Research Article

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Viral Zoonoses in Humans (Zooanthroponoses) with Similar Clinical Manifestation

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Abstract: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne human viral disease with fatality rate up to 30%. It is characterized by a sudden onset of fever, muscular pain, often progressing to hemorrhagic manifestations. Natural reservoir hosts include various wild and domestic mammals. Primary vector and reservoir hosts are ticks from the genus *Hyalomma*. Humans become infected through bite of infected tick or direct contact with tissues or body fluids of viremic animals or CCHF patients. Several hantaviruses cause hemorrhagic fever with renal syndrome (HFRS). Hantaviruses are maintained in rodents (order Rodentia, families Muridae and Cricetidae), insectivores (order Lipotyphla, families Soriciodae (shrews) and Talpidae (moles)) and also bats (order Chyroptera). Hantaviruses can be transmitted to humans, most commonly through inhalation of infested rodent excreta. Each hantavirus is associated with a specific natural reservoir. HFRS caused by Dobrava hantavirus is endemic in the Balkan countries and Alpe-Adrian region. It is usually a severe illness presented with hemorrhages, fever, acute renal failure often requiring dialysis, and has a case-fatality rate up to 10%. Located on the Balkan Peninsula, Bulgaria is known as endemic for CCHF and HFRS. Recent investigations of vectors, reservoirs and hosts are discussed.

Key words: hantavirus, Crimean-Congo hemorrhagic fever virus, hemorrhagic fever with renal syndrome, rodents, ticks

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infection. Ticks of the genus *Hyalomma* are the principal vector, and a wide range of animals can become infected. The disease exists over a large geographic area, including Africa, the Middle East, Eastern Europe and Central Asia. In animals (most of the reports involve livestock) the infection is either in-apparent or the animals exhibit minimal symptoms. However, when human cases do occur, hemorrhagic complications and high mortality rates have been described (Ergonul 2008). Over the past decade, CCHF cases in humans have emerged in Turkey and reemerged in the Balkan countries, Ukraine and Tajikistan (Maltezou *et a.l* 2010). This has prompted a renewed interest in this disease. Ever since CCHF was first described, occupational contact with infected livestock has been recognized as a common cause of the disease (Chumakov *et al.* 1968). Seroprevalence rates in livestock can be as high as 78% (Lotfollahzadeh *et al*. 2009). A recent study in Turkey found that 12% of a high-risk human population had serologic evidence of prior CCHF infection (Gunes *et al.* 2009). The reason for the apparent resurgence in CCHF activity in endemic foci is unknown, but appears to be part of a general increase in tick-borne disease in Europe and Central Asia (Heyman *et al.* 2010). Changes in the environment, land use, hunting, and animal husbandry practices all potentially impact this tick-borne disease (Ergonul *et al.* 2007).

Human CCHF infections have appeared in previously non-endemic regions in Bulgaria (Christova

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et al. 2009) and the European part of Turkey (Gargili *et al.* 2011).

In Bulgaria, CCHFV was first detected in 1952 in Stara Zagora region. Over 1500 cases have been reported in the country since then. The strains causing CCHF in the country are closely related to others in the Balkan Peninsula, belonging to lineage Europe 1.

Hemorrhagic fever with renal syndrome (HFRS) and Crimean-Congo hemorrhagic fever (CCHF) are the two viral hemorrhagic fevers spread in Europe. The two viral hemorrhagic fevers are caused by relative viruses belonging to two different genera of one viral family, the Bunyaviridae family, i.e. genus *Hantavirus* (HFRS) and genus *Nairovirus* (CCHF). Virions of both genera are enveloped, spherical particles with a diameter of 80-110 nm and a negative-sense single-stranded RNA genome with tree segments: S (small), M (medium), and L (large). Both viruses cause severe diseases with fever and hemorrhagic manifestations.

The two infections differ in their transmission routes and reservoirs.

Hantavirus infections are transmitted to humans through inhalation of rodent or some insectivore excreta. Several types hantaviruses cause HFRS: Puumala virus (PUUV), Dobrava virus (DOBV), Saaremaa (SAAV), Hantaan virus (HTNV), Seoul virus (SEOV) and Amur (AMRV). Each serotype hantavirus is associated with a specific natural reservoir: PUUV is transmitted by the bank vole, *Myodes glareolus*; DOBV is associated with the yellow-necked field mouse, *Apodemus flavicollis*; natural reservoir of SAAV and HTNV is the striped filed mouse, *Apodemus agrarius*; SEOV is transmitted by rats, *Rattus rattus* and *Rattus norvegicus*; and AMRV is associated with Korean field mouse, *Apodemus peninsulae*. HFRS caused by PUUV, SAAV or DOBV is endemic in Europe. HFRS cases in China and Korea are caused by infections with HTNV or SEOV. AMRV causes HFRS in Far East Russia.

In Europe, PUUV and SAAV infections cause a mild form of HFRS. PUUV infection was known as nephropathia epidemica in the Scandinavian countries and is characterized by fever, back pain, and renal impairment. HFRS caused by DOBV infection is endemic in the Balkan countries and is much more severe; it presents with hemorrhages, fever, acute renal failure often requiring dialysis, and case-fatality rate is up to 10%.

Hantaviruses in North and South America cause hantavirus cardiopulmonary syndrome (HCPS). They are genetically different from the Old World hantaviruses and also differ in reservoir species. The disease is associated with fever and respiratory distress symptoms, and case mortality rate is up to 30-40%.

The aim of this study was to provide insight and overview on circulation of CCHF virus in livestock and ticks as well as to screen rodents for hantaviruses and to learn which hantaviruses are in circulation.

Materials and Methods

Blood samples were collected from 392 cattle, sheep and goats in two rural farming communities in Burgas district and tested for IgG antibodies against CCHF virus by ELISA.

Ticks (623 *Hyalomma marginatum* and 522 *Rhipicephalus sanguineus*) were collected from livestock – goats, sheep and cattle, from farms located in the endemic districts where CCHF cases have been registered during the past 5 years and tested by RT-nested-PCR and real time RT-PCR as previously described (Rodriguez *et al.* 1997; Garrison *et al.* 2007).

A total of 691 rodents were collected in three different districts, two of them selected on the high incidence rate of HFRS: Pazardzhik and Smolyan and one district of low endemicity: Sliven.

RNA was extracted from lung tissue samples using QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to instructions of the manufacturer. RNA was then subjected to TaqMan RT-PCR for identification of conserved region of the S RNA segment of hantavirus genome as described (Kramski *et al.* 2007).

Results and Discussion

The results from animal study showed that, overall, 72% (282/392) of the tested animals were positive for IgG antibodies to CCHFV. Antibodies to CCHFV were found in 71% of the cattle, 74% of the sheep and 60% of goats. By looking at the age distribution of the tested animals, we found that by the time the animals were one year old almost 50% had serologic evidence of CCHF infection, and by three years over 80% had been infected.

CCHF incidence and spread increased in recent years. Sporadic cases and even outbreaks are being reported every year. The Balkan Peninsula is well-known endemic region for CCHF. Most of Bulgaria is an ecologically favourable environment for CCHF virus circulation in nature. Despite this fact, only a few CCHF cases per year are registered in the last years in Bulgaria. Although the number of cases is lower than previously reported, a spread of the disease in new areas is seen (Christova *et al.* 2009).

The seroprevalence study indicated high rates of CCHF sero-positivity among livestock. Establishment of surveillance systems to monitor the viral activity in the region and implementation of vector control plans are highly recommended.

Application of real-time RT-PCR detected CCHF virus in *H. marginatum* ticks, supporting its leading role as CCHFV vector. Notably, *H. marginatum* was the predominated species of the collected ticks exactly in the same two districts (Kardzhali and Burgas), where CCHFV was detected in *H. marginatum* ticks. The mean prevalence of CCHFV-positive *H. marginatum* ticks was 6.3%, but was up to 13.9% and 15.2% in some villages in the Kardzhali and Burgas districts.

Prevalence of CCHF virus in *H. marginatum* ticks corresponds to that described in other Balkan countries: 9.1 -10.9% for Turkey (Ergonul 2006; Yesilbag *et al.* 2013); 11 -15% for Kosovo (Duh *et al.* 2006; Sherifi *et al.* 2014), although in a recent study in Kosovo, CCHFV was not detected in ticks collected from livestock in otherwise highly endemic regions (Fajs *et al.* 2014).

CCHFV lineage Europe 2 was detected for the first time in ticks in Bulgaria. It was detected in 40% (46/114) of ticks in Kardzhali district. This could explain the low number of reported CCHF cases in Bulgaria despite the significant seroprevalence in healthy population (Christova *et al.* 2013). CCHFV lineage 2 was not detected in three of the districts tested, suggesting that endemic foci differ among lineages, which is probably related with the ecosystem favoring the vectors (*Hyalomma* spp. versus *Rhipicephalus* spp.).

A total of 691 small mammals were trapped in Pazardzhik, Smolyan and Sliven districts of Bulgaria. Of those, 234 were identified morphologically as the striped field mice *Apodemus agrarius*, 333 as the yellow-necked mice *Apodemus flavicollis*, 121 as the wooden mice *Apodemus sylvaticus* and three as the bank voles *Myodes glareolus*. Lung samples from each of these rodents were screened using TaqMan RT-PCR for DOBV and PUUV. Eight rodents were found positive for DOBV and none for PUUV. Seven of eight DOBV-positive samples originated from *A. flavicollis* and one from *A. agrarius*. Positive rodents were detected in all three investigated districts: five in the district of Pazardzhik, two in the district of Sliven and one in Smolyan. Our findings confirmed circulation of DOBV in Bulgaria. Unfortunately, the number of *M. glareoulus*, trapped in this study, was very low to learn about circulation of PUUV in the country.

The results of this study showed evidence for much higher level of CCHF virus and hantavirus circulation in the country than previously thought. Viral hemorrhagic fevers are most probably not restricted in the endemic areas but much more widespread in Bulgaria, while only the most severe cases are reported.

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