiscriminately seek to feed on any available host. This phenomenon is epidemiologically important when hunters skinning deer are bitten by dislodged ticks infected by disease agents or when milkmaids or herdsmen pull off ticks infected with these agents.[23,27,28] An equally important phenomenon in the epidemiological process of a disease agent is its transovarial transmission to the progeny of the infected tick.[23-25] This phenomenon contributes to the role of ticks as reservoirs of the disease agents.[27,29,30]

The great environmental changes in many new settlement areas in Saudi Arabia are suitable for many potential disease vectors, such as ticks of the genus Hyalomma that have become widely spread in the Kingdom.[5] An important factor in the epidemiology of tick-borne diseases is human behavior associated with availability of time and money for leisure activities, such as camping for adventure holidays in the desert. Such activities bring people into contact with foci of infection and subject them to bites by infected ticks.

Further studies are needed to investigate the occurrence and distribution of tick-borne viruses in Saudi Arabia. It is equally important that local physicians become aware of the signs and symptoms of infections with viruses commonly transmitted by ticks.

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