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別紙様式第2−1

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医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2009年3月13日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	Simian Malaria in a U.S. Traveler --- New York, 2008	公表国 米国	
販売名(企業名)	別紙のとおり					

研究報告の概要	使用上の注意記載状況・その他参考事項等
問題点：サルマラリアである*Plasmodium knowlesi*のヒトへの感染例がマレーシアおよびその周辺の広範囲において多数報告され、人畜共通感染症の病原体として新興している可能性が示されている。 4種のプラスモディウム属の赤血球内原虫（熱帯熱マラリア原虫；*P. falciparum*、三日熱マラリア原虫；*P. vivax*、四日熱マラリア原虫；*P. malaride*および卵形マラリア原虫；*P. ovale*）がヒトでマラリアを起こすことが知られている。しかし、最近のアジアからのレポートで、5番目のマラリア原虫として*Plasmodium knowlesi*が人畜共通感染症の病原体として新興している可能性が示されている。20種類以上のマラリア原虫がヒト以外の霊長類に感染するが、これまでサルマラリアのヒトへの自然感染は、公衆衛生学に重要でない稀な事象とされてきた。光学顕微鏡による観察では、多くのサルマラリア原虫はヒトにマラリアを起こす4種のマラリア原虫との鑑別はほぼ困難で、PCRやマイクロサテライト分析といった分子的技術が種の確定に必要である。 最初の*P. knowlesi*感染は、1965年に東南アジアの任務から戻ってきた米国の兵士であった。その後の報告はほとんどなく、2002年にマレーシアの研究者らが非典型的な特徴をもつ四日熱マラリア症例の増加や、より重篤な臨床症状、より高度な寄生虫血症に気付いている。nested PCR assayにより、これらのマラリア症例の50%以上が*P. knowlesi*であると確認された。最初に顕微鏡診断されていた四日熱マラリアは1例もなかった。2001〜2006年に同じ研究者らによって行われたレトロスペクティブな調査では、マレーシアのSarawak州の患者からの960検体のうち28%が*P. knowlesi*であった。以前は、そのほとんどが形態学的に四日熱マラリアと診断されていた。このグループはまた、四日熱マラリアによる重症のマラリアと考えられていた4例の異常な死亡が、後にPCRによって*P. knowlesi*と確認されたことも報告している。さらにヒトの*P. knowlesi*感染は、シンガポール、タイとミャンマーの国境、フィリピン、中国の雲南省、フィンランド（マレーシアから帰った旅行者が最初熱帯熱マラリアと誤診されていた）からも報告されている。	記載なし

報告企業の意見	今後の対応
別紙のとおり	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。

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別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	マラリアは、ハマダラ蚊によって媒介されるが、ヒトに感染すると赤血球に侵入し、増殖した後、赤血球を破壊し次の赤血球に侵入するサイクルを繰り返す。このような生活環から、稀ではあるが輸血によるマラリア感染も報告されている。 仮に、本剤の原材料であるヒト血液にマラリア原虫が混入していたとしても、当所で製造している全ての血漿分画製剤の製造工程には、約0.2μmの「無菌ろ過工程」および、マラリア原虫よりも小さいウイルスの除去を目的とした平均孔径19nm以下の「ウイルス除去膜ろ過工程」が導入されているので、これらの工程により除去されるものと考えられる。更に、これまでに本剤によるマラリアの報告例は無い。以上の点から、本剤はマラリアに対して一定の安全性を確保していると考える。

*現在製造を行っていない

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Simian Malaria in a U.S. Traveler --- New York, 2008

Four species of intraerythrocytic protozoa of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale*, and *P. malariae*) are known to cause malaria in humans. However, recent reports from Asia suggest the possibility that a fifth malaria species, *Plasmodium knowlesi*, is emerging as an important zoonotic human pathogen. Although more than 20 species of *Plasmodium* can infect nonhuman primates, until recently, naturally acquired human infections of simian malaria were viewed as rare events lacking public health significance. When viewed by light microscopy (the gold standard for laboratory diagnosis of malaria), many of the simian species are almost indistinguishable from the four *Plasmodium* species that cause infection in humans (Table). Molecular techniques, such as polymerase chain reaction (PCR) amplification and microsatellite analysis, are needed for definitive species determination. This report describes the first recognized case of imported simian malaria in several decades in the United States, diagnosed in 2008 in a patient from New York who had traveled to the Philippines. Atypical features of the parasite seen on light microscopy triggered further molecular testing, which confirmed the diagnosis of *P. knowlesi*. To date, all simian malaria species have been susceptible to chloroquine treatment. Molecular analysis of certain malaria parasites isolated from ill travelers returning to the United States from Asia or South America can more accurately assess the burden of simian malaria parasite infections in humans.

The first recognized case of naturally acquired simian malaria was a 1965 case of *P. knowlesi* infection in an employee of the U.S. Army who had returned home from an assignment in Southeast Asia (*1*); subsequent reports were few and unconfirmed. In 2002, investigators in Malaysia noted an increasing number of *P. malariae* cases with atypical features, including increased clinical severity and higher parasitemia (*2*). By using a nested PCR assay, more than 50% of these malaria cases were determined to be *P. knowlesi*, none were *P. malariae*, as originally determined by microscopy (*2*). In a retrospective evaluation by the same investigators during 2001—2006, 28% of 960 specimens from patients in Sarawak, Malaysian Borneo, were found to be *P. knowlesi*, after being morphologically diagnosed most often as *P. malariae* (*3*). The group also reported four unusual fatalities attributed to severe malaria caused by *P. malariae* that was later confirmed as *P. knowlesi* by PCR. Additional cases of naturally occurring *P. knowlesi* infection in humans have been reported from Singapore (*4*), the Thai-Burma border (*5*), the Philippines (*6*), Yunnan Province in China (*7*), and Finland, where a returning traveler from Malaysia was misdiagnosed initially as having infection with *P. falciparum* (*8*).

Case Report

In the recent U.S. case, a woman aged 50 years with no previous history of malaria who was born in the Philippines but had lived in the United States for 25 years, returned to her home country to visit friends and relatives on October 17, 2008. While there, she stayed on the island of Palawan in a cabin located at the edge of a forested area known to be a habitat for long-tailed macaques. She had not taken malaria chemoprophylaxis and had not used any mosquito-avoidance measures, both of which are recommended preventive measures for travelers to this area.

The woman returned to the United States on October 30, 2008, and noted the onset of a headache. Fever and chills ensued, and symptoms persisted for several days, after which she sought medical attention. In the emergency department, she was noted to be hypotensive and to have thrombocytopenia. Examination of thick and thin malaria smears (Figure 1) was ordered, and an initial, erroneous diagnosis of babesiosis was made by a laboratory technician. Upon review by the laboratory supervisor the following morning, the diagnosis was reassessed as malaria with 2.9% of red cells parasitized. However, the atypical appearance of the *Plasmodium* sp. seen in the smears prevented a species-specific diagnosis. The woman was treated successfully with atovaquone-proguanil and primaquine for *Plasmodium* of undetermined species.

An ethylenediaminetetraacetic acid (EDTA) blood tube and two stained smears were sent to New York state's Wadsworth Center Parasitology Reference Laboratory for confirmation of species by PCR. The Wadsworth Center confirmed the presence of atypical rings and schizonts of a *Plasmodium* species (Figure 1), but conventional PCR targeting the small subunit (SSU) of rRNA did not yield a product consistent with any of the four species of *Plasmodium* known to infect humans. The specimen also was negative for the variants of *P. ovale*, which are commonly seen in Southeast Asia. However, primers specific for the SSU rDNA of the genus *Plasmodium* yielded a 1,055-bp PCR product that was sequenced and noted to be a 99% match over its full length to the SSU rRNA gene from *P. knowlesi* (H strain) (*9*). These data confirmed that the infection was caused by *P. knowlesi*.

Reported by: *JG Ennis, AE Teal, A Habura, PhD, S Madison-Antenucci, PhD, JS Keithly, PhD, Div of Infectious Diseases, New York State Dept of Health. PM Arguin, MD, JW Barnwell, PhD, WE Collins, PhD, S Maki, MPH, L Slutsker, MD, A Dasilva, DSc, Div of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases. J Hwang, MD, EIS Officer, CDC.*

Editorial Note:

Several conditions need to coincide for simian species of *Plasmodium* to infect humans: 1) human erythrocytes must be susceptible

to invasion by simian parasites, 2) humans must be near or in forests where nonhuman simians are infected, and 3) anopheline mosquitoes that feed on both humans and nonhuman simians must be present (*10*). Many areas in Asia and South America have overlapping populations of nonhuman primates that serve as reservoirs for simian malaria and competent *Anopheles* mosquito vectors that are necessary to transmit the infection to humans (Table, Figure 2) (*1*). For *P. knowlesi* in Asia, the normal hosts are long-tailed and pig-tailed macaques and mitered-leaf monkeys, which are found with *Anopheles* mosquito vectors of the Leucosphyrus group, enabling transmission of infection (*1*). Other simian malaria species known to infect humans include *P. simium* and *P. brasilianum* in South America and *P. cynomolgi* and *P. inui* in Asia (*1,10*).

Most simian malaria infections in humans can cause mild or moderate disease but often are self-limited, not requiring antimalarial therapy (*1*). However, *P. knowlesi*, with its 24-hour asexual replication cycle, can result in large parasite burden and severe, life-threatening disease (*3*). Severe malaria imported from Asia should alert the physician to the possibility of infection with *P. knowlesi*. Health-care providers also should consider hospitalization if the patient with malaria reports travel to forested areas of Asia, where *P. knowlesi* transmission occurs. Simian *Plasmodium* species are susceptible to all available antimalarials in the United States. Although definitive diagnosis as a simian species of *Plasmodium* cannot be made in time to guide selection of antimalarials at the initiation of therapy, treatment for undetermined *Plasmodium* species will effectively treat all simian species. Use of current treatment and chemoprophylaxis guidelines are appropriate for treating and preventing simian malaria infections in humans.

Health-care providers of patients with malaria and laboratories that diagnose malaria imported from Asia or non-falciparum malaria from South America should refer appropriate specimens to a Clinical Laboratory Improvement Amendments (CLIA)–verified state health reference laboratory or CDC's Division of Parasitic Diseases Reference Laboratory for species confirmation by molecular testing. In the United States, approximately 1,500 malaria cases are reported each year, almost all imported from areas where malaria is endemic; approximately 200 of these cases are imported from Asia or South America. In the United States, the potential for not recognizing a *Plasmodium* infection of simian origin is high because diagnosis usually relies on microscopic examination of Giemsa-stained smears rather than diagnosis by molecular techniques. Only a few laboratories (including state and federal public health reference and commercial laboratories) routinely use molecular assays, and even fewer have the capacity to confirm simian species.

The substantial number of recent human cases of simian malaria reported in Malaysia and the wider region (including the travel-associated case described in this report) underscores the need to define the scope and magnitude of the problem (*2—8*). Persons wishing to send specimens for species confirmation by CDC should collect pretreatment blood in EDTA or acid citrate dextrose blood collection tubes. Instructions and specimen submission forms are available online at http://www.cdc.gov/malaria/smscs.htm. Contact information for local or state health department laboratories is available at http://www.aphl.org/aboutaphl/aboutphls/pages/memberlabs.aspx. As with all suspected cases of malaria, health-care providers with questions regarding diagnosis or treatment should call the CDC Malaria Hotline at 770-488-7788 (Monday—Friday, 8:30 a.m. to 4:30 p.m., EST). Health-care providers seeking emergency consultation after hours should call 770-488-7100 and request to speak with a CDC Malaria Branch clinician.

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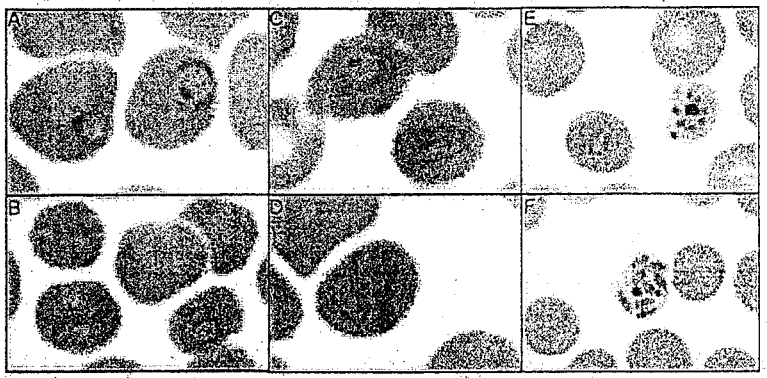
Figure 1

FIGURE 1. Giemsa-stained blood smears (1,000x magnification) from a reported case of *Plasmodium knowlesi* infection, highlighting the various features that often are mistaken for *Plasmodium malariae* or *Plasmodium falciparum — New York, 2008**



* Panel A. An infected red blood cell (RBC) with trophozoites resembling *P. malariae*. Panel B. Multiple infected RBCs, which are more commonly observed with *P. falciparum*. Panels C and D. Infected RBCs with "band-form" trophozoites resembling *P. malariae*. Panel E. RBC with eight merozoites in rosette-pattern resembling *P. malariae*. Panel F. *P. knowlesi* merozoites, although similar in appearance to *P. malariae*, are smaller and occupy less space in the infected RBC.

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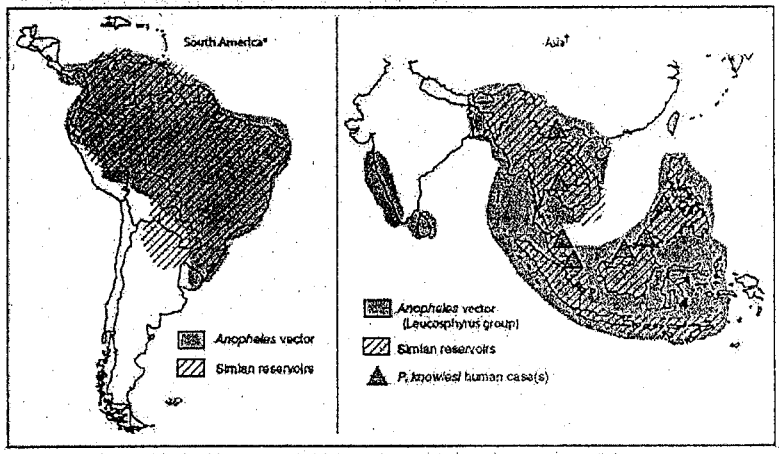
Figure 2

FIGURE 2. Overlapping distributions of competent *Anopheles* vectors and potential simian reservoirs for *Plasmodium brasilianum* and *Plasmodium simium* in South America and *Plasmodium knowlesi* in Asia



* Distribution of competent *Anopheles* and various simian reservoirs known to be infected with either *P. brasilianum* or *P. simium*.
† Distribution of *Anopheles* mosquitoes of the Leucosphyrus group and various simian reservoirs necessary for *P. knowlesi* human infection. Both single and clusters of human cases of *P. knowlesi* were reported from Malaysian Borneo, Peninsular Malaysia, China, Philippines, Singapore, and Thailand during 2001–2008.

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Table

TABLE. Simian malaria species in Asia and South America with their associated geographic distribution and morphologic similarity to one of four human *Plasmodium* species*

Simian *Plasmodium* species	Geography	Human species they resemble
Asia		
P. coatneyi	Malaysia, Philippines	*P. falciparum*
P. cynomolgi	India, Indonesia, Malaysia, Sri Lanka, Taiwan	*P. vivax*
P. eylesi	Malaysia	*P. vivax*
P. fieldi	Malaysia	*P. ovale*
P. fragile	India, Sri Lanka	*P. falciparum*
P. hylobati	Indonesia	*P. vivax*
P. inui	India, Indonesia, Malaysia, Philippines, Sri Lanka, Taiwan	*P. malariae*
P. jeffreyi	Indonesia, Malaysia	*P. vivax*
P. knowlesi	China, Indonesia, Malaysia, Philippines, Singapore, Thailand, Taiwan	*P. malariae, P. falciparum*
P. pithecl	Malaysia	*P. vivax*
P. simiovale	Sri Lanka	*P. ovale*
P. silvaticum	Malaysia	*P. vivax*
P. youngi	Malaysia	*P. vivax*
South America		
P. brasilianum	Brazil, Colombia, Mexico, Panama, Peru, Venezuela	*P. malariae*
P. simium	Brazil	*P. vivax*

*Four species of intraerythrocytic protozoa of the genus *Plasmodium* (*P. falciparum, P. vivax, P. ovale,* and *P. malariae*) are known to cause malaria in humans.

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研究報告の概要	○中国におけるヒト顆粒球アナプラズマ症の院内感染 背景：ヒト顆粒球アナプラズマ症(HGA)は、中国の新興ダニ媒介性リケッチア疾患である。劇症HGA患者との接触後に医療従事者およびその家族の感染集団を認めたため調査が行われた。 目的：安徽省における発熱性疾患の院内感染と思われる症例の感染源および伝播について検討すること。 デザイン、実施場所、および患者：発熱、出血により病院の隔離病棟へ入院し死亡した発端患者への接触後に発熱性疾患を生じ、接触が疑われた二次症例患者の抗*Anaplasma phagocytophilum*抗体、PCR測定、*A. phagocytophilum* DNA配列決定を実施した。 主な評価項目：血清学的またはPCRによりHGAの確証が得られた症例を非感染接触者と比較し、発病率、疾患相対リスク、発端患者への医療提供時の曝露についての潜在的リスクを定義した。 結果：2006年11月9日～17日の期間に、白血球減少、血小板減少を伴う発熱と血清アミノトランスフェラーゼ値上昇を発現した9名の患者が、末梢血中*A. phagocytophilum* DNAのPCRおよび*A. phagocytophilum*へのセロコンバージョンによりHGAと診断された。ダニに刺咬された患者はいなかった。9名の患者はいずれも、発端患者が死亡する直前の12時間以内に患者と接触し、その12時間の間に当該患者は大量出血があり、また気管内挿管治療を受けた。発病率は、50cm以内の接触者が32.1% vs 0% (P=0.04)、2時間以上の接触者が45% vs 0% (P=0.001)、血性分泌物への接触報告者が75% vs 0% (P<0.001)、発端患者の呼吸器分泌物への接触報告者が87.5% vs 0% (P=0.004)であった。 結論：中国におけるHGAの特定および血液や呼吸器分泌物への直接的な接触による院内HGA感染の可能性について報告する。	
報告企業の意見		今後の対応
劇症ヒト顆粒球アナプラズマ症(HGA)患者との接触後の医療従事者および家族の感染集団についてPCR等で調査した結果HGAと特定され、血液や呼吸器分泌物への直接的な接触による院内*A. phagocytophilum*感染が示唆されたとの報告である。		日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

ORIGINAL CONTRIBUTION

Nosocomial Transmission of Human Granulocytic Anaplasmosis in China

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Context Human granulocytic anaplasmosis (HGA) is an emerging tick-borne disease in China. A cluster of cases among health care workers and family members following exposure to a patient with fulminant disease consistent with HGA prompted investigation.

Objective To investigate the origin and transmission of apparent nosocomial cases of febrile illness in the Anhui Province.

Design, Setting, and Patients After exposure to an index patient whose fatal illness was characterized by fever and hemorrhage at a primary care hospital and regional tertiary care hospital's isolation ward, secondary cases with febrile illness who were suspected of being exposed were tested for antibodies against *Anaplasma phagocytophilum* and by polymerase chain reaction (PCR) and DNA sequencing for *A phagocytophilum* DNA. Potential sources of exposure were investigated.

Main Outcome Measure Cases with serological or PCR evidence of HGA were compared with uninfected contacts to define the attack rate, relative risk of illness, and potential risks for exposure during the provision of care to the index patient.

Results In a regional hospital of Anhui Province, China, between November 9 and 17, 2006, a cluster of 9 febrile patients with leukopenia, thrombocytopenia, and elevated serum aminotransferase levels were diagnosed with HGA by PCR for *A phagocytophilum* DNA in peripheral blood and by seroconversion to *A phagocytophilum*. No patients had tick bites. All 9 patients had contact with the index patient within 12 hours of her death from suspected fatal HGA while she experienced extensive hemorrhage and underwent endotracheal intubation. The attack rate was 32.1% vs 0% ($P=.04$) among contacts exposed at 50 cm or closer, 45% vs 0% ($P=.001$) among those exposed for more than 2 hours, 75% vs 0% ($P<.001$) among those reporting contact with blood secretions, and 87.5% vs 0% ($P=.004$) among those reporting contact with respiratory secretions from the index patient.

Conclusion We report the identification of HGA in China and likely nosocomial transmission of HGA from direct contact with blood or respiratory secretions.

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HUMAN GRANULOCYTIC ANAPLASMOSIS (HGA) is an emerging tick-borne infectious disease that was recognized in the United States in 1990[1] and in Europe in 1997.[2] The disease name was changed from human granulocytic ehrlichiosis to HGA in 2001 when the causative rickettsia was reclassified from the genus ehrlichia as *Anaplasma phagocytophilum*.[3] Although the clinical presentation of HGA is variable and although it may be difficult to diagnose, the annual number of infections reported in the United States since 1990 has steadily increased.[4,5] Seroepidemiological data suggest that infection rates in endemic areas are as high as 15% to 36%,[6-8] implying that the diagnosis is often missed or that infection is mild or asymptomatic. Because epidemiological, clinical, and microbiological information about HGA is limited, the disease is likely underrecognized and underreported worldwide.[7]

Despite the pathogen's global distribution, only a limited number of laboratory-confirmed cases have been reported from countries in Europe, where the median seroprevalence rate is 6.2%, similar to that in North America.[9] Serological and molecular evidence also suggests that human infection exists in Korea, Japan, and China.[10-14] Herein, we report the first cases of HGA acquired in China, as well as the unusual finding of nosocomial human-to-human transmission.

For editorial comment see p 2308.

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METHODS

Laboratory Diagnosis

Patients suspected of HGA exposure were tested for serum IgG to *A phagocytophilum* using the IgG IFA kit (Focus Diagnostics, Cypress, California), screening at a 1:64 dilution and titrating if reactive.[15] Nested polymerase chain reaction (PCR) using blood DNA (QIAamp DNA Mini Kit, QIAGEN, Hilden, Germany) was used to detect *A phagocytophilum* DNA with *Anaplasma* and *Ehrlichia* genus-common and *A phagocytophilum* species-specific *rrs* primers (16S rRNA gene),[16] and *A phagocytophilum* species-specific *groEL* primers.[17] An *A phagocytophilum rrs* plasmid and DNA from healthy people or distilled water were used as controls. Positive reactions were confirmed by direct sequencing. Polymerase chain reaction was conducted in 2 independent laboratories, the National Institute for Communicable Disease Control and Prevention in Beijing, and at the Anhui Province Center for Disease Prevention and Control in Hefei city. Each laboratory used its own primers, reagents, and patient blood DNA. All samples were tested concurrently with negative and no template controls (water) under the same conditions. Polymerase chain reaction samples from healthy people and negative controls consistently had negative results.

To exclude other infections, serological, antigen detection, and PCR diagnostic tests were conducted. These included tests on blood from the first 3 to 5 days after onset for reverse transcription (RT)–PCR of PCR for nucleic acids of Lassa fever virus, Ebola virus, Marburg virus, Hantaan virus, Junin virus, yellow fever virus, Crimean-Congo hemorrhagic fever virus, coxsackievirus, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia* species, *Ehrlichia* species, *Richettsia* species, and *Orientia tsutsugamushi*.

Tests were also conducted on oropharygeal swabs from the first 3 to 5 days after onset for influenza A virus antigens, and by PCR for influenza A viruses, influenza B virus, and influenza virus subtype H5 nucleic acids. Tests for acute-phase serum were conducted to detect IgM and IgG to severe acute respiratory syndrome virus, as well as to detect IgM or IgM plus IgG antibodies by capture enzyme-linked immunosorbent assay against Bunyaviridae, Filoviridae, Lassa fever virus, Ebola virus, Marburg virus, Hantaan virus, Junin virus, yellow fever virus, and Crimean-Congo hemorrhagic fever virus.

Epidemiological Investigation

All contacts of the index patient, including patients with similar clinical presentations and healthy persons, were interviewed before laboratory diagnostic results were obtained. A possible case of HGA was defined as a patient with a clinically compatible illness (fever, headache, chills) and laboratory findings including thrombocytopenia and leukopenia but who lacked serological or molecular tests for *A phagocytophilum*. A confirmed case was defined as a patient with a clinically compatible illness (as above) and in keeping with the US Centers for Disease Control and Prevention (CDC) criteria (http://www.cdc.gov/ncphi/disss/nndss/casedef/ehrlichiosis_2008.htm) by either seroconversion, a 4-fold increase in *A phagocytophilum* IgG antibody titer in acute and convalescent sera, or a positive PCR result for both *A phagocytophilum rrs* and *groEL* confirmed by direct sequence analysis.[15]

Contact Questionnaire

All contacts of the index patient were asked to complete a questionnaire about their health status and profession; experience with tick bites; exposure to the index patient—where, when, and how they had contact; exposure to wild animals; extent of outdoor activity; exposure to the index patient's blood and respiratory secretions or to grossly bloody oropharyngeal secretions; presence of skin lesions during exposure; whether skin surfaces were washed after exposure; whether they were exposed to the patient's stool or urine; and the timing of these events. Health care workers were asked about their use of masks and gloves.

Ethical and Human Subjects Review

The study was approved by the ethics committee of China CDC, according to the medical research regulations of Ministry of Health, China. Oral informed consent was obtained from all study participants.

Statistical Analysis

All statistical calculations were performed using Epi Info 6.04d (http://www.cdc.gov/epiinfo). To identify specific exposure risk factors, retrospective cohort comparisons were evaluated by calculating attack rates, relative risk, and 95% confidence intervals and by Fisher exact test; significance was defined as a 2-tailed $P<.05$.

RESULTS

Index Case

A 50-year-old woman with a 1-day abrupt onset of sudden fever (39.2°C), headache, myalgia, arthralgia, dizziness, and malaise presented to the village clinic on October 31, 2006, and was treated with ribavirin, cephalothin, dexamethasone, and amidopyrine for 4 days. At 9 PM on November 3, she was admitted to the local hospital because of gum bleeding, facial edema, nausea, vomiting, and oliguria, a temperature of 39.7°C, blood pressure of 85/60 mm Hg, and pulse rate of 96/min; a rash was noted over her trunk. Laboratory testing showed leukopenia (white blood cell count, 3300/μL), thrombocytopenia (platelet count, 18×10^3/μL), elevated serum aspartate aminotransferase

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(629 U/L) and alanine aminotransferase (69 U/L), elevated creatinine (2.6 mg/dL), and elevated blood urea nitrogen (48 mg/dL) levels. Dipstick urinalysis revealed 3+ hematuria and 3+ proteinuria (protein, 3 g/L). (To convert aspartate aminotransferase and alanine aminotransferase to microkat per liter, multiply by 0.0167; creatinine to micromole per liter, by 88.4; and urea nitrogen to millimole per liter, by 0.357.)

Her condition progressively deteriorated, so she was transferred to a regional hospital at 11 AM, November 4. By 7 PM, the patient became obtunded, cyanotic, and purpuric and was bleeding from her nose and mouth. This extensive mouth and nose bleeding required frequent aspiration and contaminated the working area surfaces, health care workers, and family members who were with her. Family members assisted with patient care by wiping blood from the patient's mouth and nose, rinsing and reusing the same towels. By 7:38 PM the patient developed rapidly progressive dyspnea and worsening oxygen saturation and required endotracheal intubation. The patient remained hypoxic and hypotensive with multiorgan failure and copious bleeding from the nose and mouth. Despite all efforts, the patient died at 6:45 AM, November 5, 2006. The final diagnosis was hemorrhagic fever with renal syndrome, even though no IgG antibodies to Hantaan virus were detected. A postmortem examination was not performed, and no blood or tissue samples remained for retrospective laboratory testing.

Retrospective questioning of the patient's family revealed that she was bitten by a tick 12 days before onset of fever; she had killed several mice in her home 9 days before onset, and her husband had hunted and brought home "wild animal carcasses" 3 days before onset of illness. A timeline of events is shown in the FIGURE.

Nosocomial Cases of HGA

Between November 9 and 17, 2006, 9 patients were identified at the regional hospital with fever higher than 38.0°C (9 of 9 patients), myalgia (5 of 9), diarrhea (7 of 9), leukopenia (white blood cell count, 1200-3700/μL in 9 of 9), thrombocytopenia (platelets, 39-115 × 10^3/μL in all 9), and elevated serum aspartate aminotransferase and alanine aminotransferase (7 of 9) (TABLE 1). All patients had contact with the index patient, including 5 family members, 2 physicians, and 2 nurses who had accompanied or treated her between November 4 and 5 (Figure).

The initial secondary case experienced fever on November 9, 4 days after death of the index case, followed on November 11 by another patient, on November 12 by 3 patients, and on November 14 by 3 more patients. The last patient reported illness on November 17, 12 days after the death of the index patient. The patients were between 25 and 67 years (mean, 36.2 years), and 6 were men. All were previously healthy. The average incubation period was 7.8 days (range, 4-12 days). All had fever of at least 38.5°C for 1 to 6 days (mean, 4 days). Diarrhea was characterized as 1 to 3 loose stools per day persisting for 1 to 2 days. All patients had relative bradycardia. One patient developed acute respiratory distress syndrome as a complication of *Aspergillus* pneumonia and tuberculosis during his hospitalization. The other 8 patients were mildly affected, recovered, and were discharged in good health.

Contact Investigation

The index patient had contact with 63 persons after onset of illness: 21 family members and 42 health care workers. Of the 42 health care workers, 18 were from the local hospital, including 2 from the village clinic, and 24 were from the regional hospital. Of the 21 family members, 4 had contact with the index patient in only the local hospital, 13 only in the regional hospital, and 4 in both. The 9 secondary cases occurred among the 39 health care workers and relatives with patient exposure at the regional hospital, representing an attack rate of 23%. All 9 cared for the index case in the final 12 hours of her life while she was in the critical care unit and during the endotracheal intubation procedure. No one whose only contact with the index patient was before these 12 hours was infected.

Serological and Molecular Diagnosis

Anaplasma phagocytophilum IgG seroconversions were detected for all 9 patients, and a 4-fold IgG titer increase was observed in 7 of 9 patients (Table 1). Nested PCR using genus-common *rrs* and species-specific *rrs* and *groEL* primers identified *A phagocytophilum* DNA in the blood samples from all 9 patients when they were in the acute phase, whereas all healthy and template controls had negative test results. The identity of amplicons from each of the 9 patients was confirmed by sequencing; all *rrs* sequences (206 base pairs) were identical and all *groEL* sequences (446 nt) were identical (GenBank accession numbers: *rrs* EF211110-17 and EF473210; *groEL* EF47320108 and EF473209). Although the *rrs* sequences were identical to most other human-derived strains globally, sequences from *groEL* were identical to some US strains (Wisconsin and New York) but differed from *A phagocytophilum* in China (93.6%; EU008083), Germany (99.4%; AY281850), and California (99.7%; U96727). These data support the premise that a single clone was responsible for all of the 9 secondary cases. Although peripheral blood smears were examined for all 9 patients at the time of illness, no convincing evidence of *A phagocytophilum* morulae was observed. All RT-PCR, PCR, antigen detection, and IgM antibody detection tests for microbial and viral etiologies were negative.

Risk Factors

The exposure data implicate transmission at the regional hospital, permitting focus on risk factors in 39 individuals, including 24 health care workers and 15 family members (TABLE 2). Two family members who

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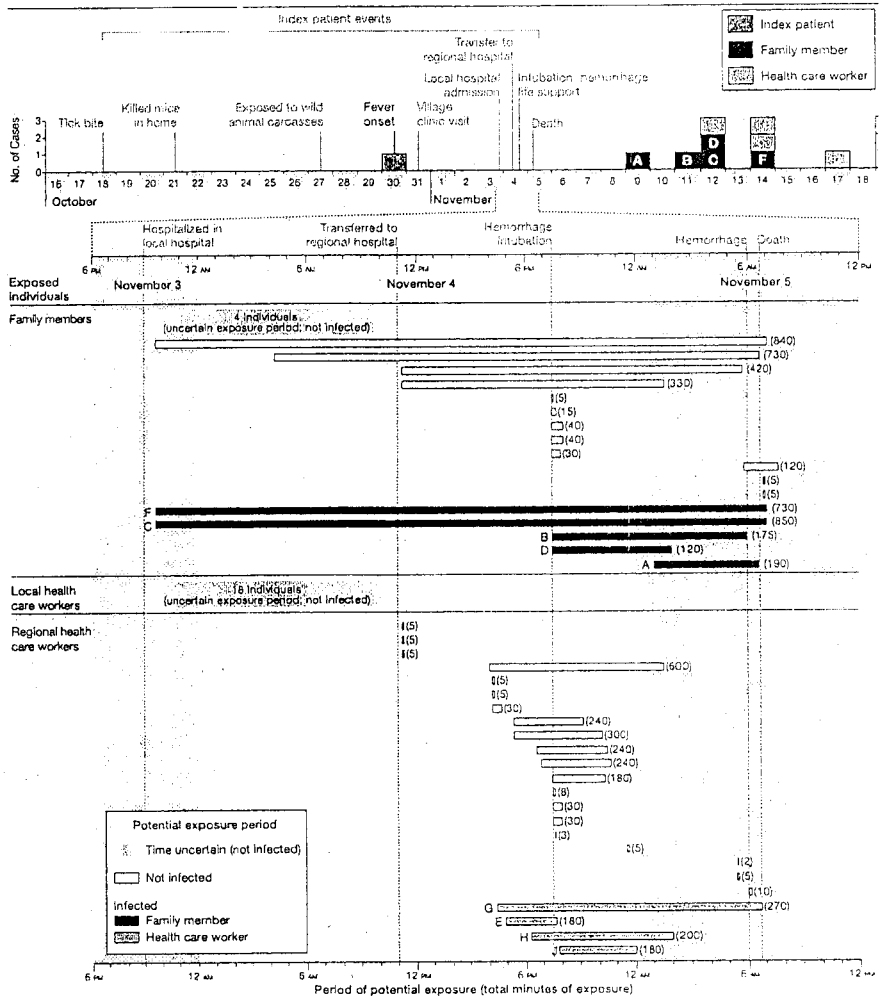
Figure. Timeline of Critical Events for the Index Patient and Direct Contact Intervals of Family Members and Health Care Workers With the Index Patient and Exposure of Patients With Nosocomial Human Granulytic Anaplasmosis



Top, epidemic curve showing progression of outbreak and key events during the index patient's illness. Bottom, each bar indicates the period of potential exposure while family members were in the hospital and while health care workers were assigned to care for the index patient. Duration of exposure in minutes is shown in parentheses and may not have occurred continuously during the exposure period. Capital letters designate the corresponding secondary cases in the top and bottom panels.

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had contact with the index case after her death were not included.

None of the 9 secondary cases reported tick bites, exposure to wild animals, or participation in hunting activity in the preceding 2 months, and only 1 reported recent outdoor activity. For all 9 secondary cases, culture serological, antigen detection, and nucleic acid detection studies for other infectious etiologies were negative.

Of 24 regional hospital health care workers who had contact with the index patient, 18 were on duty during the final 12 hours, and 4 of the 18 who were involved in the endotracheal intubation were infected. Of these 4, 3 were involved in endotracheal intubation and care during times of hemorrhage. Sixteen of 24 health care workers (67%) from the regional hospital wore masks and 9 of 24 (38%) wore gloves.

Of 17 family members who reported contact with the index patient at the regional hospital, 13 were present during endotracheal intubation, 5 of whom were infected. Of these 5 individuals, 3 reported blood contamination of skin and possible mucocutaneous exposures, suggesting direct contact with blood or respiratory secretions as the mechanism of transmission.

Among the 28 individuals who reported close contact (≤50 cm) with the index patient during the final 12 hours of her life, 9 were infected. In contrast, none of the 11 individuals who reported a physical distance of more than 50 cm from the index patient during the same time was infected. The index patient was exposed to 20 contacts for more than 2 hours, and 9 were infected, whereas none of 19 contacts exposed fewer than 2 hours was infected. All 9 infected patients reported contact with blood ($P = .002$) and 7 had contact with respiratory secretions (relative risk, 7.0; 95% confidence interval, 1.7-29.1; Table 2). Those persons with skin expsoure to blood ($P < .001$) or respiratory secretions ($P = .004$), or those with preexisting skin lesions or injuries followed by exposure to blood (relative risk, 3.6; 95% confidence interval, 1.1-7.6; $P = .02$) were significantly more likely to be infected (TABLE 3). Neither exposure to stool nor exposure to urine from the index case resulted in increased risk (0.6 and 1.1, respectively).

Table 1. Clinical, Laboratory, and Serological Findings of 9 Patients With Nosocomial Human Granulocytic Anaplasmosis

	Infected Patients								
	2	3	4	5	6	7	8	9	10
Clinical findings[a]									
Days hospitalized	19	21	19	19	19	19	21	19	36
Temperature ≥38.5°C	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Malaise	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chills	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Diarrhea	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes
Myalgia	Yes	No	Yes	Yes	No	No	Yes	No	Yes
Coryza/pharyngitis	No	No	No	No	Yes	Yes	No	No	Yes
Headache	Yes	No	No	No	No	Yes	No	No	No
Nausea	No	No	Yes	No	No	No	No	No	Yes
Edema	No	No	No	No	No	Yes	No	No	No
Gum bleeding	No	No	No	No	No	Yes	No	No	No
Dysuria	No	No	No	No	No	No	No	No	Yes
Laboratory values									
Lowest blood count, range of normal									
White blood cell, 4500-11 000/μL[a]	2600	1900	2700	2100	2500	1200	1800	3700	2200
Platelet, 150-350 × 10^3/μL	46	49	85	39	115	47	40	52	42
Highest liver enzymes, U/L									
AST, men <38; women <32	252	116	ND	77	ND	50	50	77	78
ALT, men <40; women <31	84	66	ND	64	ND	89	89	74	139
Anaplasma phagocytophilum IgG titers									
Days after onset									
0-7	<64	<64	<64	64	64	<64	<64	<64	<64
20-25	ND	64	64	128	128	128	ND	64	128
55-70	256	256	<64	256	256	<64	64	128	ND
A Phagocytophilum PCR results									
rrs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
groEL	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not done; PCR, polymerase chain reaction.
[a] Clinical findings that were documented during the course of each patient's hospitalization.

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COMMENT

Nine cases of *A phagocytophilum* infection were confirmed at the regional hospital in the Anhui Province of China in a 9-day period. All presented with HGA as described in North America and Europe[7] and fulfilled the US CDC laboratory criteria for the diagnosis of HGA.[15] The most remarkable aspect of these cases was that transmission was very unlikely to be tick-borne, but was closely associated with blood or respiratory secretion exposure from an index patient who died of a fulminant febrile illness with hemorrhage. Although the index patient can only be categorized as a possible case, clinical and historical support for the diagnosis of HGA is strong. She had a tick bite within the known incubation period and had a clinical presentation compatible with severe HGA.[4] Moreover, the epidemiological investigation of exposed individuals with HGA implicates her as the index case. Unfortunately, no tissue or serum sample is available to confirm retrospectively her diagnosis.

Human granulocytic anaplasmosis and human monocytic ehrlichiosis were initially identified with presentations now recognized as relatively uncommon for their natural histories.[16,19] Infection can be severe, with intensive care unit admission required in 7% of patients and fatalities occurring in up to 1%, yet most infections are sporadic and probably self-limited.[4] Based on the mild to moderate severity observed in 8 of the 9 secondarily infected patients, Chinese HGA conforms to the spectrum of clinical severity observed in North America.[4,7,15] The fatal outcome in the index case is clinically similar to that observed for other HGA fatalities, including exsanguination with sepsis syndrome possibly relating to cytokine overproduction, opportunistic infections, and increased HGA severity in the setting of preexisting medical conditions such as diabetes mellitus.[7,20]

A phagocytophilum transmission in China and Asia is predicated on the presence of this zoonotic agent in vector ticks and vertebrate hosts. Although studies in Asia are limited, at least 8 have examined *A phagocytophilum* infection of ticks, including 2284 *Ixodes persulcatus* ticks, of which 4.4% carried *A phagocytophilum* DNA, a prevalence similar to that in European and North American *Ixodes* species ticks.[12,14,21-27] Likewise, 9% and 24% of *Apodemus* species field mice in northern China and Korea, respectively, and 64% of *Crosidura lasiura* shrews in Korea are infected.[13,21,24,28,29] Although no proven cases of HGA have been previously identified in China, at least 1 study describes *A phagocytophilum* DNA in the blood of 4 Chinese patients with tick bites,[14,30] and seroepidemiological investigations demonstrate that 2% to 9% of febrile patients in Korea,[10,11] and between 0.5% and 6% of healthy Chinese residents have *A phagocytophilum* antibodies.[31]

Rare examples of nontick transmission of HGA exist in the literature and include direct exposure to deer blood,[32] transfusion,[33] and transplacental transmission.[34] Similarly, under the proper circumstances other rickettsial infections are transmissible via aerosol, direct contact with mucous

Table 2. Risk Factors for Acquisition of Human Granulocytic Anaplasmosis Among 39 Contacts Exposed to Index Patient While at the Regional Hospital

Exposure to Index Patient	No./Total (%)			
	Attack Rate With Exposure Factor	Attack Rate Without Exposure Factor	Relative Risk (95% Confidence Interval)[a]	*P* Value[b]
≤50 cm to nose and mouth	9/28 (32.1)	0/11 (0)		.04
>2 h	9/20 (45.0)	0/19 (0)		.001
During or after intubation	9/30 (30.0)	0/9 (0)		.09
During massive hemorrhage period	4/9 (44.4)	5/30 (16.7)	2.7 (0.9-7.9)	.17
Any direct blood contact	9/22 (40.9)	0/17 (0)		.002
Direct respiratory or tracheal secretion contact	7/13 (53.8)	2/26 (7.7)	7.0 (1.7-29.1)	.003

[a] Infinite or not able to be calculated.
[b] Fisher exact test (2-tailed).

Table 3. Risk Factors for Human Granulocytic Anaplasmosis Associated With Direct Exposure to Index Patient's Blood and Respiratory Secretions

Exposure Factor	No./Total (%)			
	Attack Rate With Exposure Factor	Attack Rate Without Exposure Factor	Relative Risk (95% Confidence Interval)[a]	*P* Value[b]
Any direct blood contact during hemorrhage				
On skin	9/12 (75.0)	0/10 (0)		<.001
Open wounds or abrasions	4/4 (100.0)	5/18 (27.8)	3.6 (1.1-7.6)	.02
Not washed timely	4/8 (50.0)	5/14 (35.7)	1.4 (0.5-3.8)	.66
Direct respiratory or tracheal secretion contact				
On skin	7/8 (87.5)	0/5 (0)		.004
Open wounds or abrasions	4/4 (100.0)	3/9 (33.3)	3.0 (1.2-7.6)	.07
Not washed timely	3/6 (50.0)	4/7 (57.1)	0.9 (0.3-2.4)	>.99

[a] Infinite or not able to be calculated.
[b] Fisher exact test (2-tailed).

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membranes or conjunctivae, or mechanical fomite transmission.[35-38] Direct exposure to small blood volumes probably carries a low risk because experimental and natural infections of white-tailed deer result in only low-level bacteremia.[39] However, it is possible that this low risk may be offset by large volumes of animal blood and tissues, such as those to which butchers are exposed.

Another factor related to transmissibility is the blood burden of *A phagocytophilum*, which appears to increase with immunosuppression resulting in absolute infected neutrophil counts as high as $2.7 \text{ to } 5.9 \times 10^9$/L.[18,40] It is unclear to what degree the sustained dexamethasone treatment of the index case contributed to transmission. The final consideration is the likelihood of health care worker and family member exposure to sufficient volumes of infectious body fluids to account for transmission. It is not unusual for occupational blood exposure to occur among those caring for patients with hemorrhage or during procedures such as intubation or surgery, for which the relative risk is 3 to 4 times higher than for other medical specialties.[41] In western societies, most family members are excluded from these events and health care workers are increasingly protected by training and barriers such as gloves, gowns, and masks.[42] However, retrospective questioning of our cases clearly indicated that both family members and health care workers not only participated in these events but were unlikely to use gloves and so reported that body surfaces were contaminated by potentially infectious fluids. Moreover, many participants did not acknowledge use of postexposure precautions, such as hand and skin washing.

Although it is likely that routine blood and body fluid precautions will protect against such future events, strict adherence to protective protocols is mandatory even if communicability is deemed unlikely. The lessons of this study remain relevant to the daily hospital and health care unit operations to prevent any additional nosocomial outbreaks of HGA. Moreover, as China advances into its future, it must also now become prepared to deal with the increasing threat that tick-borne rickettsial pathogens have been already brought to the United States and Europe.

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> Nothing is more estimable than a physician who, having studied nature from his youth, knows the properties of the human body, the diseases which assail it, the means which will benefit it, exercises his art with caution, and pays equal attention to the rich and the poor.
> —Voltaire (1694-1778)

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HUMAN GRANULOCYTIC ANAPLASMOSIS IN CHINA

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