BOURBON VIRUS: A NEWLY DESCRIBED EMERGING INFECTIOUS AGENT

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INTRODUCTION

In the recent past, novel viruses are being described in rapid succession which are adding to high morbidity, mortality and escalating healthcare cost. With increased population movement, trade and cross – cultural interaction, the implication of rapid spread of these agents among the world community is huge. Emerging infections are described as having appeared in the population for the first time (yet unrecognized), or may have existed previously and has either increased in incidence or expanded into a new ecological niche or geographic range with a significant change in its pathogenicity [1].

The increasing number of viruses being described in causing unexpected diseases of epidemic proportions among humans, livestock and wildlife is worrisome. Outbreaks by these novel agents are stretching the health resources nationally and internationally, exposing the unpreparedness and economic constraints of developing nations. The ability of identify newly emerging diseases and implement adequate control measures to restrict its spread will be effective only if implemented at a global level. Challenges include health care cost cutting measures, inadequate supply of personnel protection devices, regular surveillance, timely assessment and update of health information and willingness to share information and infrastructure [2].

Bourbon virus, first described in June 2014 and named after the Kansas County; was the suspect pathogen causing the death of a farmer who showed signs of a tick borne illness. Mr. JS, a 68-year-old, had high fever, decreased appetite, muscle aches, low red and white blood cell counts with elevated liver enzymes. He worked outdoors and often had tick bites. Initial concerns about a tick borne disease such as ehrlichiosis or Rocky Mountain spotted fever was not validated by laboratory tests and he did not respond to typical antibiotics (doxycycline) and succumbed with multi-organ failure within ten days[3].

Samples were sent to CDC laboratory in Colarado to look for Heartland virus, a tick borne infection discovered in 2009 (reported in 2012) with a similar presentation[4]. Six months later, CDC announced the identification of a new DNA virus – member of the *Orthomyxovirus* family as a subcategory that was not described earlier to cause human disease. Presumed to be transmitted by tick bite (though not proven yet), the Bourbon virus (named after the county) possess a genome similar to that of viruses in Eastern Europe, Africa and Asia[5]. Tests are ongoing to determine if prior undiagnosed, but similar cases may have been caused by Bourbon virus.

Course of description of the virus

Evaluation of the suspected patient sample was negative for most tick borne virus serology, including for Heartland virus antibodies and RNA. PRNT cell culture wells, however, showed heterologous (non-Heartland) viral plaques leading to suspicion of a novel virus (Figure 1). Inoculation of blood samples and serum samples on cell line revealed substantial cytopathic effect 3 days post inoculation which was reproducible on repeated isolation.

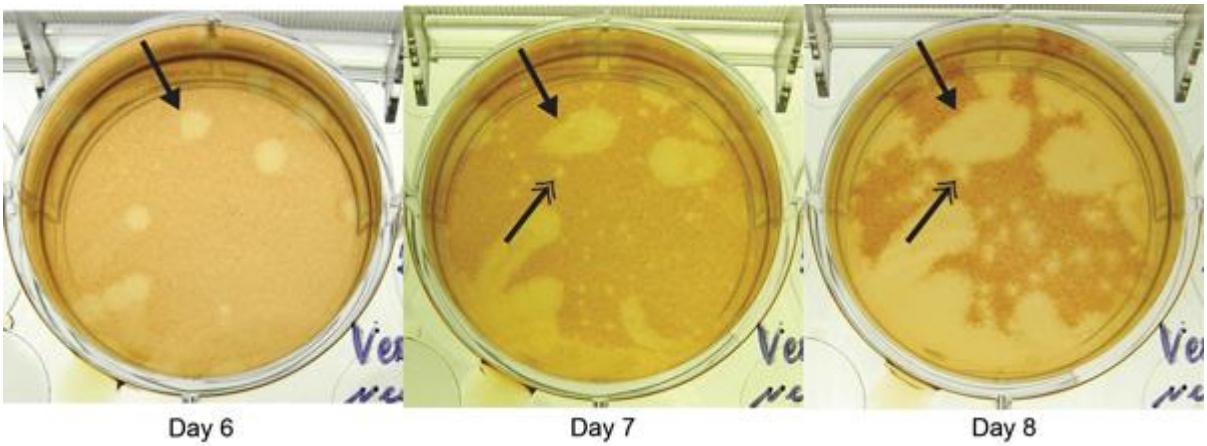


Figure 1:*Plaque reduction neutralization test of patient sample for Heartland virus, showing images of the same well obtained days 6, 7, and 8 post inoculation at a dilution of 1:20. Arrows with single heads indicate appearance of a novel virus plaque beginning at day 6. Arrows with double heads indicate development of a typical Heartland virus plaque, apparent on day 7 and more evident on day 8, generated from a control strain added to each well in defined quantities to identify Heartland virus–specific antibodies in the patient sample.(Courtesy: CDC http://dx.doi.org/10.3201/eid2105.150150)*

Further studies by electron microscopy (Figure 2)and next generation sequencing (NGS)[6] helped to place the novel RNA virus in the family of Orthomyxoviridae with 70% overall average nucleotide sequence percentage identity with Dhori virus. RT_PCR based studies confirmed the patient blood and serum samples as the source of this newly described virus.

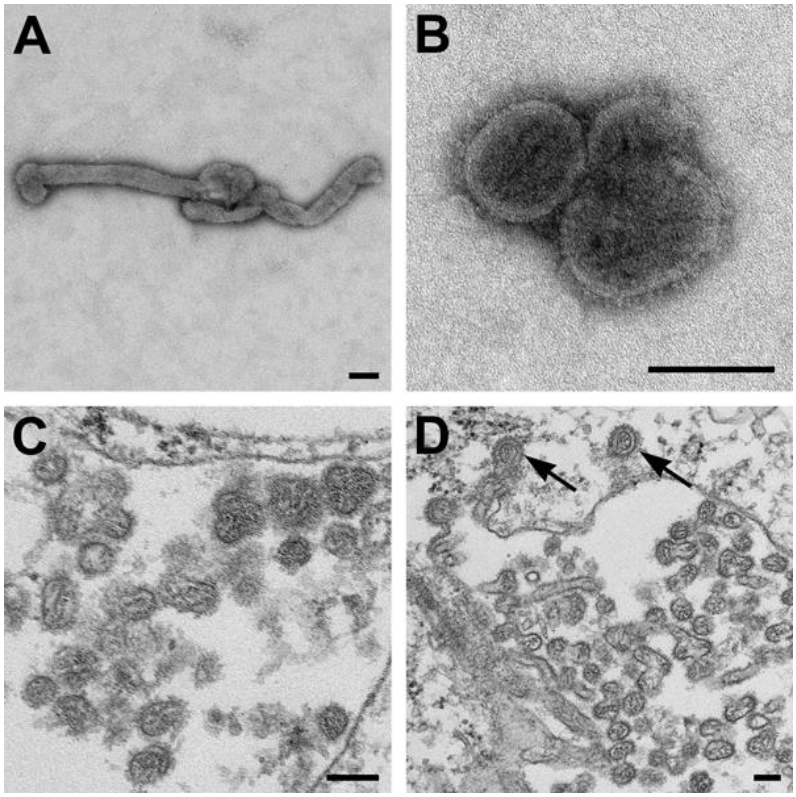


Figure 2.*Electron microscopic images of novel Thogotovirus isolate. Filamentous (A) and spherical (B) virus particles with distinct surface projection are visible in culture supernatant that was fixed in 2.5% paraformaldehyde. Thin-section specimens (C and D), fixed in 2.5% glutaraldehyde, show numerous extracellular virions with slices through strands of viral nucleocapsids. Arrows indicate virus particles that have been endocytosed. Scale bars indicate 100 nm. . (Courtesy: CDC http://dx.doi.org/10.3201/eid2105.150150)*

PHYLOGENETIC ANALYSES

Phylogenetic analyses (Figure 3) indicated that Bourbon virus is most closely related to Dhori and Batken viruses. However, the branch lengths suggest a relatively distant evolutionary distinction of Bourbon virus from Dhori and Batken viruses, which have only been described in the Eastern Hemisphere. Dhori, Batken, and Thogoto viruses have been identified in various hard tick species. Based on the observation that onset of illness in the patient was in late spring and a history of finding an embedded tick before becoming ill ,support the notion that Bourbon virus might be transmitted by ticks.

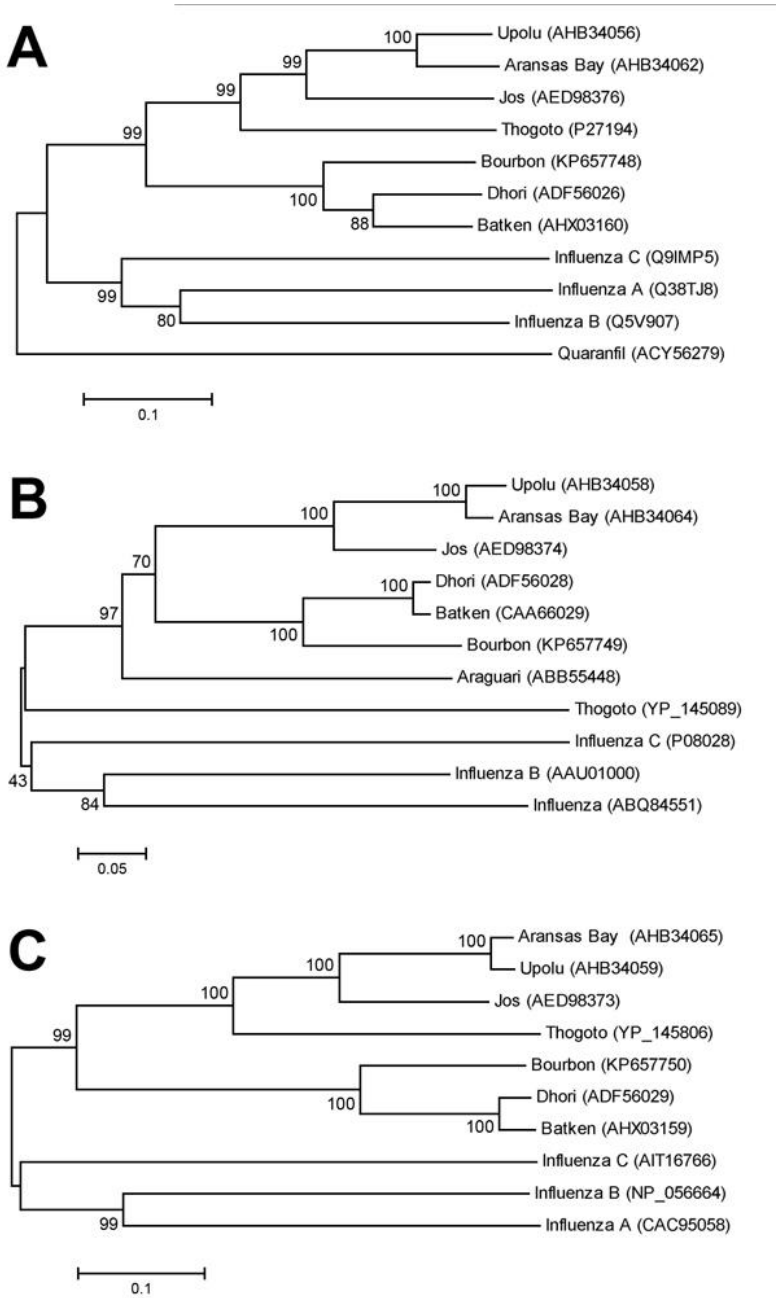


Figure 3: *Phylogenies of deduced amino acid sequences of representative genes of Bourbon virus in comparison to homologous sequences of selected orthomyxoviruses. A neighbor-joining method was used for inference of each phylogeny with 2,000 replicates for bootstrap testing. Values at nodes are bootstrap values. A) PA polymerase subunit, (segment 3). B) Nucleocapsid protein (segment 5). C) Membrane protein*

(segment 6). GenBank accession numbers appear next to taxon names. Scale bars indicate number of amino acid substitutions per site.(Courtesy: CDC http://dx.doi.org/10.3201/eid2105.150150)

The history of tick bite, geographical location (Kansas is endemic for tick borne fever), clinical presentation with leucopenia and thrombocytopenia were consistent with tick borne illness. Serology was negative for common tick borne pathogens like *Ehrlichia chaffensis*, *Rickettsia*, and Heartland virus with non-responsive to doxycycline. Although it is unclear what role the novel virus of the *Thogotovirus* species played in the death of the patient, the high level of viremia, as shown by multiple isolations from the blood of the patient 2 days before his death, suggests that this might have contributed to the death of the patient.

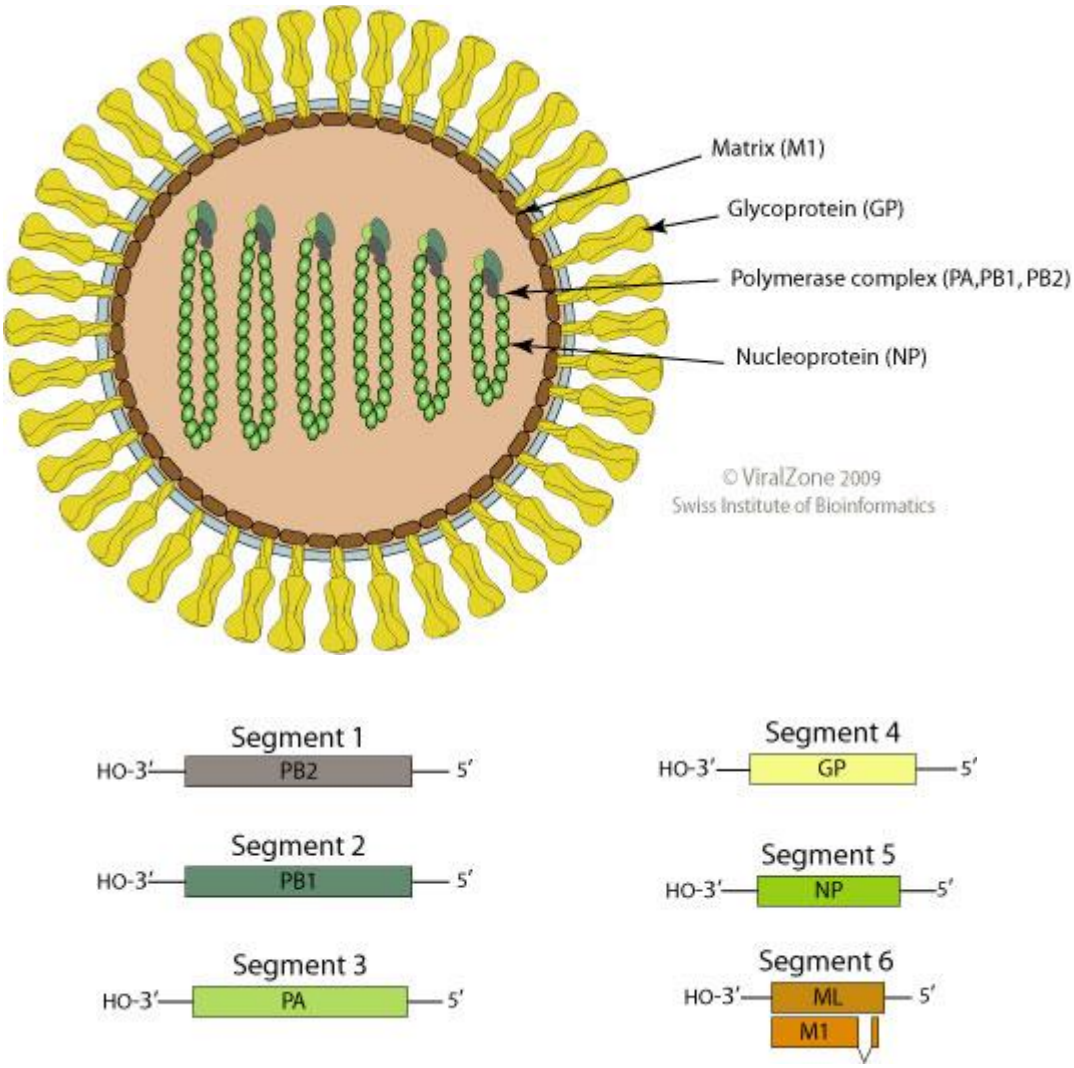
THOGOTOVIRUS: AN OVERVIEW

First isolated in Kenya and named after the Thogoto forest[7],the genus *Thogotovirus* (family *Orthomyxoviridae*) contains more than 6 distinct viruses, including *Araguari, Aransas Bay, Dhori, Jos, Thogoto*, and *Upolu* viruses[8]. These viruses have been primarily associated with either hard or soft ticks and have a wide geographic distribution. The hosts include mammals with *Dhori* and Bourbon viruses shown to infect humans. Zoonotic transmission by vector (tick bite) from animal reservoir is suspected. The virus is able to replicate in vertebrate and vector (tick) cells. Experimental transmission from infected to uninfected ticks has been shown as a result of cofeeding on uninfected guinea pigs[9]. Thogotovirus has been described in Southern Europe (Portugal), Egypt, Eastern Russia, India, Africa and North Americas.

VIROLOGY

Thogoto viruses are spherical, enveloped single stranded RNA viruses with a segmented genome. Virions are 80-120 nm in diameter with a total genome size of approximately 10Kb. The 6-7 segments of genome code for 7-9 proteins with each segment ranging from 0.9 to 2.3 Kb[10]. Viral RNA polymerases (PA, PB1 and PB2) transcribes one mRNA from each gnome segment . Splicing of segment 6 mRNA gives rise to mRNA coding for the matrix protein M1.

The virus entry into host cell is mediated by the viral surface glycoproteins attaching to sialic acid receptors on host. Clarithrin mediated endocystosis is followed by membrane fusion (viral- endosomal membranes) to liberate the viral RNA segments which further migrate to the nucleus. Within the host nucleus, transcription of genomic segments by viral polymerases generates mRNAs which are capped and polyadenylated. Viral replication occurs with generation of new viral proteins. High levels of M1 protein induce genome export from nucleus. Viral assembly occurs in the cytoplasm which then buds out from the plasma membrane to infect other cells[11, 12].



Though this is the first reported incidence of Bourbon virus infection, researchers assume that milder versions of the infection may have been existing for a while. No antiviral agents or vaccines are recommended and the best defense is personal protection. Long sleeve clothing, anti-insecticide sprays; avoiding bushy and wooden areas are effective to avoid tick bite. Performing tick checks after spending time outdoors is a recommended safety measure.

The recent reporting of emerging tick associated viral infections presenting with fever and thrombocytopenia with rapid health deterioration points to worrisome public health burden of the future. The extent of the disease and its prevalence and spread is yet to be completely unraveled. The use of novel techniques of pathogen identification like NGS sequencing along with traditional microbiological techniques has made great inroads in rapid diagnosis of novel infectious agents. Understanding the biology of Bourbon virus is currently ongoing with work planned to identify extensively its geographicl distribution, viral characterization, its potential reservoir and vectors. This information will be crucial is mapping the preventive methods which can prevent future outbreaks and help curb the morbidity and mortality associated with it.

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