Table. Number of reported hemotrophic mycoplasma infections, China, 1994–2007*

Year	Species				
	Human	Cow	Swine	Sheep	Fox
1994	200	NR	NR	NR	NR
1995	331	132	NR	231	NR
1996	1,229	259	147	NR	NR
1997	2,262	69	1,282	126	NR
1998	740	64	127	115	NR
1999	3,861	1,460	397	2,493	954
2000	1,971	2,920	140	NR	371
2001	329	329	7,775	NR	16,697
2002	126	NR	17,068	NR	17,068
2003	880	84	600,033	1,877	31,208
2004	4	625	15,604	206	NR
2005	451	119	27,268	2,916	20
2006	4	75	15,916	536	465
2007	452	3	1,686	53	60

*NR, no record.

ease in domestic animals (e.g., pigs) and humans has reached an alarming level (Table). Human infection rates in certain areas in China have been high; for example, in Inner Mongolia, samples collected from 1,529 randomly selected persons during 1994–1996 showed that 35.3% of the local population, 57.0% of local pregnant women, and 100% of newborns of infected mothers were positive for hemotrophic mycoplasma infection (*2*). Infections in animals in China have been recognized since 1995, and the number of cases has been increasing rapidly. For example, >600,000 pigs infected with *M. suis* were reported in 2003 (Table). These infections have had a large economic impact on regions where the infection is endemic (*8*). Infections in other animals, including cows, sheep, and foxes, were also common, indicating a high prevalence of the bacteria in China. However, because of the lack of in vitro cultivation systems that assist in characterizing pathogens, progress in species identification and molecular characterization of these pathogens has been slow. Thus far, names of hemotrophic mycoplasma species have been based on the hosts from which they were identified. Due to the zoonotic nature of these pathogens, more in-depth studies on these microorganisms are needed.

This work was supported by the Young Distinguished Scholar Grant from the National Natural and Scientific Committee, People's Republic of China (grant no. 30625029).

Zhe Hu,[1] Jigang Yin,[1] Kefei Shen, Wei Kang, and Qijun Chen

Author affiliations: Jilin University, Changchun, People's Republic of China (Z. Hu, J. Yin, K. Shen, W. Kang, Q. Chen); and Swedish Institute for Infectious Disease Control and Karolinska Institutet, Stockholm, Sweden (Q. Chen)

DOI: 10.3201/eid1507.090174

References

1. Hoelzle LE. Haemotrophic mycoplasmas: recent advances in *Mycoplasma suis*. Vet Microbiol. 2008;130:215–26. DOI: 10.1016/j.vetmic.2007.12.023
2. Yang D, Tai X, Qiu Y, Yun S. Prevalence of *Eperythrozoon* spp. infection and congenital eperythrozoonosis in humans in Inner Mongolia, China. Epidemiol Infect. 2000;125:421–6. DOI: 10.1017/S0950268899004392
3. Neimark H, Hoff B, Ganter M. *Mycoplasma ovis* comb. nov. (formerly *Eperythrozoon ovis*), an eperythrocytic agent of haemolytic anaemia in sheep and goats. Int J Syst Evol Microbiol. 2004;54(Pt 2):365–71. DOI: 10.1099/ijs.0.02858-0
4. Pitcher DG, Nicholas RA. Mycoplasma host specificity: fact or fiction? Vet J. 2005;170:300–6. DOI: 10.1016/j.tvjl.2004.08.011
5. Stoffregen WC, Alt DP, Palmer MV, Olsen SC, Waters WR, Stasko JA. Identification of a haemomycoplasma species in anemic reindeer (*Rangifer tarandus*). J Wildl Dis. 2006;42:249–58.
6. Henderson JP, O'Hagan J, Hawe SM, Pratt MC. Anaemia and low viability in piglets infected with *Eperythrozoon suis*. Vet Rec. 1997;140:144–6.
7. Hoelzle LE, Hoelzle K, Helbling M, Aupperle H, Schoon HA, Ritzmann M, et al. MSG1, a surface-localised protein of *Mycoplasma suis* is involved in the adhesion to erythrocytes. Microbes Infect. 2007;9:466–74. DOI: 10.1016/j.micinf.2007.01.004
8. Wu J, Yu J, Song C, Sun S, Wang Z. Porcine eperythrozoonosis in China. Ann N Y Acad Sci. 2006;1081:280–5. DOI: 10.1196/annals.1373.038

[1]These authors contributed equally to this article.

Address for correspondence: Qijun Chen, Swedish Institute for Infectious Disease Control and Karolinska Institutet, Nobels väg 16, Stockholm 171 81, Sweden; email: qijun.chen@smi.se

Sensitivity of Andes Hantavirus to Antiviral Effect of Human Saliva

To the Editor: Hantaviruses cause 2 severe and often fatal human diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Rodents are the natural hosts for hantaviruses that cause HFRS and HCPS, and humans are usually infected by aerosolized virus-contaminated rodent excreta (*1,2*). Except for Andes virus (ANDV), human-to-human transmission of hantaviruses does not seem to occur. ANDV clearly is transmitted directly

from human to human (*3*), but exactly how this occurs or why other pathogenic hantaviruses are not transmitted between humans is not known.

ANDV antigen has been detected in the secretory cells of the salivary glands of humans (*4*). The risk for infection with ANDV is higher in people having sex or involved in deep kissing with an infected person than in other contacts (*5*), suggesting that transmission of ANDV needs close person-to-person contact. Therefore, one can speculate that ANDV is likely to be secreted into saliva and that saliva is involved in human-to-human transmission. Hantaviruses can be transmitted through saliva between the natural hosts (*6,7*), indicating that hantaviruses can withstand the antiviral effects of saliva or can interfere with production of saliva and thereby inhibit its antiviral effect.

We recently showed that saliva from Puumala hantavirus (PUUV)–infected humans contains viral RNA (*8*). This finding suggests that PUUV, and perhaps other hantaviruses, might be secreted into human saliva. However, we found no evidence of replicating virus in the saliva samples (*8,9*); neutralizing antibodies or salivary components may have inactivated the virus. We therefore analyzed the effect of saliva on the prototype hantavirus, Hantaan virus (HTNV). Our analysis shows that although HTNV is sensitive to the overall antiviral capacity of human saliva from healthy donors, it is insensitive to the antiviral effects of certain salivary components, i.e., histatin 5, lactoferrin, lysozyme, and secretory leukocyte protease inhibitor (*9*), which are known to have antiviral effects against other viruses.

We tested the hypotheses that ANDV might be less sensitive than HTNV and PUUV to the antiviral effect of human saliva. Saliva from healthy persons with no evidence of seropositivity against hantavirus was pooled and preincubated at different concentrations with 10,000 focus-forming units of ANDV (strain Chile-9717869), HTNV (strain 76-118), or PUUV (strain Kazan E6) for 1 hr (*9*). The virus plus saliva mixtures were then titrated on Vero E6 cells. Virus without saliva was used as a control. The medium used for dilution of saliva and virus was Hank's balanced salt solution (Invitrogen, Paisley, UK) supplemented with 2% fetal calf serum, 2% HEPES, 100 U of penicillin/mL, and 100 μg of streptomycin/mL. Because of a cytopathic effect on the cells, we could not test saliva concentrations >50% (*9*). After incubation, titers in samples incubated with saliva were calculated and compared with titers from virus incubated without saliva.

The different hantaviruses clearly differed in their sensitivities to human saliva. At a low concentration (12.5% saliva), we observed a slight effect on HTNV, even though we saw no effect on ANDV and PUUV. ANDV was the only virus that resisted higher concentrations of saliva (25% and 50%), and an antiviral effect was clearly observed on HTNV and PUUV at these saliva concentrations (Figure).

Our finding that ANDV is less sensitive than HTNV and PUUV to the antiviral effect of human saliva might explain why ANDV, but not HTNV or PUUV, is transmitted between humans. Saliva might be the preferred route of transmission for ANDV between humans, as it is for the long-tailed rice rat (*Oligoryzomys longicaudatus*), the natural host for ANDV (*10*). However, transmission of ANDV between rodents, from rodents to humans, and between humans differs. Replicating hantaviruses have not been isolated from saliva of patients with HFRS or HCPS. In patients who have seroconverted, hantavirus-specific antibodies are likely to be present and might efficiently neutralize the virus, including ANDV. If this is the case, the interval might be short between excretion of the virus into the saliva and seroconversion, enabling the infected person to transmit hantavirus to other humans. Ferres et al. showed that in persons who developed HCPS after human-to-human transmission of ANDV, viremia preceded onset of disease and detection of ANDV-specific antibodies by up to 2 weeks (*5*). Sampling of saliva from healthy household contacts to ANDV-infected persons, with subsequent virus isolation attempts, might show whether human saliva is the mode of ANDV transfer during human-to-human transmission.

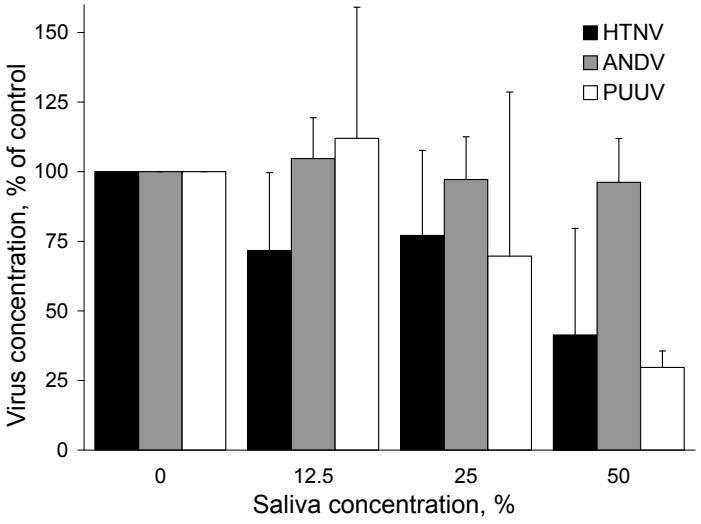

Figure. Antiviral effect of human saliva against Hantaan virus (HTNV), Andes virus (ANDV), and Puumala hantavirus (PUUV). Data represent mean + SD of 3 independent experiments.

This study was funded by the Swedish Society of Medicine, the Swedish Society for Medical Research, the Swedish Medical Research Council (project 12177), and European Union grant GOCE-2003-010284 EDEN (Emerging Diseases in a changing European eNvironment).

This paper is catalogued by the EDEN Steering Committee as EDEN0146 (www.eden-fp6project.net).

Jonas Hardestam, Åke Lundkvist, and Jonas Klingström

Author affiliations: Karolinska Institutet, Stockholm, Sweden, and Swedish Institute for Infectious Disease Control, Solna, Sweden

DOI: 10.3201/eid1507.090097

References

1. Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. Lancet Infect Dis. 2003;3:653–61. DOI: 10.1016/S1473-3099(03)00774-6
2. Schönrich G, Rang A, Lütteke N, Raftery MJ, Charbonnel N, Ulrich RG. Hantavirus-induced immunity in rodent reservoirs and humans. Immunol Rev. 2008;225:163–89. DOI: 10.1111/j.1600-065X.2008.00694.x
3. Montgomery JM, Ksiazek TG, Khan AS. Hantavirus pulmonary syndrome: the sound of a mouse roaring. J Infect Dis. 2007;195:1553–5. DOI: 10.1086/516793
4. Navarrete M, Pizarro E, Méndez C, Salazar P, Padula P, Zaror L, et al. 2007. Hantavirus ANDV distribution in human lungs and salivary glands. In: Abstracts of the VII International Conference on HFRS, HPS and Hantaviruses; 2007 June 13–15; Buenos Aires, Argentina. Abstract 103.
5. Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. J Infect Dis. 2007;195:1563–71. DOI: 10.1086/516786
6. Yanagihara R, Amyx HL, Gajdusek DC. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (*Clethrionomys glareolus*). J Virol. 1985;55:34–8.
7. Hardestam J, Karlsson M, Falk KI, Olsson G, Klingström J, Lundkvist Å. Puumala hantavirus excretion kinetics in bank voles (*Myodes glareolus*). Emerg Infect Dis. 2008;14:1209–15. DOI: 10.3201/eid1408.080221
8. Pettersson L, Klingström J, Hardestam J, Lundkvist Å, Ahlm C, Evander M. Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome. Emerg Infect Dis. 2008;14:406–11. DOI: 10.3201/eid1403.071242
9. Hardestam J, Petterson L, Ahlm C, Evander M, Lundkvist Å, Klingström J. Antiviral effect of human saliva against hantavirus. J Med Virol. 2008;80:2122–6. DOI: 10.1002/jmv.21332
10. Padula P, Figueroa R, Navarrete M, Pizarro E, Cadiz R, Bellomo C, et al. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol. 2004;78:11972–9. DOI: 10.1128/JVI.78.21.11972-11979.2004

Address for correspondence: Jonas Klingström, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden; email: jonas.klingstrom@smi.se

Spread of Cantagalo Virus to Northern Brazil

To the Editor: *Cantagalo virus* (CTGV) is a strain of *vaccinia virus* (VACV; *Poxviridae*) that was isolated from pustular lesions on dairy cattle and dairy workers in Rio de Janeiro State, Brazil, in 1999 (*1*). Subsequently, similar lesions caused by poxviruses have been reported in cattle and humans in all 4 states of the southeast region of Brazil and in Goiás State in central-western Brazil (online Technical Appendix, panel A, available from www.cdc.gov/EID/content/15/7/1142-Techapp.pdf)(*2*–*7*). Etiologic agents were VACV strains, most of which were genetically related to CTGV, such as Araçatuba and Passatempo viruses (*2*,*4*), with the exception of Guarani P1 virus, which was isolated in Minas Gerais State in 2001 and is phylogenetically close to VACV strain WR (*5*). Reisolation of Guarani P1 virus has not been reported. All VACV isolates related to CTGV share 2 molecular signatures: an 18-nt deletion in the A56R gene, which encodes viral hemagglutinin (*1*–*7*), and a 15-nt deletion in K2L gene, which encodes serine protease inhibitor-3 (*8*,*9*). Other VACV strains unrelated to CTGV were isolated from rodents in Brazil before 1999, but reisolation of these viruses has not been described (8). Although CTGV-like disease has not been reported in the northern, northeastern, and southern regions of the country, rapid interstate spread of CTGV infection is of concern. We report an episode of CTGV infection in Tocantins State, northern Brazil (online Technical Appendix, panel A).

In September 2008, teat and udder lesions were found on 15 of 356 febrile (39.5°C–40°C) cattle on a dairy farm in the municipality of Muricilândia. Small papules progressed to vesicles and pustules (online Technical Appendix, panel B), which usually healed in 3–4 weeks. New lesions subsequently appeared on previously healthy cows on the same farm, and muzzle lesions developed on suckling calves. Dairy workers reported fever and lesions on their hands and neck. The farm was quarantined for 3 weeks until the condition was diagnosed.

Four scab samples were sent for virus identification by PCR. Parts of the samples were used to infect BSC-40 cells and for DNA isolation by phenol-chloroform extraction, as described (*6*). After 48 hours, a strong cytopathic effect suggested poxvirus infection. The PCR used unambiguously differentiates CTGV-related infections from other orthopoxvirus diseases, including cowpox virus and several VACV strains (*6*). The reverse primer targets nucleotide sequences flanking the deletion signature of the hemagglutinin gene from CTGV-related viruses. Therefore, a specific annealing site for the reverse primer is produced when these external sequences are contiguous, as occurs in CTGV (*6*).