医薬品 研究報告 調査報告書

			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 4. 27	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況		公表国
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		ABC Newsletter #15. 2010 Apr 23; 15.	ニュージーランド・オーストラリア

研究報告の概要

○ニュージーランドの血液銀行は慢性疲労症候群(CFS)の既往を持つ供血者の供血延期を開始し、オーストラリア当局は、供血ガイドラインの見直しを行っている
ニュージーランドの決定は、前立腺癌と関連性があるXMRVが、健常集団と比較してCFS患者の血中に非常に多く認められたという米国の試験を受けてなされた。他の科学者は、この結果を確認することができなかったが、米国保健当局は、CFSとXMRV間の関連の可能性について調査を行っており、カナダ血液サービスはすでにCFSの診断を受けた供血者からの供血を無期限延期としている。一方、オーストラリア赤十字血液サービスは、独自にリスク分析を行い、完全に回復するまでのCFS患者からの供血を延期することを現行のガイドラインで求めている。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
ニュージーランドの血液銀行は慢性疲労症候群の既往を持つ供血者の供血延期を開始し、オーストラリア当局は、供血ガイドラインの見直しを行っているとの報告である。 XMRVの病原性の有無は未だ定かではない。XMRVはマウス白血病ウイルスと類縁な脂質膜を持つ大型RNAウイルスである。この性状から本製剤の製造工程でウイルスが不活化・除去されると期待しうることから、本製剤の安全性は確保されていると考える。	注目すべきウイルスとして今後も引き続き、新たなウイルス等に関する情報の収集に努める。

GLOBAL NEWS

Members of the Dutch Parliament met last week to discuss the cost of blood in that country, and one of their topics was the transparency of operations at Sanquin, the foundation responsible for managing the blood supply in the Netherlands. Last August, a benchmark report compared the price of blood products in a number of European countries, and it concluded that prices in the Netherlands were higher than those in Ireland, Belgium, France, and Finland. In response, the Minister of Health wrote a letter in which he indicated a number of steps that would improve transparency at Sanquin, and he also announced a follow-up study that would focus on the current law on blood supply. The meeting this week was also attended by representatives from patient organizations, donor organizations, physicians, the Plasma Proteins Therapeutics Association, the Dutch Red Cross, and Sanquin. (Source: *PPTA Leadership Briefing*, 4/16/10) ♦

INFECTIOUS DISEASE UPDATES

CFS

Blood banks in New Zealand will begin deferring any potential blood donor who has a record of chronic fatigue syndrome (CFS), and officials in Australia are reviewing donation guidelines there. The decision in New Zealand was made in the wake of a US research study that found xenotropic murine leukemia virus-related virus (XMRV), a virus that has been linked to prostate cancer, in the blood of far more people with CFS than the healthy population. Other scientists have been unable to confirm those results, but health authorities in the US are investigating the possible link between CFS and XMRV, and Canadian Blood Services (CBS) has already instituted a lifetime deferral for potential blood donors who have been diagnosed with CFS (see *ABC Newsletter*, 4/9/10). The national medical director for New Zealand's blood banks, Peter Flanagan, said the New Zealand Blood Service (NZBS) reviewed the issue at a meeting held earlier this month and decided that the present exclusion of blood from people still suffering from CFS or patients who had been diagnosed in the past two years "should be extended to also exclude donors who report ever having been diagnosed with chronic fatigue syndrome." He admitted that the decision was made despite a lack of good scientific data on the issue. Meanwhile, the Australian Red Cross Blood Service is conducting its own risk analysis, and it says existing donor guidelines require people with CFS to defer giving blood until they make a full recovery. It said it collects more than 500,000 blood donations each year, but only 70 donors with CFS have been deferred in the past two years. The blood service said in a statement that it "currently defers donors who suffer from [CFS and] before we can accept their blood again, they need to bring us a letter from their treating physician advising us that they are completely recovered." (Sources: www.stuff.co.nz, 4/21/10; www.heraldsun.com.au, 4/20/10) ♦

We Welcome Your Articles

We at the *ABC Newsletter* welcome freelance articles on any subject relevant to the blood banking community. Writers are encouraged to submit short proposals or unsolicited manuscripts of no more than 1,100 words. While ABC cannot pay for freelance pieces, the writer's name and title will be included at the end of the story, brief news item, or commentary. If proposing a story, please write a few paragraphs describing the idea and sources of information you will use, your present job and background, and your qualifications for writing on the topic. ABC staff cannot guarantee all stories will be published, and all outside writing will be subject to editing for style, clarity, brevity, and good taste. Please submit ideas and manuscripts to Editor Robert Kapler at rkapler@americasblood.org. You will be sent a writer's guide that provides information on style conventions, story structure, deadlines, etc.

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	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm223277.htm	公表国	
販売名（企業名）	－			http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm223232.htm	米国	

研究報告の概要

米国食品医薬品局生物製剤評価・研究センターおよび米国国立衛生研究所臨床センターの研究者が、慢性疲労症候群（CFS）と診断された患者37名と健常血液ドナー44名から採取した血液サンプルを検査したところ、CFS患者37名のうち32名のサンプル（87%）、および健常血液ドナー44名のうち3名のサンプル（7%）において複数の異なるマウス白血病ウイルス（MLV）遺伝子配列を特定した。本研究は、MLV様ウイルスの遺伝的変異体であるXMRV（異種指向性マウス白血病ウイルス関連ウイルス）がCFS患者の血液中に存在することを明らかにした過去の研究結果を裏付けており、CFSの診断と血液中のMLV様ウイルス遺伝子配列の存在との間に強い関連性があることを実証している。さらに、ごく一部の健常血液ドナーにおいてMLV様ウイルス遺伝子配列が検出された。CFSとの統計的関連性は強いとはいえ、これらのレトロウイルスがCFSの原因であることを証明するものではない。XMRVやその他のMLV関連ウイルスがCFSを引き起こす可能性を確定するためには、今後も研究を継続する必要がある。

「MLVやXMRVは血液製剤や組織由来製剤によって伝播するか？」については、これらウイルスが血液やヒトの組織によって伝播する可能性があるかどうか、そして、これらのウイルスが疾患を引き起こすかどうかを調査するためには追加研究を行う必要がある。FDA、NIH、CDCおよびその他の科学機関の研究者は、血液中のXMRVやMLV関連ウイルスの検出用として多くの研究所が使用している試験の能力を検証するため、複数の研究を実施中である。これらの研究は、XMRVが血液や組織のレシピエントに伝播する可能性だけでなく、XMRVと疾患との関連性についてもより詳しく調べるために、感受性が高く、特異的なXMRV試験の開発や標準化を目的としている。

報告企業の意見	今後の対応
慢性疲労症候群の原因である可能性があるXMRVやその他のMLVの血液からの検出に関する情報である。 現時点で疾患の原因として特定されておらず、検出法についても検討中との情報であった。	今後ともXMRVやその他のMLVに関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
［患者への説明］
本剤の投与にあたっては、疾病の治療における本剤の必要性とともに、本剤の製造に際し感染症の伝播を防止するための安全対策が講じられているが、ヒト血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。

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U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

News & Events

FDA Note to Correspondents

For Immediate Release: August 23, 2010
Media Inquiries: Shelly Burgess, 301-796-4651, shelly.burgess@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

Study: Presence of murine leukemia virus related gene sequences found in CFS patients

Researchers have found murine leukemia viruses (MLV) related gene sequences in blood samples collected from patients diagnosed with chronic fatigue syndrome (CFS) and some healthy blood donors, according to a study published online today by the scientific journal Proceedings of the National Academy of Sciences (PNAS).

Investigators from the U.S. Food and Drug Administration's Center for Biologics Evaluation and Research and the National Institutes of Health Clinical Center, in collaboration with a physician scientist at Harvard Medical School, examined blood samples from 37 patients diagnosed with CFS and from 44 healthy blood donors.

MLV is a type of retrovirus known to cause cancer in mice. Several different MLV gene sequences were identified in samples from 32 of the 37 patients with CFS (87 percent) and 3 of the 44 (7 percent) healthy blood donors. Investigators performed DNA sequencing on all positively amplified samples to confirm MLV like gene sequences.

This study supports a previous investigation [Lombardi et al. Science October 23, 2009 326: 585] that showed XMRV, a genetic variant of MLV-like viruses, to be present in the blood o people with CFS. The study demonstrates a strong association between a diagnosis of CFS and the presence of MLV-like virus gene sequences in the blood. The study also showed tha MLV-like viral gene sequences were detected in a small fraction of healthy blood donors. Although the statistical association with CFS is strong, this study does NOT prove that these retroviruses are the cause of CFS. Further studies are necessary to determine if XMRV or other MLV-related viruses can cause CFS.

A previous study, published in 2009, reported finding XMRV infections in a high percentage of CFS patients and a small percentage of healthy blood donors. However, several other studies from the United States (including a recent report from the Centers for Disease Control and Prevention), the United Kingdom, and the Netherlands have found no evidence of XMRV or other MLV-like viruses in the blood of people with CFS.

For more information:

- Murine Leukemia Virus Gene Sequence Study - Questions and Answers[1]
- Xenotropic Murine Leukemia Virus-related Virus - Overview[2] (CDC)
- Xenotropic Murine Leukemia Virus-related Virus - Questions and Answers[3] (CDC)

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Links on this page:

1. http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm223252.htm
2. http://www.cdc.gov/xmrv/index.html
3. http://www.cdc.gov/xmrv/questions-answers.html

U.S. Food and Drug Administration

Vaccines, Blood & Biologics

New study on the detection of murine leukemia virus-related virus gene sequences in the blood of patients with chronic fatigue syndrome (CFS) and healthy blood donors - Questions and Answers

Questions and Answers

1. What are murine leukemia viruses?

Murine leukemia viruses (MLV) are retroviruses known to cause cancer in certain mice. In 2006, investigators found that a type of MLV, called xenotropic murine leukemia virus-relate virus (XMRV), could potentially infect humans. XMRV is one of a number of MLVs that appear to be transmitted to humans.

2. What is CFS?

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms and the absence of other causes. It's unknown what causes CFS.

3. Has MLV or XMRV previously been associated with CFS or other disease?

A previous study, published in the journal [Lombardi et. al. *Science* October 23, 2009 326: 585], reported finding XMRV in a high percentage of CFS patients and a small percentage of healthy blood donors. However, other studies conducted in the U.S., Netherlands, and UK did not detect evidence of XMRV or other MLV-related viruses in CFS patients.

XMRV was first identified in tissue samples from some prostate cancer patients in 2006. However, one subsequent study failed to find XMRV in prostate cancer tissues, and another study found the virus only rarely in such tissues.

4. What did the new study evaluate?

Investigators from the Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research, the National Institutes of Health (NIH) Clinical Center, and Harvard Medical School have published a study in the scientific journal *Proceedings of the National Academy of Sciences* that examines the presence of MLVs in blood collected from two groups -- patients diagnosed with CFS and healthy blood donors.

This study tested blood samples collected from the New England area in the mid-1990s from 37 patients diagnosed with CFS, as well as samples from 44 healthy blood donors collectec in the Clinical Center Blood Bank, NIH, between 2003 and 2006. Investigators performed DNA sequencing on each sample that produced positive product for verification of MLV-like gene sequences. Diverse MLV gene sequences, similar to that of the recently discovered XMRV, were identified in samples from 32 of the 37 patients with CFS (86.5%) and 3 of the 44 (6.8%) healthy blood donors that were tested.

Follow-up samples were collected from 8 of the CFS patients in 2010, and 7 of these again tested positive for MLV-like gene sequences.

5. What did the new study conclude?

This study supports a previous investigation[Lombardi et al. *Science* October 23, 2009 326: 585]that showed XMRV, a genetic variant of MLV-like viruses, to be present in the blood of people with CFS. The study demonstrates a strong association between a diagnosis of CFS and the presence of MLV-like virus gene sequences in the blood. The study also showed tha MLV-like viral gene sequences were detected in a small fraction of healthy blood donors. Although the statistical association with CFS is strong, this study does NOT prove that these retroviruses are the cause of CFS. Further studies are necessary to determine if XMRV or other MLV-related viruses can cause CFS.

6. Are there studies that support different conclusions?

Some previous studies from the United States (including a study by the Centers for Disease Control and Prevention), the United Kingdom and the Netherlands reported finding no evidence of XMRV or other MLV-related infections in people with CFS. These different findings could be caused by a variety of factors (for example, difference in study populations), and underscore the need for additional studies and standardized methods.

7. Can MLV or XMRV be transmitted by blood or tissue products?

Additional research is needed to investigate the possibility that these MLV-related viruses and XMRV may be transmitted by blood or human tissue and are capable of causing disease. Investigators at FDA, NIH, CDC and other scientific institutions are in the process of conducting studies to verify the capabilities of the tests used by the different laboratories for the detection of XMRV or MLV-related viruses in blood. These studies are intended to develop and standardize a highly sensitive and specific XMRV test to better study its association with disease, as well as the possibility that XMRV can be transmitted to blood or tissue recipients.

8. What are the implications for blood donors?

At present, FDA does not have a donor policy specific to XMRV or other MLVs. There is currently no evidence that XMRV or MLVs are transmitted by transfusion in humans or that XMR or other MLVs cause human disease. FDA regulations require that donors be in good health at the time of donation.

9. Does FDA agree with the AABB recommendation to discourage donation by people with history of CFS?

FDA does not object to the AABB recommendation. The AABB recommendation is consistent with a long-standing position of the Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America that individuals with CFS voluntarily should not donate blood.

10. How are the differences between the CDC and FDA study results being evaluated?

Differences in the results could reflect differences in the patient populations that provided the samples. Alternatively, undefined differences in the method of sample preparation could be contributing to the discordant test results. All of the scientists involved are working collaboratively to design experiments to quickly answer this scientifically puzzling question. An independent investigator at the National Heart, Lung, and Blood Institute (NHLBI) set up a test set of 36 samples, including known positives and presumed negatives. Both the FDA/NIH and CDC labs participated in this test, and the results showed that both labs were able to detect XMRV present at low levels in blinded samples. Additionally, the CDC laboratory provided 82 samples from their published negative study to FDA, who tested the samples blindly. Initial analysis shows that the FDA test results are generally consistent with CDC, with no XMRV-positive results in the CFS samples CDC provided (34 samples were tested, 31 were negative, 3 were indeterminate).

11. What do these findings mean to CFS patients and clinicians who treat them?

Although this study found MLV-like viral gene sequences in a high percentage of CFS patients, this does not prove that these retroviruses are the cause of CFS or of any other disease. Moreover, other studies have not found evidence of such retroviruses in patients with CFS. Further studies are necessary to determine if XMRV or other MLV-like viruses are reproducibly associated with CFS, and if so whether the virus is a causative agent or a harmless co-traveler. The different findings from various studies reinforce the need for more research--including careful analysis of other cohorts of CFS patients from different geographic regions, studies of larger populations of healthy people, and testing of transmissibility of the agents through blood transfusions in animal models. FDA, NIH, and CDC have and will continue to collaborate with other agencies and groups involved in this research.

Links on this page:

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	新医薬品等の区分	総合機構処理欄
				2010. 5. 18	該当なし	

一般的名称	人血清アルブミン	研究報告の公表状況		公表国
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		ProMED 20100513.1557, 2010 May 13, 情報源: WHO Global Alert and Response (GAR) Disease Outbreak News	WHO

研究報告の概要

○南アフリカのリフトバレー熱(RVF)
2010年5月10日の時点で南アフリカ保健省は、18人の死者を含む186人のRVF症例を報告している。主要な感染経路は、感染した家畜の血液や組織に触れることであるが、蚊に刺されることも感染原因となる。世界保健機関(WHO)は、南アフリカへの旅行に対して規制の勧告は行っていないが、特に農場や動物保護区に行く者は、動物組織や血液との接触を避け、未殺菌、非加熱ミルクや生肉の摂取をしないことを勧めている。そして、全旅行者に対し、長袖長ズボンの着用や防虫剤、蚊帳を使用するなどして、蚊や吸血昆虫に刺されないよう注意を呼びかけている。また、ドイツ保健当局は、4月に、南アフリカ旅行から帰国したドイツ人のRVF検査確定症例を報告したが、その後の追加検査により、この症例はRVFではなくリケッチア感染であったと報告した。

使用上の注意記載状況・その他参考事項等

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赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
南アフリカでは2010年5月10日現在、18名の死者を含む186名のリフトバレー熱症例が報告されているとのことである。 リフトバレー熱ウイルスはブニヤウイルス科の脂質膜を持つウイルスである。これまで、本製剤によるリフトバレー熱ウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考える。	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。

World Health Organization

Rift Valley fever in South Africa- update 2

12 May 2010 -- On 11 May 2010 Bernhard-Nocht-Institute for Tropical Medicine in Germany reported that additional laboratory analyses conducted both in Germany and South Africa on the German tourist who was preliminarily diagnosed with Rift Valley Fever (RVF) following her return from South Africa, was in-fact infected with Rickettsia and not with RVF virus.

Rickettsia, commonly known as tick fever is a bacterium which can cause many diseases that are transmitted by blood-sucking parasitic arthropods such as fleas, lice and ticks. Symptoms of rickettsial infections include rash, fever, and flulike symptoms. African tick bite fever is caused by *rickettsia africae* and tends to be a milder illness, with less prominent rash and little tendency to progress to complicated disease. All rickettsial diseases respond to treatment with antibiotics such as doxycycline and tetracycline

As of 10 May, the Government of South Africa has reported 186 confirmed cases of RVF in humans, including 18 deaths, in Free State Province, Eastern Cape Province, Northern Cape Province, Western Cape, and North West Province. RVF is a viral disease that primarily affects animals (such as cattle, buffalo, sheep, goats and camels). The disease can also affect humans. The main mode of transmission of RVF is via direct or indirect contact with the blood or organs of infected animals. Human infections have also resulted from the bites of infected mosquitoes. There is evidence that humans may become infected by ingesting the unpasteurized or uncooked milk of infected animals.

WHO advises no international travel restriction to or from South Africa. However, WHO recommends that visitors to South Africa, especially those intending to visit farms and/or game reserves, avoid coming into contact with animal tissues or blood, avoid drinking unpasteurized or uncooked milk or eating raw meat.

All travelers should take appropriate precautions against bites from mosquitoes and other blood-sucking insects (including the use of insect repellents, wearing long-sleeved shirts and trousers, and sleeping under mosquito nets). Travel medicine professionals and travel medicine services should be aware of the current RVF situation in South Africa in order to provide advice and care accordingly.

For more information

Department of Health, South Africa

National Institute for Communicable Diseases (NICD)

Robert Koch Institute

Rift Valley fever: WHO fact sheet

Protection against vectors [pdf 5-48kb]
International Travel and Health

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	新医薬品等の区分	総合機構処理欄
				2010. 5. 17	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		Amitai Z, Bromberg M, Bernstein M, Raveh D, Keysary A, David D, Pitlik S, Swerdlow D, Massung R, Rzotkiewicz S, Halutz O, Shohat T. Clin Infect Dis. 2010 Jun 1;50(11):1433-8.	イスラエル

研究報告の概要

○イスラエル中央部の都市の学校における大規模Q熱アウトブレイク

背景:2005年6月28日に、イスラエル中央部の都市部の、全寮制高校の生徒および職員322名に多くの発熱性疾患症例が報告された。その後の調査で、その2週間前のQ熱アウトブレイクが確認された。

方法:Q熱疾患の危険因子を特定するため、症例対照研究を行った。環境サンプルを採取し、*Coxiella burnetii*(*C.burnetii*)の感染源と伝播経路を確認した。

結果:2005年6月15日～7月13日の間に、303名中187名(62%)が体調の不具合を報告した。検査を実施した164名中144名(88%)に、*C.burnetii*感染の血清学的証拠が明らかとなった。学生であること、学校の食堂で定期的に食事をしたこと、6月の宗教上の休日期間ならびにその前の週末に寮生活を行ったことは、いずれもQ熱感染の重大なリスク因子であった。PCR検査により学食の空調から*C.burnetii* DNAが検出され、空調を介して病原体に空気感染したことが示唆された。

結論:インフルエンザのオフシーズンにおいて、インフルエンザ様疾患のアウトブレイクの調査を行う際には、*C.burnetii*感染を強く疑うことが必要である。

報告企業の意見	今後の対応
イスラエル中央部の都市の全寮制高校における大規模Q熱アウトブレイクの原因調査を行ったところ、学食の空気調節システムから*Coxiella burnetii* DNAが検出され、空気調節システムによる空気感染が示唆されたとの報告である。	日本赤十字社では、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。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(17)

MAJOR ARTICLE

A Large Q Fever Outbreak in an Urban School in Central Israel

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Background. On 28 June 2005, numerous cases of febrile illness were reported among 322 students and employees of a boarding high school located in an urban area in central Israel. Subsequent investigation identified a large outbreak of Q fever which started 2 weeks earlier. We describe the investigation of this outbreak and its possible implications.

Methods. We conducted a case-control study to identify risk factors for Q fever disease. Environmental sampling was conducted to identify the source and the mode of transmission of *Coxiella burnetii*, the infectious agent.

Results. Of 303 individuals, 187 (62%) reported being ill between 15 June and 13 July 2005. Serological evidence for *C. burnetii* infection was evident in 144 (88%) of the 164 tested individuals. Being a student, dining regularly at the school dining room, and boarding at school during a June religious holiday and the preceding weekend were all significant risk factors for contracting Q fever. *C. burnetii* DNA was detected using polymerase chain reaction on samples from the school dining room's air conditioning system, supporting contribution of the air conditioning system to the aerosol transmission of the infectious agent.

Conclusions. We report a large outbreak of Q fever in an urban school, possibly transmitted through an air conditioning system. A high level of suspicion for *C. burnetii* infection should be maintained when investigating point source outbreaks of influenza-like disease, especially outside the influenza season.

Q fever is a worldwide-distributed bacterial zoonosis caused by *Coxiella burnetii*. The most common reservoirs are domesticated ruminants, but other mammals, birds, and arthropods are also naturally infected [1, 2]. *C. burnetii* is often excreted in milk, urine, and feces of infected animals and is present in high numbers within the amniotic fluid and the placenta during parturition [2]. Viable bacterium may be present in the soil for months or years, and inhalation of contaminated aerosols is the major mode of transmission [2, 3]. In humans, acute infection may present as a self-limited influenza-like illness, hepatitis, and/or atypical pneumonia [4, 5]. About 60% of infections may be asymptomatic [4], especially among female persons [4, 6] and children aged <15 years [7].

Most reports of Q fever outbreaks are from rural areas and are associated directly or indirectly with farms or farm animals [2, 3]. Nevertheless, urban outbreaks have been described after exposure to slaughterhouses [8, 9], animal research laboratories [10], parturient cats [11], contaminated straw [12], and following windborne spread of *C. burnetii* from farmlands [13]. In some urban outbreaks, the source of the infection was never determined [14, 15].

In Israel during 1998–2004, the average annual incidence of Q fever was 0.6 cases 100,000 persons (20–70 cases per year) (Israel Ministry of Health, personal communication). Only a few outbreaks were reported, with the majority occurring in rural or adjacent areas

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following outbreaks of Q fever in livestock, and all were relatively limited in scale [15–17].

We report a very large urban outbreak of Q fever in a boarding high school in Israel. This outbreak is unique in its magnitude and setting, because there was no proximity to livestock or their products.

METHODS

Epidemiologic investigation. On 28 June 2005, 2 reports of a possible outbreak of febrile illness in a religious boarding high school in the center of the largest urban area in Israel were received at the Tel Aviv District Health Department. Initial investigation identified a large outbreak of influenza-like illness which started 2 weeks earlier, had already peaked, and was later confirmed to be due to acute *C. burnetii* infection.

We conducted a case-control study to identify risk factors for contracting Q fever. All school students and employees were asked to fill out a short questionnaire, including demographic characteristics, medical history, school boarding history, in-school dining habits, and contact with pets at school. Those who reported being ill during the previous 2 months were asked to specify the date of onset of illness, duration, symptoms and signs, and use of health services. All students and employees were referred for Q fever testing. In several cases, primary practitioners were contacted for additional information. Regional and reference laboratories were queried about additional Q fever cases from the school surroundings during the same time period.

Human serologic testing. Serum samples were tested for antibodies to *C. burnetii* with use of several laboratory methods. Indirect immunofluorescent assays were performed at the Israeli Reference Laboratory for Rickettsial Diseases in Ness-Ziona [18]. Complement fixation tests were performed by the Tel Aviv Medical Center's Clinical Virology Unit with use of the standard complement fixation microtiter method (Lennette and Schmidt) [19]. Qualitative enzyme immunoassays were performed by Clalit Health Services community laboratories with use of the PANBIO Q fever DIP-S-TICKS test. Quantitative tests were performed in various laboratories in western Europe.

Case definitions. A "clinical case" was defined as a patient with symptoms compatible with Q fever, with illness onset from 1 June through 31 July 2005 and no other likely cause for his/her illness.

A "confirmed case" was defined as anyone with immunoglobulin (Ig) M and IgG indirect immunofluorescent assay titers $\geq$100 to phase II antigen, or IgG titers $\geq$800 and IgM titers <100 in a "clinical case" that was tested at least 4 months after illness [8, 20]. Using complement fixation test, a phase II titer $\geq$256 was considered to represent a confirmed case.

A "probable case" was defined as phase II IgM titer $\geq$100 and IgG titer <100 by indirect immunofluorescent assay, a phase II titer <256 but $\geq$32 by complement fixation test, or a positive or borderline laboratory result of qualitative enzyme immunoassay or other quantitative tests. A "possible case" was defined as a "clinical case" with no serologic testing. A "non-case" (control) was defined as negative serologic results for Q fever.

Environmental and veterinary investigation. A comprehensive environmental inspection of the school grounds was conducted by environmental health inspectors, a veterinarian, and an air-conditioning system specialist for a possible source of infection. Two weeks after the last reported case, environmental samples were collected from the air-conditioning systems. The samples included 8 gauze pads that were used to swab the dining room's and synagogue's air-conditioning systems and 4 samples from the 2 fiberglass filters from the inlet of the dining room's air-conditioning unit. All samples were prepared for DNA extraction.

Serum samples of male and female feral cats trapped in the Tel Aviv area for routine neutering by municipality veterinarians were tested for Q fever by complement fixation test [21]. Samples that reacted nonspecifically were retested by indirect immunofluorescent assay (*C. burnetii* spot IF; BioMérieux). In addition, endometrial tissue proximal to the cervix was collected from each of the spayed female cats and was processed for DNA extraction.

DNA was extracted by use of the DNeasy DNA purification kit (Qiagen). Polymerase chain reaction (PCR) assay was performed as described by Stein and Raoult [22].

All tests were performed in the Kimron Veterinary Institute (Bet Dagan, Israel). Filter samples from the dining room's air-conditioning system were also sent to the Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention.

Data analysis. Data were analyzed with Excel (Microsoft) and SPSS, version 10 (SPSS), software. The prevalence of possible risk factors for contracting Q fever in cases (confirmed cases with and without probable cases) and controls was compared using the Fisher's exact test. Odds ratio (ORs) and 95% confidence intervals (95% CI) were calculated. All significant risk factors were tested for colinearity.

RESULTS

The school setting. The school, a religious boarding high school for boys, is located in central Tel Aviv in a densely populated area. During June 2005, 271 students aged 14–20 years (mean age $\pm$ standard deviation, 16.9 $\pm$ 1.5 years) and 51 employees attended the school. Eighty-four students boarded at the school regularly. Some of the others, who resided in different cities in Israel, stayed over during certain weekends and holidays. A weekend occurred on 10–11 June 2005, and 12–13 June was a special Jewish holiday (Shavuot). The em-

ployees were mainly men (84%) aged 33–92 years (mean age ± standard deviation, 55.4 ± 13.8 years) from various cities in central Israel.

Outbreak description. Of the 322 individuals who attended the school during June 2005, 187 reported being ill from 1 June through 31 July 2005, including 179 (96%) students and 8 (4%) employees (19 individuals were excluded from further analyses because of lack of information). The clinical attack rate was 62% (70.5% and 16% among students and employees, respectively). Attack rates were similar in different grades and ranged between 67% and 74.5%.

Information on date of illness onset was available for 155 (83%) individuals. The epidemic curve (Figure 1) correlates to a point source epidemic. The earliest and the latest date of illness onset were 15 June and 13 July, respectively. The majority of cases reported onset during 19–26 June. Assuming an incubation period of 14–21 days [1, 2], the presumed exposure occurred around 5 June. The reported illness duration was 1–21 days (mean duration ± standard deviation, 7 ± 3 days).

The dominant clinical presentation (Table 1) was fever (98%), headache (90%), and weakness (80%). Only 21% had cough, and none reported symptoms consistent with hepatitis. One hundred forty-one individuals (79%) visited their primary practitioner during their illness. Thirty-one individuals underwent chest radiography examination, and 7 (4%) received a diagnosis of pneumonia. Five patients were hospitalized (2 students and 3 employees) for pneumonia ($n = 2$, 1 of which was a man aged 92 years, the oldest patient in our exposed population), perimyocarditis ($n = 1$), perimyocarditis and pneumonia ($n = 1$), and observation ($n = 1$). Duration of hospitalization ranged between 1–7 days. No deaths occurred. Only 3 individuals were treated with doxycycline during illness. Of note, no additional cases of acute Q fever were diagnosed in the neighborhoods surrounding the school during the same time period.

Serologic results. Results of serologic tests were available for 164 individuals (151 [59%] students and 13 [26.5%] employees). One hundred eight (66%) were "confirmed cases" (103 students and 5 employees), 36 (22%) were "probable cases" (35 students and 1 employee), and 20 (12%) were "non-cases" (13 students and 7 employees). Sixty-five individuals met the criteria for a "possible case" (63 students and 2 employees).

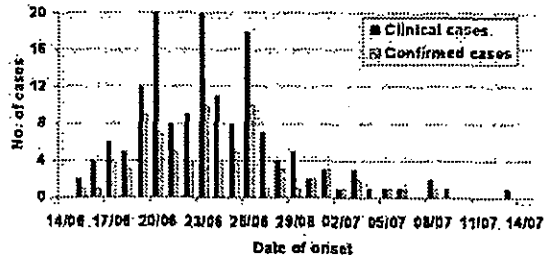

Figure 1. Epidemic curve of all clinical cases and confirmed symptomatic cases. Clinical cases were defined as individuals who reported symptoms compatible with Q fever with illness onset from 1 June through 31 July, with other etiologies ruled out. Confirmed symptomatic cases included any clinical case with positive serologic test results for Q fever.

Table 1. Symptoms of All Clinical Cases and Confirmed Symptomatic Cases

Symptom	No (%) of cases	
	All clinical cases	Confirmed cases
Fever	184 (98)	92 (98)
Headaches	166 (90)	85 (92)
Sweats	81 (49)	45 (53)
Weakness	145 (80)	78 (87)
Chills	60 (35)	36 (42)
Vomiting	30 (17)	22 (24)
Myalgia	39 (23)	22 (26)
Cough	38 (21)	22 (24)
Sore throat	42 (23)	23 (26)
Chest pain	21 (13)	13 (15)

Eighty-six percent and 81% of the confirmed and probable cases, respectively, were clinically ill. All of the non-cases were asymptomatic. The incubation period and the clinical presentation of the confirmed cases resembled that of all clinical cases (Figure 1 and Table 1).

The exact attack rate could not be determined, because everyone was not tested for Q fever; therefore, we estimated a range. The lower limit was 144/303 (47.5%), including confirmed and probable cases. The upper limit was 209/303 (69%), which also included the possible cases. This was based on the observation that all serologically tested clinical cases were either confirmed or probable cases.

The symptomatic to asymptomatic ratio among serologically positive individuals (85:15) is biased, because symptomatic individuals were more likely to be tested. Given that all tested symptomatic individuals had positive results, the numerators were more likely to be near 187 versus 116−20 (all symptomatic individuals vs the asymptomatic minus the seronegative individuals), which translates to a ratio of 66:34 or even higher.

Risk factors. Table 2 summarizes the prevalence of several possible risk factors in confirmed cases and controls. Being a student (OR, 11.09; 95% CI, 3.07–40.07), boarding at school during the June holiday (OR, 13.9; 95% CI, 4.45–43.45), and dining regularly at the school dining room (OR, 8.57; 95% CI, 2.05–35.79) were significantly associated with contracting Q fever. When probable cases were included in the univariate analysis, boarding at school during the weekend before the June holiday was also significantly associated with Q fever infection (OR, 3.18; 95% CI, 1.09–9.22). Because all of the above significant risks factors were statistically associated with each other, we did not perform multiple logistic regression analysis.

Table 2. Risk Factors for Acquiring Q fever

Factor	No (%) of persons		OR (95% CI)
	Cases	Controls	
Status in school (student vs employee)	103 (95)	13 (65)	11.09 (3.07–40.07)
Boarding at school on a regular basis	32 (30)	3 (15)	2.45 (0.67–8.95)
Boarding at school during Shavuot holiday	91 (92)	9 (45)	13.9 (4.45–43.45)
Boarding at school during the weekend before the holiday	48 (59)	6 (35)	2.67 (0.9–7.92)
Boarding at school during the weekend after the holiday	33 (41)	6 (35)	1.29 (0.43–3.83)
Eating at the school dining room (frequently vs seldom or never)	96 (96)	14 (74)	8.57 (2.05–35.79)
Contact with pets on school ground	0 (0)	0 (0)	

NOTE. CI, confidence interval; OR, odds ratio.

Environmental and veterinary investigation. Numerous stray cats were seen in the schoolyard, especially in proximity to the kitchen and the garbage cans which were located outside the dining room. The dining room had its own air-conditioning system, with inlet that drew air from the dining room and outlet that emitted the cooled air back to the room. The air-conditioning ducts were located on the dining room's roof and could be accessed by animal secretions. One of the 4 filter samples, as well as 1 of the 8 gauze swabs taken from the inlet of the dining room's air-conditioning unit, had positive results for Q fever by PCR. Similar positive PCR results were obtained by the Centers for Disease Control and Prevention on filter samples.

Serum samples of 65 feral cats were tested for Q fever serology. Nine cats (14%) had positive results; 2 (10%) of 20 were caught within a 2-km radius of the school, whereas the other 7 (15%) of 45 were from other parts of the city. Forty feline uterine specimens were tested by PCR, and all were found to have negative results.

DISCUSSION

We describe a Q fever outbreak that was unusual in its magnitude and place of occurrence. It represents 1 of the largest outbreaks described in the literature and the largest to occur in a densely populated urban area located far away from livestock farms [3]. The clinical attack rate was remarkably high (62%), with the serological attack rate estimated to be even higher (69%). This is a conservative estimate because asymptomatic individuals, who could have been serologically positive (if tested), were not included and the pre-existing immunity in this particular population was assumed to be very low (based on research that found 14% seropositivity to Q fever among adults residing in the Northern part of Israel, which is a more rural area) (A.K., unpublished data). The symptomatic to asymptomatic ratio was estimated to be 66:34, higher than that reported elsewhere (40:60) [1, 4].

The high attack rate and symptomatic to asymptomatic ratio might be explained by a large inoculum of bacteria and effective modes of transmission. The demonstration of the presence of *C. burnetii* by PCR in the samples from the dining room's air-conditioning system supports an effective aerosol transmission. A similar phenomenon was described in an outbreak in a cosmetics factory where all the exposed workers were symptomatic [23]. The high proportion of symptomatic infection can also be attributed to the male predominance of the exposed population [4, 6] and to the fact that none of the students were aged <14 years [7].

Notable is the low clinical attack rate among the school employees, compared with the students (16% vs 70.5%), which we think is attributable to their lower exposure to the infectious agent. An alternative explanation could be a higher pre-existing immunity among the employees. However, even if the pre-existing immunity was 14% (A.K., unpublished data), this would have changed the calculated clinical attack rate among employees by 2% only (from 16% to 18%).

The dominant clinical presentation was an influenza-like illness, and the working diagnosis of the majority of the primary physicians was a viral infection. Seven patients (4%) received a diagnosis of pneumonia, and none exhibited overt signs of hepatitis. Because of the delayed notification of the Tel Aviv District Health Department and the subsequent delay in the laboratory confirmation of *C. burnetii* infection, the outbreak investigation had little effect on the clinical management during the acute illness. Thus, laboratory and imaging tests were not conducted routinely but were rather conducted on the basis of clinical judgment, and only 3 individuals were treated with doxycycline.

Geographic variation in the clinical presentation of Q fever is well described [2]. In a recent review of 100 hospitalized patients with acute Q fever from Israel [24], the most common presentation was an acute febrile illness with few physical findings. Rare but severe manifestations of the disease are myocarditis and pericarditis, each described in ~1% of patients [1]. Two patients in the present study were hospitalized for myopericarditis. Thus, the clinical presentation in the present study is consistent with that described in the literature.

Most reported large Q fever outbreaks have occurred in or adjacent to rural areas as a result of direct or indirect exposure to infected livestock, especially to parturition products, as is the case in an outbreak in the Netherlands [25]. Urban outbreaks have been typically linked to farm animals that were brought to slaughterhouses [8, 9], animal research laboratories [10], urban farmers' markets [26], contaminated livestock products [23], or windborne aerosols carried long distance from neighboring farms engaged in outdoor lambing and calving [13]. Some urban outbreaks have been linked to parturient dogs [27] and cats [11, 28], and in some the source was never determined [14, 15].

The source of infection in the present outbreak was not clearly defined. However, the findings that being a student, dining at the school's dining room, and boarding during the June holiday were significantly associated with contracting the disease support the hypothesis that the transmission of the infection occurred in the dining room. The positive PCR results from the dining room's air-conditioning system further suggest that the air-conditioning system contributed to the aerosol transmission of the agent, although we could not prove whether the primary source of infection was the dining room or the air-conditioning system. The fact that the environmental samples were taken 2 weeks after the last reported case and mainly from the inlet of the air-conditioning system could explain why only 2 inlet samples of 12 total samples had positive results for *C. burnetii* by PCR. No new cases appeared a month after the initial case (Figure 1), and no other cases were diagnosed in the vicinity of the school, pointing to a limited exposure, both in time and space.

The air-conditioning system could have been contaminated by the numerous stray cats seen in the schoolyard. We were unable to demonstrate that cats from the school vicinity were more likely to be seropositive for Q fever than cats from different areas of the city. Nevertheless, the cat sampling showed that *C. burnetii* is endemic in feral cats in the school's surroundings. To our knowledge, no similar surveys were previously conducted among cats in Tel Aviv.

The magnitude of the present outbreak is impressive, given the yearly incidence of Q fever in Israel (0.6 cases 100,000 persons) and in comparison with other outbreaks described in nonrural areas. It demonstrates that *C. burnetii* can be effectively transmitted to a large number of people through a common exposure.

This outbreak raises the issue of underdiagnosis of Q fever, especially when a primary practitioner treats a sporadic case that manifests as an influenza-like illness. In our study, the working diagnosis of the majority of the physicians was a viral infection. This also implies that there could be a delay in outbreak investigations with implications on the probability of revealing their sources. A high index of suspicion is required when dealing with a relatively prolonged febrile disease, even with no history of exposure to farm animals. A cluster of febrile patients, especially if occurring outside the influenza season, should raise the possibility of Q fever, and rapid investigation into the etiology and source of infection should be made by public health authorities.

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Potential conflicts of interest. All authors: no conflicts.

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医薬品 研究報告 調査報告書

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販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			

研究報告の概要

○カラカス(ベネズエラ)の学校における、経口感染による急性シャーガス病(CD)の大都市でのアウトブレイク

背景:*Trypanosoma cruzi*(*T. cruzi*)は、媒介動物の糞便で汚染された食物によって経口感染する。経口感染による急性CDの小規模流行の疫学的・臨床的な特徴については、ほとんどわかっていない。

方法:学校コミュニティに影響を及ぼした急性CDのアウトブレイク時において、コホート疫学研究を実施した。症状と感染源を特定するため、統一的問診を計画した。すべての患者から心電図データを入手し、免疫酵素的および間接血球凝集検査によって、特異的血清抗体を評価した。一部の症例においては、寄生虫血症を直接的または培養、動物接種試験、PCR法により検査した。

結果:曝露された1000名中103名に感染が確認された。感染者のうち、75%に症状があり、その20.3%は入院を必要とした。また59%は心電図異常を示し、44名に寄生虫血症が認められ、子供1名が死亡した。臨床的な特徴は、ベクターを介した感染で見られるものとは異なった。子供は感染率が有意に高かった。疫学研究では、汚染した生グアバジュースが唯一の感染原因とされた。

結論:当該アウトブレイクは、大都市部で、主に若年齢を中心とした中流層の、健康に問題のない集団に感染するという、先例のない公衆衛生的非常事態を招いた珍しいものであった。迅速な診断と処理により、高い死亡率は回避された。しかし*T. cruzi*の食物を介する感染は、現在認識されるより頻繁に起こる可能性がある。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ベネズエラの大都市におけるシャーガス病のアウトブレイクについての疫学研究を行ったところ、*Trypanosoma cruzi*の食物を介する感染は、現在認識されるより頻繁に起こる可能性が示されたとの報告である。	日本赤十字社は、輸血感染症対策として献血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、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

MAJOR ARTICLE

Large Urban Outbreak of Orally Acquired Acute Chagas Disease at a School in Caracas, Venezuela

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(See the editorial commentary by Miles, on pages 1282–1284.)

Background. *Trypanosoma cruzi* oral transmission is possible through food contamination by vector's feces. Little is known about the epidemiology and clinical features of microepidemics of orally acquired acute Chagas disease (CD).

Methods. A case-control, cohort-nested, epidemiological study was conducted during an outbreak of acute CD that affected a school community. Structured interviews were designed to identify symptoms and sources of infection. Electrocardiograms were obtained for all patients. Specific serum antibodies were assessed by immunoenzimatic and indirect hemagglutination tests. In some cases, parasitemia was tested directly or by culture, animal inoculation, and/or a polymerase chain reaction technique.

Results. Infection was confirmed in 103 of 1000 exposed individuals. Of those infected, 75% were symptomatic, 20.3% required hospitalization, 59% showed ECG abnormalities, parasitemia was documented in 44, and 1 child died. Clinical features differed from those seen in vectorial transmission. The infection rate was significantly higher among younger children. An epidemiological investigation incriminated contaminated fresh guava juice as the sole source of infection.

Conclusions. This outbreak was unique, because it affected a large, urban, predominantly young, middle-class, otherwise healthy population and resulted in an unprecedented public health emergency. Rapid diagnosis and treatment avoided higher lethality. Food-borne transmission of *T. cruzi* may occur more often than is currently recognized.

The burden of illness associated with Chagas disease (CD) remains the second highest among all of the endemic tropical diseases in Latin America and results in an annual loss of >2 million disability-associated life years (DALYs) [1, 2]. Although Chile, Uruguay, and Brazil have been certified as free of vectorial transmission by domiciliary *Triatoma infestans* [1], eradication appears to be an impossible task because of the complexity of the zoonotic life cycle of its causative agent, *Trypanosoma cruzi*. In addition to vectorial transmission, other secondary mechanisms of infection include congenital, transfusional, organ transplantation–related, and oral transmission. A sparse number of outbreaks of orally acquired human CD have been reported from Brazil [3–7], Argentina [8], and Colombia [9].

Venezuela has a successful CD vector control program that is based on the improvement of rural housing and vector control [10, 11]. However, epidemiological data suggest a reemergence of the infection [12–14]. At the capital, Caracas, which is a densely populated cosmopolitan city surrounded by mountains covered by tropical forests, the local sylvatic triatomine vector, *Panstrongylus geniculatus,* has been recorded since 1920 [15]; it was reported inside the houses in 1986 [16] and captured in the wild or within households show-

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ing a high rate (76.1%) of *T. cruzi* infection [17]. However, vectorial transmission has not been reported in this city.

The current study describes the largest known outbreak of orally acquired CD to date in the American continent, which involved numerous children and personnel from an urban school in Caracas.

METHODS

On 6 December 2007, trypomastigotes of *T. cruzi* were detected on peripheral blood smears from a 9-year-old student (index case), who was admitted to the Hospital Universitario de Caracas (Caracas, Venezuela) with a 3-week history of fever of unknown origin (FUO). Twenty persons from the patient's school were hospitalized with similar symptoms and were later found to have circulating trypomastigotes and/or serological test results positive for CD. The municipal health authorities were contacted at once, and they reported an unexpected simultaneous sharp increase in medical consultations and absenteeism among school personnel from 30 October through 25 November 2007.

The center involved (Unidad Educacional "Andrés Bello") is located in the Municipality of Chacao, in the eastern part of Caracas, with predominantly middle-class inhabitants. All of the food and beverages consumed by the students and personnel were supplied by the same caterer that supplied other municipal schools, with the exception of breakfast, which was prepared under unsupervised sanitary conditions, located in a distant slum on the western mountain slopes of the city. A multidisciplinary task force was summoned to analyze the epidemiological situation with the aim of controlling the outbreak [18]. A case-control, cohort-nested, epidemiological outbreak study was designed to assess the extent of the outbreak and to identify possible sources of infection. Cases were classified as "suspected" or "confirmed" in accordance with a consensus document prepared by the interdisciplinary group, based on World Health Organization recommendations [19]. A suspected case patient was any person with an epidemiological link to the institution involved from 10 October through 1 November 2007 who developed FUO of >5 days duration and other clinical manifestations. A confirmed case patient was any suspected case patient or asymptomatic person with the epidemiological link who, in addition, exhibited blood parasites or specific anti–*T. cruzi* antibodies by 2 different serological techniques: enzyme-linked immunosorbent assay (ELISA) and indirect hemaglutination (IH) or ELISA and Western blot (WB) tests.

The study population consisted of all students, teachers, workers from the school, external persons involved with the preparation or transportation of food consumed in the school, and any person considered to be a "school contact" potentially at risk. Blood samples for diagnosis were initially collected from 11 December through 14 December 2007, as an emergency intervention, with the aim of identifying infected persons and immediately starting antiparasitic treatment of any individual affected by a severe, potentially lethal, acute illness in the context of a large outbreak that occurred at a critical time of the year (3 days before a prolonged Christmas and new year vacation). During a second sampling that was performed 6 weeks later, 21 January through 25 January 2008, all participants undertook a detailed clinical and epidemiological questionnaire on CD risk factors (eg, exposure to vectors, transfusions, infected relatives, contact with animal reservoirs, and ingestion of food and/or beverages in the school). Case patients were compared with control subjects from the same cohort of exposed individuals.

The study was performed under the supervision of the Ethical Committee of the Tropical Medicine Institute. Informed written consent was obtained from each participant or from their legal guardians.

For the first 43 symptomatic patients, fresh and Giemsa-stained peripheral blood smears were reviewed for trypomastigotes. In addition, 2 mL of blood were cultured in biphasic medium and checked periodically over at least 3 months. Mice were inoculated intraperitoneally with 300 μL of blood and examined each week [19].

All serum samples were screened for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against a crude extract of *T. cruzi* epimastigotes [20] with use of an ELISA developed in house [21] and an IH test [22]. The immunodiagnosis of CD was based on the positivity of at least 2 specific serological tests [19]. Those samples with ELISA results positive for IgG and negative IH results were also tested with WB tests [23].

A representative number of 150 blood samples were randomly evaluated by a polymerase chain reaction (PCR). For the DNA extraction, 5 mL of blood was mixed with an equal volume of 6M guanidine HCl /0.2M EDTA (GE) [24]. The amplification reactions were targeted to the 330–base pair minicircle fragment of the *T. cruzi* kinetoplastid DNA [25].

Conventional 12-lead electrocardiogram (ECG) recordings were obtained from confirmed or suspected case patients and treated with either benznidazole (Rochagan; Roche Laboratories) at a dosage of 6 mg/kg/day for 60 days or nifurtimox (Lampit; Bayer Laboratories) at a dosage of 8 mg/kg/day for 90 days [19, 26].

The dependent variable or main outcome was based on serological status. Epidemiological exposure was evaluated using χ^2 or the Student's t test depending on the binary or continuous independent distribution of the variable. Only variables significantly associated in the univariate regression were included in the multivariate regression, using $P < .05$ as the entry criteria. The relationship between risk factors and final outcome (*T.*

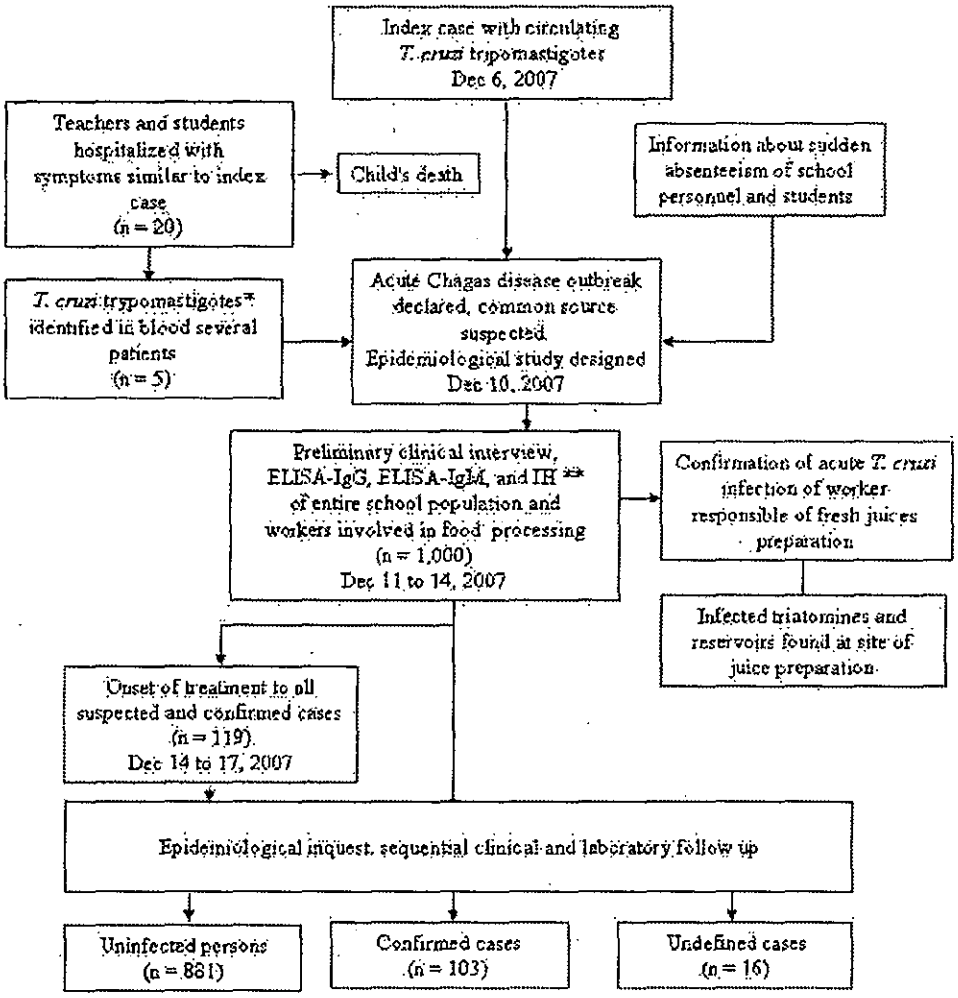

Figure 1. Study profile and major outcomes of the epidemiological investigation of the outbreak of acute Chagas disease, Caracas, Venezuela, 2007. *Parasitemia investigation by direct techniques. **Parasitemia, Western blot, and polymerase chain reaction tests performed on a more limited group of exposed individuals (see Methods). ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IH, indirect hemaglutination; *T. cruzi*, *Trypanosoma cruzi*.

cruzi infection) was estimated by means of the paired odds ratio (OR), with 95% confidence intervals (CIs). Stata, version 6.0 for Windows (Stata), was used as the basic statistical software for all calculations.

RESULTS

Figure 1 depicts the general outline of the study. Because the outbreak occurred in a well-off urban area of the city with no current vectorial transmission, a food-borne mechanism was presumed to be the cause. Date of exposure was estimated to occur between 10 October and 25 October 2007, based on previous reports of orally acquired infections with documented incubation periods of 5–20 days [5].

The demographic characteristics of the entire exposed population ($n = 1000$) are shown in table 1. No statistically significant differences were found in the attack rates among the sexes. Although, as a whole, age was not associated with the main outcome, a more meticulous revision of age distribution of those infected revealed a bimodal distribution curve, with a reverse trend, in which the OR for CD decreased with age for children but increased with age for adults. As depicted in Tables 1 and 2, significantly different attack rates were observed among students and teachers in relation to their school attendance (morning vs afternoon shifts; 65 cases [17.9%] among 363 subjects vs 10 cases [2.6%] among 385 subjects; OR, 3.19 [95% CI, 2.1–4.8; $P < .001$). The difference between the attack rate among students of the morning shift (22.5%) and the attack rate among children of the afternoon shift (2.4%) was statis-

Table 1. Demographic Characteristics and Rates of Infection of 1000 Individuals Exposed to Infection at a Public School Community of Caracas, Venezuela, Affected by a Large Outbreak of Orally Acquired Acute Chagas Disease in December 2007

Variable	No. (%) of exposed individuals (n = 1000)	
	Study population	Infected subjects
Age		
≤18 years old	795 (79.5)	77 (9.6)
>18 years old	205 (20.5)	26 (12.6)
Sex		
Male	455 (45.1)	50 (10.9)
Female	545 (54.9)	53 (9.7)
Students		
Kindergarten	65 (8.7)	15 (23.1)
1st grade	63 (8.4)	13 (20.6)
2nd grade	54 (7.2)	7 (12.9)
3rd grade	61 (8.1)	9 (14.7)
4th grade	66 (8.8)	7 (10.6)
5th grade	92 (12.3)	9 (9.7)
6th grade	82 (10.9)	7 (8.5)
7th grade	96 (12.8)	6 (6.2)
8th grade	89 (11.9)	0 (0)
9th grade	79 (10.5)	4 (5.1)
Subtotal	747 (74.7)	77 (10.3)
Nonstudents		
Personnel	165 (16.5)	25 (15.2)
Food handlers	16 (1.6)	1 (6.2)
Other contact	72 (7.2)	0 (0)
Subtotal	253 (25.3)	26 (10.2)
Shift		
Morning	363 (36.3)	65 (17.9)
Afternoon	385 (38.5)	10 (2.6)
Both	252 (25.2)	28 (11.1)

tically significant ($P < .05$). Although the absolute number of infected children was higher (77 of 103 infected subjects), the maximum infection rate (15.2%) was observed among the school employees. One of the 16 workers who were involved directly in the preparation or transportation of luncheons showed evidence of acute *T. cruzi* infection, with serological test results positive for specific IgM and IgG (Table 1).

A significant positive correlation was found between ingestion of guava juice and risk of infection (OR, 3.5 [95% CI, 1.85–6.7]) (Table 2). The epidemiological interviews revealed that, except for the guava juice, all other beverages were made in the early morning. The guava fruits, in contrast, were boiled the night before and left to cool inside a large uncovered pot before blending in the morning. Once in the school, the juice was delivered to the morning shift, first to school personnel, then to kindergarten students, and then to students in ascending grades. Some personnel and students of the afternoon shift customarily consumed any remaining juice.

Of those infected, 75% were symptomatic, 20.3% required hospitalization, and a 5-year-old child died of acute chagasic myocarditis. Most patients reported fever that lasted >7 days, abdominal pain, headache, dry cough, and myalgia; to a lesser degree, they reported diarrhea, facial edema, malaise, arthralgias, dyspnea, and tachycardia (Table 3). In the univariate regression analysis, the following symptoms showed a significant association with a higher risk of serologically confirmed infection: fever, arthralgias, skin lesions (rash, erythema nodosum, or facial edema), and cardiovascular abnormalities. However, on the multivariate analysis, only fever and cardiovascular abnormalities showed statistical significance.

In 61 (59%) of the 103 confirmed cases, ≥1 abnormality was noticed on the ECG recordings. T wave abnormalities were significantly more common among patients ≤18 years of age, whereas supraventricular arrhythmias and microvoltages were predominant among adults (Table 4), who more frequently developed severe clinical cardiological manifestations that required hospitalization.

Among 1000 persons evaluated, 103 individuals had anti–*T. cruzi* IgG antibodies by ELISA, and 90 (87.3%) were also IgM positive. The specific IH test was concordant in 99 (96.1%) of 103 individuals, whereas the remaining 4 individuals had positive WB results.

Because of logistic constraints, parasitemia could be assessed in only 43 patients by parasitological methods. Of these, 13 (30.2%) had positive fresh-stained blood smear results, in vitro culture, or mice inoculation.

Sixteen individuals with ELISA results positive for anti–*T. cruzi* IgG antibodies but negative IH results nevertheless received a full course of antiparasitic treatment. During follow-up, they became IgG seronegative while remaining persistently negative according to both IH and WB results. Five such patients developed clinical signs, as well as ECG abnormalities. Because these patients did not fulfill World Health Organization criteria for the CD diagnosis, they were considered to have undefined cases (Figure 1).

Samples of 150 persons were randomly chosen to be tested by specific PCR targeted at the *T. cruzi* kinetoplastid DNA. The reaction was positive in 35 (79.5%) of 44 serologically confirmed cases. All 106 seronegative individuals tested were also negative by PCR. A collateral survey performed at the site where the incriminated juice was processed revealed the presence of infected *P. geniculatus* and domestic rats.

As part of an ongoing cooperative study with the Instituto López Neyra in Granada, Spain, 3 parasite isolates obtained from patients, as well as from 1 infected triatomine captured at the juice preparation site, were typed using *T. cruzi* ribosomal and mini-exon gene markers. Preliminary results revealed a great genetic homogeneity, with all of the isolates belonging to the *T. cruzi* I lineage. Furthermore, homology analysis of the

Table 2. Univariate and Multivariate Logistic Regression Analysis of Risk Factors Associated with *Trypanosoma cruzi* Transmission during an Outbreak of Orally Acquired Chagas Disease, Caracas, Venezuela, 2007

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*
Age				
≤18 Years	0.85 (0.79–0.91)	.01	0.7 (0.73–0.87)	.001
>18 Years	1.03 (1.0–1.07)	.02	1.03 (1.1–1.05)	.01
Worker vs student	1.3 (0.83–2.06)	.24	...	...
Shift (morning vs afternoon)	3.19 (2.1–4.8)	.001	4.7 (2.6–8.3)	.001
Any fresh beverage	2.17 (0.77–6.1)	.14	...	...
Guava juice	3.5 (1.85–6.7)	.001	3.2 (1.4–7.1)	.004
Passion fruit juice	0.95 (0.59–1.62)	.95	...	...
Melon juice	1.16 (0.76–1.7)	.47	...	...
Lemon-starch drink	1.03 (0.68–1.52)	.85	...	...
"Chicha"	0.77 (0.51–1.18)	.24	...	...
Oat meal drink	1.37 (0.9–2.0)	.13	...	...
Tamarind juice	0.6 (0.39–0.94)	.60	...	...
Mango juice	0.79 (0.5–1.1)	.26	...	...
Papaya juice	1.32 (0.8–2.0)	.19	...	...
Pineapple juice	0.72 (0.4–1.9)	.12	...	...

NOTE. CI, confidence interval; OR, odds ratio.

sequence of an amplified polymorphic mini-exon from *T. cruzi* RNA confirmed that all parasite isolates from the patients were identical, which was consistent with a common source of infection.

DISCUSSION

Thanks to a coordinated program in the Southern Cone countries, the transmission of CD has been successfully interrupted in Uruguay and Chile, as well as in at least 8 of the 12 states of Brazil in which CD is endemic [19, 27]. However, the persistence of numerous sylvatic foci and the wide distribution of vectors and reservoirs, together with a progressive reduction in the availability of the vector's natural source of blood (birds and mammals) in intervened forested areas, is driving originally wild triatomines to invade human dwellings [28, 29]. Once domiciliation has occurred, *P. geniculatus* may feed abundantly on domestic reservoirs, as well as on humans. As part of their nocturnal activity, vectors circulate widely inside the house and can thereby eventually contaminate unprotected food and beverages with their feces. There is also the possibility of trans-

Table 3. Univariate and Multivariate Logistic Regression Analysis According to Symptoms and Serological Test Results for 1000 Individuals Exposed during an Outbreak of Orally Transmitted Acute Chagas Disease in Caracas, Venezuela, 2007

		Serological test results		Univariate analysis		Multivariate analysis	
Symptom	No. (%) of subjects (n = 1000)	Percent positive/percent negative	P^a	OR (95% CI)	*P*	OR (95% CI)	*P*
Fever	190 (19.0)	46.6/15.7	.001	4.6 (3.0–7.1)	.001	5.4 (3.9–9.6)	.001
Artralgias	18 (1.8)	6.6/1.2	.001	5.7 (2.1–15.4)	.001	3.3 (0.4–26.2)	.250
Skin lesions[b]	30 (3.0)	11.4/2.0	.001	6.2 (2.9–13.4)	.001	2.2 (0.7–6.9)	.140
Cardiovascular	4 (0.4)	1.9/0.2	.001	8.6 (1.2–62.0)	.030	4.3 (1.2–12.8)	.040
Gastrointestinal	84 (8.4)	11.4/8.0	.230	1.4 (0.7–2.8)	.240	...	...
Respiratory	49 (4.9)	7.6/4.5	.170	1.7 (0.7–3.7)	.170	...	...
Unspecific	26 (2.6)	4.7/2.3	.140	2.0 (0.7–2.8)	.150	...	...

NOTE. CI, confidence interval; OR, odds ratio.

[a] By χ^2 analysis.

[b] Rash, erythema nodosum, and facial edema.

Table 4. Basal Electrocardiogram (ECG) Abnormalities by Age Group for 61 Infected Patients from an Outbreak of Orally Acquired Acute Chagas Disease in Caracas, Venezuela, 2007

ECG abnormality	Age group		Total	P^a
	≤18 Years (n = 48)	>18 Years (n = 13)		
ST abnormality	30	4	34	.028
T abnormality	39	1	40	<.001
Supraventricular arrhythmia	3	6	9	.002
Ventricular arrhythmia	2	0	2	.897
Microvoltage/decrease amplitud QRS	0	3	3	.007
QTc prolongation	2	0	2	.897
Fascicular block	3	2	5	.621
AV block	2	0	2	.897

[a] Yates corrected χ^2 analysis.

mission by food contamination with urine or anal secretions of infected marsupials [30].

The genetic homogeneity and lack of significant genetic intralineage polymorphism observed in all of the isolates thus far typed from the current outbreak is consistent with a common source of infection. Moreover, the confirmation of an acute infection in the woman responsible for the preparation of the juice lends further support to evidence that indicates short-term exposure, as do the logistic regression analysis results, which incriminated the guava juice as the possible source of contamination. We therefore postulate that, during the night, infected triatomines might have contaminated the unprotected pot where the guava juice was left before being blended in the early morning. Once the juice arrived at the school, it was first served to the teachers and afterwards served to the students, progressing from the lower to the higher grades of the morning shift. Any remaining juice was later shared by the teachers and students of the afternoon shift. This sequence of events could explain the relatively high attack rate observed among school personal (15.2%) and the significant decrease in the attack rate among students in the ninth grade (5.1%), compared with that among kindergarten students (23.1%). The significant difference in the attack rates found between students of the morning (22.5%) and afternoon shifts (2.4%) suggests that the concentration of the inoculum may have been different for both groups, perhaps reflecting a steady decrease in the survival of infecting metacyclic trypomastigotes [31].

Orally transmitted CD episodes have been described previously, all of which have been reported in South America [3–9, 32–34]. Distinctive epidemiological features included a lower number of infected persons (37 cases being the maximum number reported in any outbreak); relatively high lethality (up to 35.2%, with an average rate of 7.1%); a preponderance of cases occurring among adults; and occurrence in remote rural areas or in urban communities where fruits obtained from areas of endemicity, such as açai *(Euterpe oleracea)*, piassava *(Leopoldinea piaçaba)*, and sugar cane, were consumed. The present outbreak is unique in that it affected a large, predominantly young, healthy urban population and was associated with high rates of parasitemia and morbidity but a very low mortality rate (0.97%). The latter probably relates to prompt diagnosis and treatment. It is the first time that contaminated guava juice has been incriminated as the source of infection. Moreover, this represents a genuine urban oral CD outbreak, because the *T. cruzi* strain that was involved in the outbreak originated from an inner-city household, where peridomestic triatomines and rodent reservoirs allowed the maintenance of transmission.

One crucial problem was the overwhelming amount of clinical cases that required diagnostic confirmation. Serological testing with the ELISA was very useful for this purpose, and the assessment of both IgG and IgM anti–*T. cruzi* antibodies for all members of the exposed population enabled us to demonstrate the infection in the early phase. The concurrent onset of symptoms in most cases and the fact that specific IgM antibodies were demonstrated in a high percentage of cases (87.3%) further suggests that exposure to the infecting inoculum was recent [35] and singular or short-lived.

Of the 103 individuals in whom *T. cruzi* parasitemia was determined by parasitological methods and/or PCR, 44 (40.7%) had positive test results. This is probably one of the highest rates of parasitemia ever documented in any orally transmitted CD outbreak.

Although 75% of the infected individuals were symptomatic, the predominant clinical manifestations observed (fever, headache, and myalgias) are all highly unspecific. Indeed, dengue, mononucleosis, hepatitis, and intoxications were among the causes contemplated initially. Clinical findings such as facial edema, gingivitis, and dry cough are probably the consequence of the penetration of the parasite throughout the oral cavity, lips or pharyngeal mucosa. These latter manifestations, along

with other unexpected findings, such as erythema nodosum, anasarca, and lower limbs edema, are not described in vectorial transmission and even in prior reports of orally-acquired CD. They may be related to the host's immune inflammatory response conditioned by the genetics of each individual or by a high parasite load [36]. On the other hand, the findings of acute myocarditis were observed in an unusually high proportion (59%) of confirmed cases.

The diagnosis of acute CD requires a high index of suspicion by the clinician, especially when patients are seen away from the traditional areas of endemicity. In countries in which CD occurs, this condition must be considered in the differential diagnosis of FUO, because food-borne acute CD may occur more often than is currently recognized.

Progressive environmental changes that affect the ethology and ecology of potential *T. cruzi* reservoirs and vectors, together with an increase in human populations surrounded by intervened forests, have favored the urbanization and domiciliation of the cycle maintained by *P. geniculatus*, thus affecting the poor populations of the misery belts around most Latin American cities and middle-class populations, under the concept of the "edge-mediated effects" [37]. This new situation imposes necessary changes in the strategy of CD control programs, which until now have been limited to vector control activities in rural Latin American communities in areas of endemicity.

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