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Original article

Phylogeny of tick-derived Crimean-Congo hemorrhagic fever virus strains in Iran



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ABSTRACT

The presence of Crimean-Congo hemorrhagic fever virus (CCHFV) in Iran was assessed by collecting ticks from Golpayegan, Isfahan Province. Real time RT-PCR was used to detect the CCHFV RNA in the tick population and the origins of the viral sequences were determined. The CCHFV RNA was detected in 5.2% of 492 ticks collected from livestock in different regions of Golpayegan. The tick species that tested positive for the presence of CCHFV RNA included *Hyalomma*, *Rhipicephalus* and *Haemaphysalis* species. Phylogenetic analysis using the partial S-segment indicated that eight sequences clustered in clade IV (Asia-1) and three other sequences aligned within clade VI (Europe) with other CCHFV strains from Kosovo (Kosova1917) and Russia (Kashmanov).

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1. Introduction

Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic viral disease transmitted by ticks. The virus has a wide distribution, and is detected in at least 30 countries, from Asia to Africa and Europe. The virus, CCHFV belongs to the genus Nairovirus,

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family Bunyaviridae (Whitehouse, 2004). CCHFV is a single-stranded RNA virus with a segmented negative-sense genome consisting of a small (S), a medium (M), and a large (L) segment (Zehender et al., 2013). CCHFVs are grouped into seven distinguishable clades based on the S-segment genome analysis: West-African viruses in clade I, Central African in clade II, South African and West African viruses in clade III, Middle East and Asian viruses in clade IV, European viruses in clade V and Greek viruses in clade VI. The clade IV are divided into two distinct clades, Asia-1 and Asia-2 (Fajs et al., 2014; Hewson et al., 2004).

The main routes of infection transmission include bites from infected ticks or by direct contact with virus-contaminated

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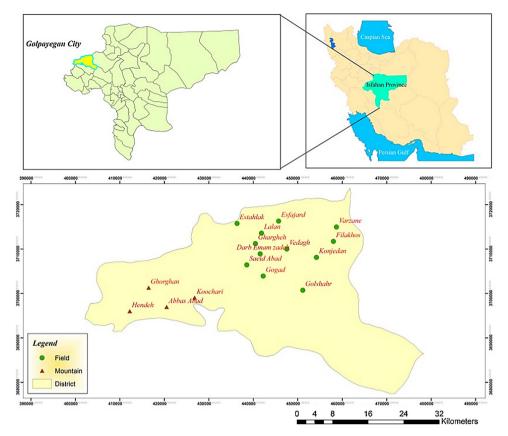


Fig. 1. The map of Golpayegan city in the province of Isfahan.

tissues or blood (Champour et al., 2014b). In addition to zoonotic transmission, CCHFV can spread from person-to-person by nosocomial transmission (Bokaie et al., 2008; Whitehouse, 2004). CCHF infection is symptomatic in human cases, in which manifestations include an acute febrile illness followed by a fatal hemorrhagic syndrome with mortality rates of up to 50% (Chinikar et al., 2013a). In domestic animals, the infection has no obvious clinical signs. Shepherds, campers, agricultural workers, veterinarians, abattoir workers, and other persons in close contact with livestock are considered to work in high risk professions (Ergönül, 2006).

CCHF virus was first isolated from adult *Hyalomma* ticks in the 1960s. It has been detected in at least 31 species of ticks in the Ixodidae (hard ticks) and Argasidae (soft ticks) (Ergönül, 2006; Kayedi et al., 2015; Mehravaran et al., 2013).

Outbreak reports often increase when ticks are in the peak of activity. Despite that *Hyalomma* spp. ticks play a critical role in the epidemiology of CCHF (Chen, 2013), the role of ticks of other genera however, i.e., *Rhipicephalus*, *Boophilus*, *Dermacentor*, *Haemaphysalis*, and *Ixodes* spp. should not be neglected (Tahmasebi et al., 2010).

CCHF cases were previously reported from different parts of Iran (Sureau et al., 1980), and it was considered to be one of the country's major public health concerns. CCHF virus infection has been reported in 27 of the 31 provinces of Iran; with Sistan va Baluchistan, Isfahan, Fars, and Khuzestan being the most affected provinces (Chinikar et al., 2010a).

Since understanding the epidemiology of CCHFV is the *sine qua non* to develop and implement surveillance strategies for its control. The main objectives of this study were to investigate tick distribution and prevalence of CCHFV in Golpayegan city between July 2014 and June 2015, and to undertake phylogenetic analysis of tick-derived CCHFV strains.

2. Materials and methods

2.1. Study area

Isfahan province has been considered to have a high prevalence of CCHF infection during the past 15 years. Isfahan province is located in center of Iran, bordering with nine other provinces of Iran. Golpayegan (50°18′E, 33°27′N and 1818 m above sea level) is one of the most important cities in Isfahan province, consisting of three townships and 38 villages. The economy relies mainly on agriculture and livestock husbandry; as a result, the majority of residents have regular direct contact with livestock. This causes a high risk of exposure to CCHF virus (Fig. 1).

2.2. Sample collection and preparation

In this study, 16 villages were randomly selected for sample collection in two different ecological regions (plain and mountain). Twelve villages were in the plain area and four villages were in the mountain area. Ticks were collected from sheep, cattle and goats with a total population of 437 livestock investigated. In each village, livestock were checked randomly and ticks collected. The entire body of each animal was checked, particularly the ears, nape of the neck, chest, perineum, scrotum, and the tail base were inspected for the presence of ticks. Of the 437 animals investigated, 81 (18.5%) were infested by ticks. A total of 492 ticks were collected and kept alive in separate labeled vials and then transferred to the Medical Entomology Laboratory, School of Public Health, Tehran University for morphological identification and sex determination.

2.3. RNA extraction and RT-PCR

From 492 ticks, 210 ticks were chosen (based on genus, species and sampling site) for detection of the CCHFV genomic RNA. Ticks

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