Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death

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Summary

Background *Rickettsia helvetica* is the only non-imported rickettsia found in Scandinavia. It was first detected in *Ixodes ricinus* ticks, but has never been linked to human disease. We studied two young Swedish men who died of sudden cardiac failure during exercise, and who showed signs of perimyocarditis similar to those described in rickettsial disease.

Methods Samples from the heart and other organs were analysed by PCR and DNA sequencing. May-Grünwald-Giemsa, Grocott, and acridine-orange stains were used for histopathological examinations. Staining of *R helvetica* grown on shell-vials in vero cells, and the early descriptions of *R rickettsii* by H T Ricketts and S B Wohlbach served as controls. Immunohistochemistry was done with *Proteus* OX-19 rabbit antisera as the primary antibody. The structure of rickettsia-like organisms was investigated by transmission electron microscopy. Serological analyses were carried out by indirect immunofluorescence with *R helvetica* as the antigen.

Findings By use of a semi-nested PCR, with primers specific for the 16S rRNA and 17-kDa outer-membrane-protein genes, and sequence analysis of the amplified products, genetic material from *R helvetica* was detected in the pericardium and in a lymph node from the pulmonary hilum in case 1, and in a coronary artery and the heart muscle in case 2. A serological response in case 1 revealed an endpoint titre for *R helvetica* of 1/320 (1/256 with *R rickettsii* as the antigen). Examination of PCR-positive tissue showed chronic interstitial inflammation and the presence of rickettsia-like organisms predominantly located in the endothelium. These organisms reacted with *Proteus* OX-19 antisera, and their size and form were consistent with rickettsia. Electron microscopy confirmed that the appearance of the organisms was similar to that described for spotted-fever rickettsia.

Interpretation *R helvetica*, transmitted by *I ricinus* ticks, may be an important pathogen in the aetiology of perimyocarditis, which can result in sudden unexpected cardiac death in young people.

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Introduction

Infectious perimyocarditis is caused by viruses or bacteria, although rarely by rickettsiae. Cases of pleuropericarditis, caused by *Rickettsia conorii* or *R typhi*, have been reported from France and Spain,[1–8] and cases of rickettsial myocarditis have occurred in Italy.[9,10] The severe sequelae of rickettsial diseases were described as early as 1922 by Wohlbach and colleagues, who were studying louse-borne typhus at St Stanislaus Hospital in Warsaw, Poland.[11]

We examined two cases of sudden cardiac death in young Swedish men who had signs of chronic perimyocarditis at necropsy. Microscopic investigation of the hearts from these men revealed myocardial changes similar to those described in US soldiers in the southwest Pacific who contracted scrub typhus (caused by *Orientia tsutsugamushi*) during World War II.[12–14] *R helvetica* has been detected in *Ixodes ricinus* in Sweden at a prevalence of around 22%.[15,16]

Patients and methods

Patients

We studied the cases of two young men who died of sudden unexpected cardiac failure during exercise. Histological examination of cardiac tissue showed low-grade interstitial perimyocarditis with fibrosis and infiltration by mononuclear cells. Culture and serology for microbes commonly associated with this disorder were negative or inconclusive in both cases. Both men had lived in suburban areas of Sweden where tick bites are common; however, whether the two patients had actually been bitten was not confirmed.

Case 1—A 19-year-old male ice-hockey player died suddenly during training in October, 1997. An abnormal electrocardiogram (ECG) during rest had been noted when the man joined the ice-hockey team. This finding led to examination by a cardiologist in September, 1996. The ECG showed T-wave inversion in leads II and V4-6. ECG during physical work was normal up to 230 W. Echocardiography was normal. Since the beginning of 1997 he had had attacks of tachycardia and faintness for periods of 5–10 s during and after exercise. A 48 h ECG in July, 1997, showed sinus tachycardia during exercise, and frequent ventricular premature beats, periods of ventricular tachycardia, and frequent periods of second-degree atrioventricular block with Wenckebach phenomena at night. Myocardial scintigraphy revealed decreased perfusion over the apex of the left ventricle. A β-receptor blocker (metoprolol 50 mg daily) was given in a regular dose. There was a strong suspicion that the patient had myocarditis. Infectious causes were not investigated. The patient disregarded strong advice not to do physical exercise, and died suddenly during training. Shortly before death he had complained of feeling feverish, and because of a slight increase in monocytes in the peripheral blood, mononucleosis was suspected. No palpable enlarged lymph nodes were observed.

Case 2—A 33-year-old healthy man died unexpectedly during a rink-bandy (type of ice-hockey) match. The patient went into ventricular fibrillation from which he could not be revived, despite hospital attention.

Primer	Gene	Nucleotide sequences (5′ to 3′)	Product size (bp)
Ric Ric-U8	16SrRNA	CTAGAACGAACGCTATCGGTAT TGCGTTAGCTCACACCTTCAGG	1385
Ric Rt-Ric	16SrRNA	TTTCATCGTTTAACGGCGTGGACT TTTCATCGTTTAACGGCGTGGACT	757
RpCS.877 RpCS.1258	CS	GGGGGCCTGCTCACGGCGG ATTGCAAAAAGTACAGTGAACA	381
17-up 17-low	17 kDa OMP	AAAATTCTAAAAACCAT TCAATTCACAACTTGCC	532
17-up Rj5	17 kDa OMP	AAAATTCTAAAAACCAT CGCCATTCTACGTTACTACC	351

Table 1: **Details of primers used to amplify rickettsial genes**

Primer	Denaturation (ºC)	Annealing (ºC)	Extension (ºC)	Number of cycles
Ric Ric-U8 RtRic	94	65	72	35
RpCS.877 RpCS1258	94	48	72	35
17-up 17-low Rj5	93	45	72	35

Table 2: **Details of PCR cycles used to amplify rickettsial genes**

Methods

In case 1, DNA was extracted in duplicate[15] from lung, pericardial fluid, visceral pericardium, coronary artery, ascending aorta, liver, and a lymph node from the pulmonary hilum. In case 2, DNA was extracted in triplicate from tissue samples from visceral pericardium, blood, muscle of the left ventricle, coronary artery, lung, lymph node from the pulmonary hilum, pleura, and small intestine.

All samples were examined for rickettsial DNA by PCR of the 16S rRNA, citrate synthase, and 17 kDa outer-membrane-protein (OMP) genes. The thermal conditions and primers used for amplification of the different genes are shown in tables 1 and 2. A semi-nested procedure was carried out, with the primer pair Ric-RicU8 followed by Ric-RtRic for 16S rRNA and the primer pairs 17up-17low and 17up-Rj5, respectively, for the 17 kDa OMP gene.[16] The amplified products were analysed on a 1·5% agarose (Kodak) gel in 0·5×Tris-borate-edetic acid buffer. *R prowazekii* and *R canada* were chosen as positive controls.

PCR-derived fragments of the 16S rRNA, citrate synthase, and 17 kDa OMP gene were sequenced by a direct solid-phase procedure.[17]

Vero-cell-grown rickettsia isolates were purified and placed on slides as spots.[16] Serum samples were tested in dilution from 1/10 to 1/2560. IgG antibodies were detected by γ-chain-specific antibody to human immunoglobulin conjugated with fluorescein isothiocyanate (Dakopatts, Denmark). Serum samples from 15 patients with tick bites who were screened for borrelioses were included as controls. The serological result was confirmed at the WHO Collaborating Center for Tropical Diseases, Rickettsial and Ehrlichial Research Laboratories, University of Texas, USA, with *R rickettsii* as the antigen.

All formalin-fixed and paraffin-embedded samples were examined with routine histological stains (haematoxylin and eosin, phosphotungistic acid-haematoxylin, van Gieson, MGG, and acridine orange). For detection of rickettsia, MGG staining was used. For confirmation of the presence of intracellular bacteria, Gimenez or Grocott stains were chosen. The presence of rickettsia-like organisms in the blood and the lung tissue in case 2 was also confirmed with acridine-orange staining. Stained cultures of our own isolated *R helvetica* grown on shell-vials in vero cells, and the descriptions by H T Ricketts and S B Wohlbach in their studies on Rocky Mountain spotted fever served as references for the detection of riskettsia-like organisms.[19,20] Heart sections from young people who had died and skeletal muscle from case 1 served as negative controls for MGG staining. Deparaffinised, trypsin-digested sections from the thickened perimyocardial tissue from both cases were incubated with antserum to *Proteus* OX-19 (Difco Laboratories, Detroit, Michigan, USA) at 37ºC for 1 h and IgG antibodies were visualised by γ-chain-specific antibody to rabbit immunoglobulin conjugated with fluorescein isothiocyanate (Dacopatts, Denmark).

Pathological perimyocardial tissue from the left ventricle from case 1 was fixed in 1% glutaraldehyde sodium cacodylate buffer (pH 7·4) overnight then treated with 1% osmium tetroxide$_4$ in distilled water. The bacteria and cell material were then dehydrated in an ethanol gradient and embedded in Agar 100 resin (Agar Scientific Ltd, Stanstead, UK). 50 nm sections were cut, stained with uranyl acetate and lead citrate, and inspected in a Philips TEM 420 transmission electron microscope.

Results

At necropsy in case 1, there were signs of acute heart failure with heavy congestion in the internal organs. The tonsils were slightly enlarged. In the liver hilum, one large lymph node was detected. In the pericardium and in each pleural cavity, 30 mL and 150 mL, respectively, of clear straw-coloured serous fluid was found. The heart weighed 417 g and had a normal configuration. There was a greyish thickening of the visceral pericardium over the apex of the left ventricle, comprising the anterior wall and lateral walls, and the back of the apex. In these regions, fibrotic tissue seemed to infiltrate the outer layer of the myocardial wall, corresponding to the areas of diminished perfusion

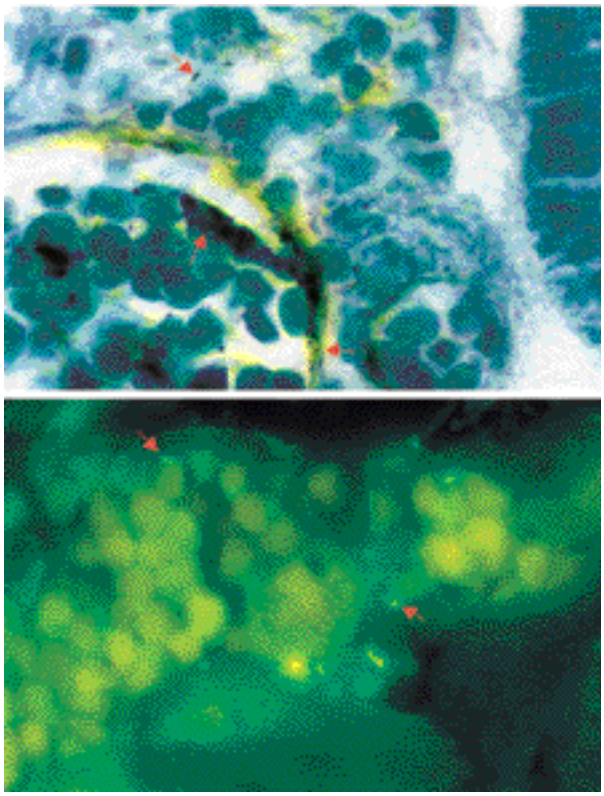

Figure 1: **Vasculitis in fibrotic perimyocardial tissue in case 1**
Top photograph shows rickettsia-like coccobacillary organisms inside and outside the damaged vessel wall (arrows). Note extravascular erythrocytes and endothelial stripping from the vessel wall with accumulation of mononuclear cells (arrow). MGG stain.
Lower photograph shows brightly immunofluorescent coccobacillary rickettsia-like organisms in the endothelial layer of a venule, and outside the vessel in the fibrotic tissue (arrows). Inside the venule are autofluorescent erythrocytes.
Both photographs reduced by 62% from original magnification (×1000).

found previously. The heart was contracted in systole, with the left-ventricular wall measuring 20 mm. The intraventricular septum was 20 mm thick, and the right-ventricular wall measured 6–7 mm. There was no sign of hypertrophic or arrythmogenic right-ventricular cardiomyopathy. The coronary arteries and the heart valves were normal. The spleen was enlarged, weighing 365 g, and showed signs of infection, with a grey-red cut surface. The liver capsule was thickened in some areas. Bacterial cultivation was inconclusive.

Toxicological examinations, including those for narcotic drugs and anabolic steroids, were negative. At microscopy, a low-grade diffuse interstitial perimyocarditis was seen, with fibrosis, infiltration of mononuclear cells, myocytolysis, and areas with swollen degenerated myocyte nuclei. No ischaemic myocardial changes were observed. In the heart and in pulmonary lymph nodes, rickettsia-like microorganisms were found in macrophages and the endothelium by staining with May-Grünwald-Giemsa (MGG) stain, and Grocott silver stain. These microorganisms differed from the larger and darker blue mast-cell granules in that they were small and purple-red in colour (figure 1). Immunohistochemistry with anti-*Proteus* OX-19 antisera as the primary antibody confirmed the finding (figure 2). In the lungs, acute congestion, oedema, and solitary round cells were seen. The patient was diagnosed as having diffuse, interstitial, mononuclear, low-grade perimyocarditis in a process of healing.

Both samples of a lymph node from the pulmonary hilum were positive for 16S rDNA; this result was confirmed by amplification with the 17 kDa OMP gene primers. When all samples from the first extraction were diluted 1 in 100, those from the visceral pericardium and ascending aorta were positive for 16S rDNA, as confirmed by amplification of the citrate synthase gene. Diluted samples from the second extraction yielded a 16S rDNA-positive coronary-artery sample. No diluted samples were positive for the 17 kDa OMP gene.

At necropsy in case 2, the internal organs showed signs of heavy congestion. Severe, dry, recent, and widespread miliary tubercular pericarditis and pleurisy were observed. Over the apex of each lung, a solitary scarred and indrawn area pathognomonic for tuberculosis was seen. The lymph nodes of both pulmonary hila were enlarged, and there were signs of acute tracheobronchitis. The heart weighed 394 g and was of normal size and configuration. The heart valves

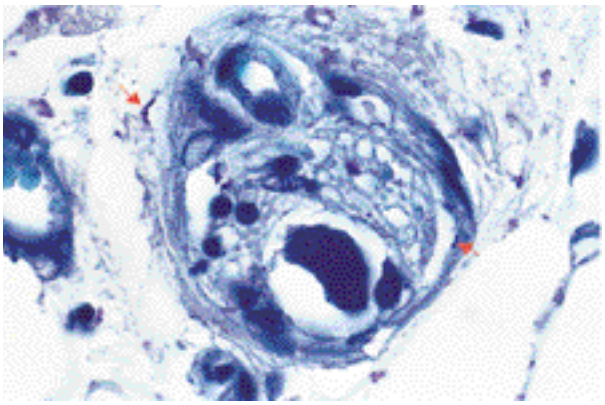

Figure 2: **Chronic myocardial vasculitis in case 1, showing rickettsia-like coccobacillary organisms in the vessel wall (arrows)**
MGG stain. Reduced by 62% from ×1000.

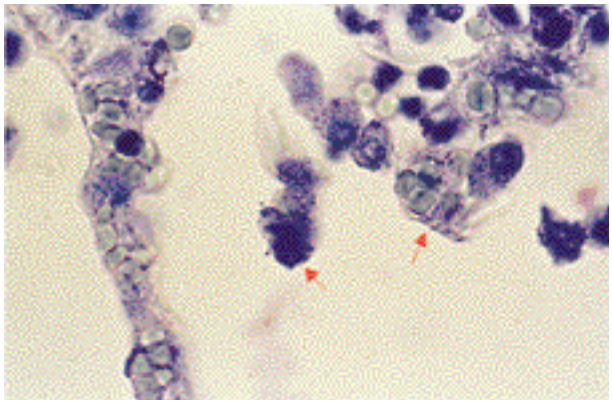

Figure 3: **Interstitial mononuclear pneumonitis with septal and alveolar involvement in case 2**
Note capillaritis and rickettsia-like organisms in the walls of a capillary (arrow). Rickettsia-like organisms are also present in the alveolar macrophages (arrow). MGG stain. Reduced by 62% from ×1000.

and coronary vessels were normal. Macroscopically and microscopically there were signs of severe, persisting, diffuse interstitial and mononuclear perimyoendocarditis with chronic nodular vasculitis in smaller vessels and large numbers of macrophages and endothelial cells filled with rickettsia-like organisms as shown by Gimenez and MGG stains (figure 3). Mast cells with abundant granules larger than the rickettsia-like structures were also present. Similar inflammatory changes were noted in lymph nodes, a coronary artery, and the lungs, where mononuclear pneumonitis was also observed (figure 4). The spleen was enlarged, weighing 237 g, with a grey-red cut surface, indicating an infectious state. Microscopic investigation of heart and lung tissues and cultivation of these tissues for tuberculosis were negative. Growth of ordinary bronchial non-pathogenic bacteria was observed. The diagnosis given was chronic, persisting, diffuse interstitial perimyoendocarditis and pleurisy in a process of healing.

Triplicate samples from the coronary artery and heart muscle proved positive for the 17 kDa OMP gene. One of three blood samples was also positive for this gene. A partial sequence from both strands of the 16S rRNA, citrate synthase, and 17 kDa OMP gene fragments

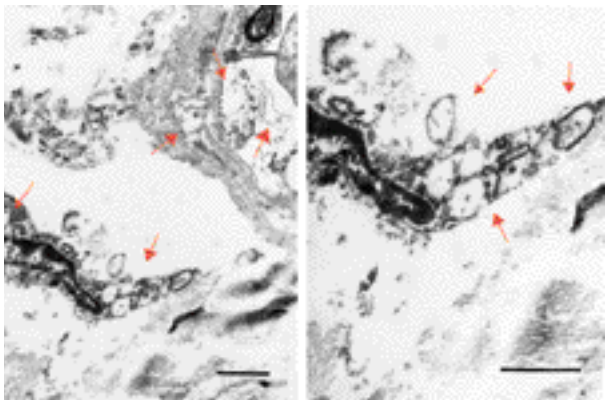

Figure 4: **Transmission electron micrographs of fibrotic perimyocardial tissue from case 2**
Left: perivascular monocyte/macrophage with rickettsia in cytosol (arrow). A cluster of rickettsiae is also located close to the cell (arrow). Rickettsiae are also located free in the vessel, as well as in the endothelium and perithelial (arrows). Reduced by 62% from ×18 000. Bar, 1·0 μm. Right: details of the rickettsia close to the monocyte/macrophage (arrows). Reduced by 62% from ×54 000. Bar, 0·5 μm.

showed 100% homology with the published sequences from *R helvetica* (GenBank accession numbers L36212, U59723, and AF 181036).

An endpoint titre of 1/320 was found for case 1. In case 2 the result was inconclusive, possibly due to haemolysis of the serum collected after death. The 15 control samples were negative or showed titres below 40 when tested by immunofluorescence with *R helvetica* as the antigen.

The light-microscopic observations of rickettsia-like organisms in the perimyocardial tissue of case 1 were verified by electron microscopy. Figure 5 shows several rickettsiae in the endothelium of myocardial vessels, and in the periendothelial and fibrotic tissue outside the vessels.

Discussion

In these two patients, *R helvetica* has been linked to human disease.[21] The relation between *R helvetica* and myocarditis is suggested by positive PCR results with primers for three different genes, documentation of a seroresponse and histopathological change in accordance with riskettsioses, and demonstration of rickettsia-like organisms in the tissues.

Two rickettsiae known to be pathogenic (*R conorii* and *R typhi*) cause the same pattern of disease as shown in our patients. The histological findings in these cases are also similar to those described in other, more fulminant rickettsial diseases. For example a 1996 case report described a patient who died of ventricular arrhythmia due to *R rickettsii*: at necropsy, microscopic examination of many organs, including the heart, showed diffuse vasculitis with disruption of the vessel wall by a predominantly mononuclear-cell infiltrate.[22] All organs examined were positive by immunohistochemistry for riskettsia of the spotted-fever group.

The methods used in diagnosing rickettsial disease are isolation of the organism, PCR, immunohistochemistry, and serology. Isolation is laborious, time consuming, and less sensitive than other methods. *R helvetica* has been isolated only twice, the first time after inoculation into voles (*Microtus pennsylvanicus*), and the second by the shell-vial technique with vero cells,[23,24] For the genus rickettsia, in which few ambiguities in the genetic code are detectable between species, designing of specific primers is difficult. We used genus-specific primers and DNA sequencing of the amplified product. This approach had the advantage of eliminating the most common cause of contamination, namely the positive controls, and also allowed use of species other than *R helvetica* as controls, further lowering the risk of contamination. In some cases duplicate samples, for example the blood in case 2, give different results (ie, one positive, one negative); this discrepancy could be explained by the bacterial load being close to the detection limit. Serology based on cross-reactivity has been known about and used for a century in the Weil-Felix reaction; different *Proteus* strains can discriminate between rickettsiae of the spotted-fever and typhus groups. Nowadays serology is done by microimmunofluorescence. This method is well established, but can give non-specific results, owing to cross-reactivity of antibodies with lipopolysaccharide (common to all rickettsiae of the spotted-fever group.)[18] By this method, a strong serological reaction was seen in case 1. However, because the patient died, titre changes were not possible to follow.

Infectious diseases that show slowly progressive and long courses, due to low virulence of the organism, may result in various late symptoms such as gastric ulcer caused by *Helicobacter pylori*,[25] ischaemic heart disease linked to *Chlamydia pneumoniae*,[26,27] and the tubercula caused by mycobacteria. Our cases showed atypical symptoms of rickettsial disease: neither had fever or rash. Necropsy showed involvement of the heart in both cases; the presence of fibrosis and of low-grade perimyocarditis undergoing healing showed that the infection was slow and persistent. Spotted-fever infections caused by *R conorii* cause signs and symptoms typical of mononucleosis, miliary pericarditis, and pleuritis seen in tuberculosis. In rickettsioses, persistent infection and late sequelae are also well known. For example, Brill-Zinsser disease is a recrudescence of epidemic louse-borne typhus fever occurring in a mild form years after the primary infection.

Seven new rickettsiae causing disease have been identified in the past 14 years; *R helvetica*, isolated from *I ricinus* ticks, seems to be the eighth. Without any signs of primary infection, this spotted-fever rickettsia seems able to cause the same pathological changes in the heart as have been described for other rickettsiae and *O tsutsugamushi*.[12]

A serosurvey in France showed a seroprevalence of about 14% for a spotted-fever group rickettsia other than *R conorii*. The region studied was the same as that in which *R helvetica* was first isolated from *I ricinus* ticks in France.[23]

A previous study showed that about 20% of *I ricinus* ticks in Sweden are infested with *R helvetica*, a figure close to that found in epidemiological studies in ticks in southern Europe.[28] Continuing studies in Sweden indicate seroconversion to *R helvetica* in people exposed to ticks in endemic areas (unpublished data).

The two cases described in our report showed histopathological changes in the tissue similar to those found in known pathogenic rickettsial disease. Although cause and effect cannot be proven from our study, we believe that the spotted-fever rickettsia *R helvetica* is associated with perimyocarditis and possibly other vascular diseases.

Contributors

O Lindquist did the necropsies, identified the index cases, and recorded histopathological changes; K Nilsson and C Påhlson developed and carried out PCR, immunohistochemistry, and serology assays; all investigators contributed to the writing of the paper.

Acknowledgments

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References

1 Pons B, Martin L, Equy C. Rickettsial pericarditis. *Gaz Med Fr* 1965; **72:** 3445–55.

2 Morichaeu-Beauchant G, Brisou J, Dussouil P, de Larroy R, Charpentier. Pulmonary rickettsiosis and autochthonous pleuropericardial rickettsiosis. *J Fr Med Chir Thorac* 1967; **21:** 157–65.

3 Warembourg H, Bertrand M, Vasseur P. Acute benign idiopathic pericarditis: review of 34 cases. *Lille Med* 1968; **13:** 166–75.

4 Grand A, Gallet J. Murine typhus revealed by percarditis. *Mal Coeur Vaiss* 1972; **65:** 637–46.

5 Grand A, Guillermet FN, Despret P, Kreuter M, Hintzy L. Acute percarditis caused by *Rickettsia conorii*. *Arch Mal Coeur Vaiss* 1975; **68:** 1225–31.
6 Pieron R, Coppin M, Develoux M, Mafart Y, Lesobre B. Rickettsial pericarditis and pleurisy. *Semin Hop* 1976; **52:** 621–25.
7 Pieron R, Lesobre B, Mafart Y, Meyniel D, Coppin M. Pulmonary, pleural and pericardial manifestations of rickettsioses. *Poumon Coeur* 1976; **32:** 161–67.
8 Moreno Castillo JL, Sanchez Roman J, Pamies Andreu E, Morales Sandino M, Vazquez Garcia F, Torronteras Santiago R. Pleuripericarditis caused by *Rickettsia mooseri*: an unusual manifestation of murine typhus. *Rev Clin Esp* 1984; **173:** 125–27.
9 Salvi A, Della Grazia E, Silvestri F, Camerini F. Acute rickettsial myocarditis and advanced atrioventricular block: diagnosis and treatment aided by endomyocardial biopsy. *Int J Cardiol* 1985; **7:** 405–09.
10 Marcon G, Callegari E, Scevola MI, et al. Acute rickettsial myocarditis: description of a clinical case and review of the literature. *G Ital Cardiol* 1988; **18:** 72–75.
11 Wohlbach SB, Todd JL, Palfrey FW. The etiology and pathology of typhus. Cambridge, MA: Harvard University Press, 1922.
12 Allen AC, Spitz S. A comparative study of the pathology of scrub typhus (Tsutsugamushi disease) and other rickettsial diseases. *Am J Pathol* 1945; **21:** 603–81.
13 Levine HD. Pathological study of thirty-one cases of scrub typhus fever with especial reference to the cardiovascular system. *Am Heart J* 1946; **31:** 314–28.
14 Smadel JS. Scrub typhus. In: Beeson PB, McDermott W, eds. Cecil-Loeb's textbook of medicine, 11th edn. Philadelphia: WB Saunders Company, 1963: 140–43.
15 Nilsson K, Jaenson TGT, Uhnoo I, et al. Characterization of a spotted fever group rickettsia from *Ixodes ricinus* ticks in Sweden. *J Clin Microbiol* 1997; **35:** 243–47.
16 Nilsson K, Lindquist O, Liu AJ, Jaenson TGT, Friman G, Påhlson C. *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *J Clin Microbiol* 1999; **37:** 400–03.
17 Hultman T, Ståhl S, Hornes E, Uhlén M. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Res* 1989; **17:** 4937–46.
18 Teysseire N, Raoult D. Comparison of Western immunoblotting and microimmunofluorescence for diagnosis of Mediterranean spotted fever. *J Clin Microbiol* 1992; **30:** 455–60.
19 Ricketts HT. A micro-organism which apparently has a specific relationship to Rocky Mountain spotted fever: a preliminary report. In: contribution to medical science by Howard Trevor Ricketts—1870–1910. Published as a tribute to his memory by his colleagues under the auspices of the Chicago Pathological Society. Chicago: University of Chicago Press, 1911: 368–72.
20 Wohlbach BS. Studies on Rocky Mountain spotted fever. *J Med Res* 1919; **41:** 1.
21 Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev* 1997; **10:** 694–719.
22 DiNubile MJ. Reliving a nightmare: a hard (and tragic) lesson in humility. *Clin Infect Dis* 1996; **23:** 160–64.
23 Parola P, Beati L, Cambon M, Raoult D. First isolation of *Rickettsia helvetica* from *Ixodes ricinus* ticks in France. *Eur J Clin Microbiol Infect Dis* 1998; **17:** 95–100.
24 Burgdorfer W, Aeschlimann A, Peter O, Hayes SF, Philip RN. *Ixodes ricinus*: vector of a hitherto undescribed spotted fever group agent in Switzerland. *Acta Trop Med* 1979; **36:** 357–67.
25 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; ii: 1311–14.
26 Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997; **350:** 430–36.
27 Gurfinkel E, Bozovich G, Daroca A, Beck E, Mautner B, for the ROXIS Study Group. Randomised trial of roxithromycin in non-Q-wave coronary syndromes: ROXIS Pilot Study. *Lancet* 1997; **350:** 404–07.
28 Peter O, Burgdorfer W, Aeshlimann A. Epidemiological survey in a natural focus of rickettsia transmitted by *Ixodes ricinus* in Switzerland. *Am Parasitol Hum Comp* 1981; **56:** 1–8.