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# Trace element balance is changed in infected organs during acute *Chlamydophila pneumoniae* infection in mice

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**Abstract** Most infectious diseases are accompanied by changed levels of several trace elements in the blood. However, sequential changes in trace elements in tissues harbouring bacterial infections have not been studied. In the present study the respiratory pathogen *Chlamydophila pneumoniae* (*C. pneumoniae*), adapted to C57BL/6J mice, was used to study whether the balance of trace elements is changed in infected organs. Bacteria were quantitatively measured by real-time PCR in the blood, lungs, liver, aorta, and heart on days 2, 5, and 8 of the infection. Concentrations of 13 trace elements were measured in the liver, heart, and serum by inductively coupled plasma mass-spectrometry (ICP-MS). Infected mice developed expected clinical signs of disease and bacteria were found in lungs, liver, and heart on all days. The number of bacteria peaked on day 2 in the heart and on day 5 in the liver. The copper/zinc (Cu/Zn) ratio in serum increased as a response to the infection. Cu increased in the liver but did not change in the heart. Iron (Fe) in serum decreased progressively, whereas in the heart it tended to increase, and in the liver it progressively increased. *C. pneumoniae* may thus cause a changed trace element balance in target tissues of infection that may be pivotal for bacterial growth.

**Keywords** Bacterial infection · Heart · Liver · Serum · Trace elements

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## Introduction

The host's normal response to infection (the acute-phase response) includes increased synthesis of metal-binding proteins and a concomitant flux of trace elements between blood and tissues (Beisel 2004). The most consistent responses involve a decrease in plasma levels of iron (Fe) and zinc (Zn) and an increase in copper (Cu) (Pekarek 1981; Ilback et al. 2003b; Beisel 2004). An increased Cu/Zn quotient in blood can also be used to indicate infection before the development of clinical disease (Ilback et al. 2003b).

Epidemiological studies support the possibility that increased serum Cu and decreased Zn concentrations may increase the risk of cardiovascular

disease (Alissa et al. 2006). Moreover, many bacteria need Fe for their growth and multiplication and excessive Fe in specific tissues has been shown to promote bacterial infection in those tissues (Al-Younes et al. 2001; Lounis et al. 2001). Among microorganisms with the potential of stimulating the development of chronic inflammation existent in atherosclerotic plaques, interest has primarily been focused on *Chlamydophila pneumoniae* (*C. pneumoniae*) during the past 10 years (Leinonen and Saikku 2002; Belland et al. 2004; Campbell and Kuo 2004).

The obligate intracellular bacterium *C. pneumoniae* is a respiratory pathogen that is estimated to cause about 10% of all community-acquired pneumonia (Kuo et al. 1995). Infection and re-infection are common with approximately 50% of the population being seropositive by the age of 20 years (Grayston 2000). These bacteria have unique biphasic lifecycles, making them particularly prone to establish chronic infections that are unsusceptible to antibiotic treatment (Grayston 2000). *C. pneumoniae* is believed to be spread from the respiratory tract throughout the body by alveolar macrophages (Moazed et al. 1998) and some studies have shown that cardiovascular patients have a higher prevalence of *C. pneumoniae* in peripheral blood mononuclear cells (PBMC) than healthy blood donors (Sessa et al. 2001; Smieja et al. 2002). Mice and rabbits that spontaneously develop atherosclerosis show an accelerated disease process when infected with *C. pneumoniae*, including development of larger and more unstable plaques compared with individuals that are not infected (Campbell and Kuo 2004; de Kruif et al. 2005).

Although changes in some essential trace elements (i.e., Cu, Fe, and Zn) have been described in the blood in various bacterial infections, changes in these and other trace elements have not been studied in *C. pneumoniae* infection. Moreover, to the best of our knowledge, trace element changes in infected tissues during the course of the disease have previously not been studied in any bacterial infection.

The aim of this study was to determine the pattern of changes in a broad range of both essential and non-essential trace elements in serum and in infected organs during the course of acute *C. pneumoniae* infection. Moreover, we wanted to study the sequential distribution of *C. pneumoniae* in different organs.

## Materials and methods

### Mice

Adult female C57BL/6J mice were purchased from Charles River (Copenhagen, Denmark) and maintained at the Animal Department, Biomedical Centre, Uppsala, Sweden. Mice were housed in hygienic barriered cages (TouchSLIMLine, Tecniplast, Scanbur BK A/S, Denmark) at a temperature of 20 ± 1°C and a relative humidity of 50 ± 2%. Water and regular chow diet were supplied ad libitum.

The animal experiments described in this publication took into account all ethical aspects of the welfare of animals following the recommendations in "Guide for the Care and Use of Laboratory Animals" of the Swedish National Board for Laboratory Animals (CFN). The study was approved by the local Ethical Committee for Experimental Use at the Faculty of Medicine, Uppsala University.

### Experimental design

Adult female mice with a mean weight of 24.5 g on day 0 were used in this experiment. The clinical isolate G-954 of *C. pneumoniae* was propagated in Hep-2 cells and stored in sucrose–phosphate–glutamate (SPG) solution at –70°C. A total of 24 mice were divided into four groups; each group containing six mice. On day 0 three groups of mice were infected with $5 \times 10^8$ ifu in 30 µl SPG. The remaining group of mice was sham-inoculated with 30 µl SPG to serve as a control group.

On day 0, mice were sedated using Fluothane (Astra Läkemedel, Södertälje, Sweden) and administered intranasally (i.n.) 30 µl of the inoculum or SPG. Control mice and infected mice were kept in separate cages. Six infected mice were sacrificed on each of days 2, 5, and 8. On each day, sham-inoculated mice ($n = 2$) were concomitantly sacrificed to serve as a healthy control group (total $n = 6$). Body weight and body temperature changes were recorded during the course of the infection.

### Tissue sampling and tissue preparation

Mice from each group were anaesthetized on days 2, 5, and 8 using Fluothane. Blood was collected in

heparinized tubes using heart puncture with a sterile syringe. The thoracic cavity was opened and lungs, liver, aorta, and heart were excised. A sample from the blood and tissue samples of the lungs, liver, aorta, and heart were frozen at –70°C and later DNA was extracted for determination of *C. pneumoniae*. Serum was separated from the remaining whole blood by centrifugation. Serum and the remaining tissue samples of the liver and heart were stored at –20°C until determination of trace elements.

### DNA extraction for *C. pneumoniae* measurements

DNA was extracted from two pieces of each organ, i.e. one piece weighing around 5 mg and the other 15–20 mg. This approach was used to investigate whether the size of the sample influenced the rate of positive *C. pneumoniae* results with the PCR method applied. DNA was also extracted from 200-µl blood. Extraction was carried out with the QiaAmp DNA mini kit (Qiagen) according to the manufacturer's protocol. DNA was eluted in 50 µl buffer AE and then diluted 1:10 in double distilled water and stored at 4°C until further analysis was performed, i.e. within a few days. In each step of extraction a negative (no template) control was processed in the same way as the samples. DNA concentration was measured for each sample with NanoDrop ND-1000, NanoDrop Technologies Inc, Wilmington, DE, USA.

### Real-time polymerase chain reaction (real-time PCR)

DNA samples were subjected to real-time PCR, amplifying a fragment of the *C. pneumoniae ompA* gene (Kuoppa et al. 2002). The PCR mix consisted of 25 µl with 1× TaqMan Universal PCR master mix (Applied Biosystem), 900 nM forward primer (Thermo Hybaid, Germany), 300 nM reverse primer (Thermo Hybaid, Germany), 300 nM probe (Scandinavian Gene Synthesis, Sweden) and 10 µl sample. All samples were run in duplicates, both in full and diluted (1:10) concentration in double distilled sterile water. In all runs at least two no template controls were included. Positive controls consisting of plasmids were included in each run. Plasmid controls were made using a TOPO TA Cloning kit (Invitrogen) with an insert consisting of the gene fragment made with the primers in the PCR reaction. A plasmid standard curve was constructed and used for quantification of each sample. Because a dilution of possible inhibitors can be assumed to increase the accuracy of the *C. pneumoniae* detection, all samples that were positive in dilution 1:10 were used for quantification of *C. pneumoniae*.

### Assessment of trace elements in the heart, liver, and serum

To determine the elements aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), mercury (Hg), selenium (Se), vanadium (V), and zinc (Zn), in heart, liver, and serum, samples were decomposed using ultra-pure nitric acid (Chemscan AS, Elverum, Norway) in a steel bomb (MeAna-Konsult, Uppsala, Sweden). Serum and tissue samples of about 100 mg were weighed (wet weight) and put in quartz tubes. Then 0.5 ml of 65% nitric acid/100 mg sample wet weight was added to the serum and 0.5 ml of 30% ultrapure hydrogen peroxide was also added to the liver and heart samples (Merck, Darmstadt, Germany). Tubes were sealed with a Teflon lid and put into steel bombs, which were sealed with exactly the same momentum. The bombs were then heated in an oven to 180°C for 4 h. After decomposition, an internal standard (indium) was added to a final concentration of 10 µg/l and the samples diluted in 10 ml of high purity water from an ElgaStat UHP (Elga Ltd., High Wycomb, Buckshire, England). The water quality was maintained at more than 18 Mohm-cm. All handling of the samples was done in a clean room. The trace element content of the samples was subsequently measured by inductively coupled plasma mass-spectrometry (ICP-MS; Perkin-Elmer SCIEX ELAN 6000, Perkin Elmer Corp., Norwalk, CT, USA). For quality control, every fifth sample was checked against reference materials Seronorm Trace Element Human Whole Blood (batch OK0336), Serum (batch MIO181) (Sero AS, Bilingstad, Norway) and Bovine Muscle (Community Bureau of Reference, Brussels, Belgium). All reference material measurements for each element were within 8% variation of the stated value and the precision of the measurements was within 5% variation.

### Statistical analysis

Because the experimental design involves one control group and three infected groups, analysis of variance (ANOVA) was applied. For the primary ANOVA, the Kruskal–Wallis test was used to determine whether there were any differences between the four independent groups. In the case of rejecting the null hypothesis in the ANOVA Dunn's method for multiple comparisons was adopted to establish which of the groups differed significantly. This method relies on comparisons of mean ranks. The test statistic is actually the difference between the mean ranks of the groups divided by an adequate standard error. Data are in figures expressed in median and interquartile range (25–75 percentile range). Asterisks (*) in the figures denote a significant difference ($P < 0.05$) between non-infected and infected mice.

## Results

### Clinical response to infection

Mice responded to the infection with expected clinical signs of disease, including ruffled fur and inactivity, as well as a concomitant decrease in body weight (Fig. 1) and body temperature (Fig. 2). Signs were most pronounced on day 5 and at that time one mouse was sacrificed for ethical reasons. Clinical signs then gradually resolved until day 8.

### Tissue distribution of *C. pneumoniae*

The sequential spread of *C. pneumoniae* in the body after the i.n. inoculation was followed in the lungs,

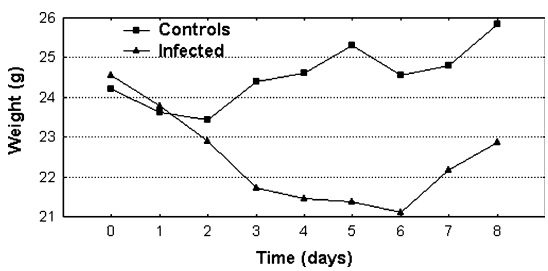


**Fig. 1** Mean body weight of infected and control mice during the time of the study

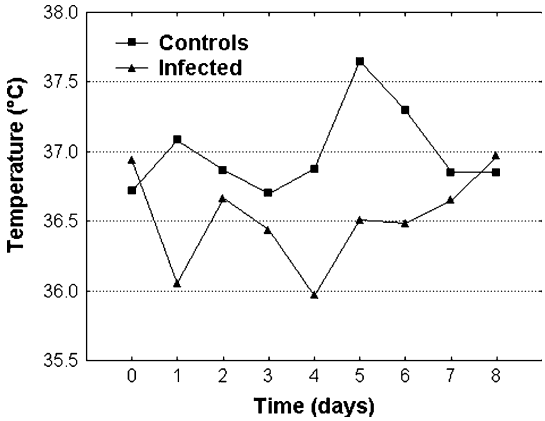


**Fig. 2** Mean rectal temperature of infected and control mice during the time of infection

blood, heart, aorta, and liver. *C. pneumoniae* was found in the blood in three mice on day 2 but in none of the mice on days 5 and 8. The number of mice that were positive for *C. pneumoniae* in different organs on days 2, 5, and 8 are shown in Fig. 3.

The number of *C. pneumoniae* in the heart and liver on days 2, 5, and 8 are shown in Figs. 4 and 5. It was noteworthy that the bacterial counts in the heart peaked on day 2, whereas in the liver they peaked on day 5.

The variation in number of bacteria within each group, as well as the variation between each day of

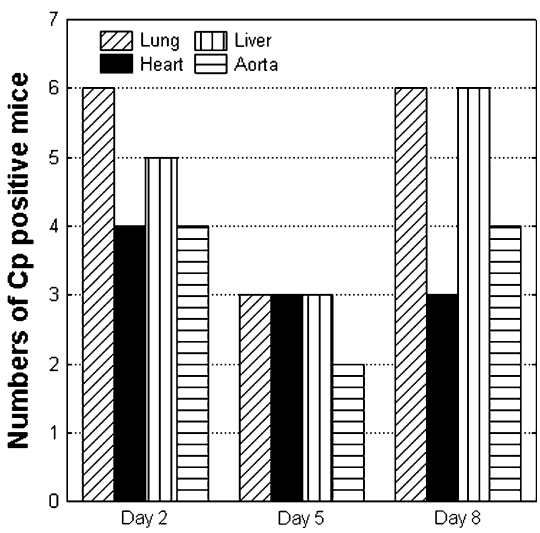


**Fig. 3** Infected mice positive for *C. pneumoniae* in lung, heart, liver, and aorta on days 2, 5, and 8. Total number of animals on day 2 = 6, day 5 = 5, and day 8 = 6

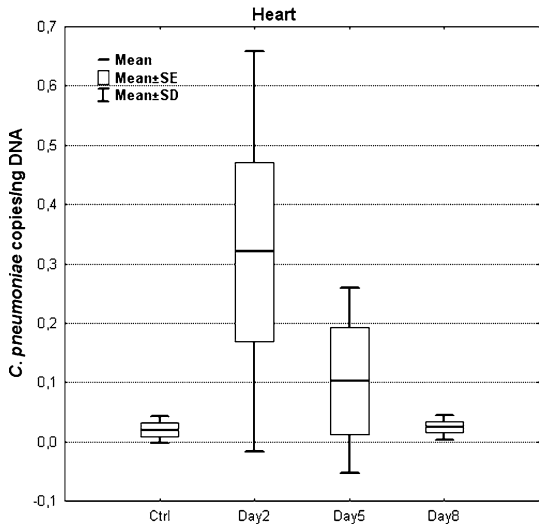


**Fig. 4** *C. pneumoniae* copies/ng DNA in heart biopsies on days 2, 5, and 8 in infected and control mice

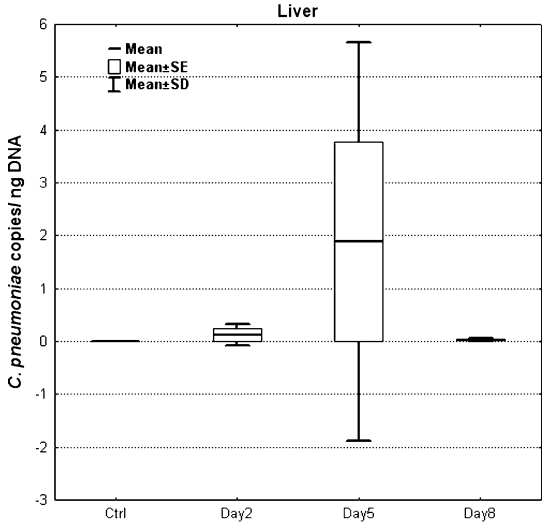


**Fig. 5** *C. pneumoniae* copies/ng DNA in liver biopsies on days 2, 5, and 8 in infected and control mice

sampling, was great. The size of the tissue sample did not seem to have a significant impact on the detection of *C. pneumoniae*, both regarding positivity and number of bacteria in the biopsy specimen. In organs with low copy number of bacteria there were some cases when only one of the biopsy specimens was positive for *C. pneumoniae*. Moreover, some of the control samples tended to show a minor positivity and a cut-off was made on number of *C. pneumoniae* copies/ng DNA in order to make all control samples negative. The reason for the positivity detected in some control organs may be a combination of the very high sensitivity of the PCR method and the problems of tissue preparation and contamination in an animal setting for infected animals. All no-template controls for DNA extractions, as well as for real-time PCR were negative.

## Trace elements in the heart, liver, and serum

Results of all 13 trace elements in the heart, liver, and serum are shown in Tables 1, 2, 3, but only Cu, Zn, and Fe are specifically discussed because of their known association with bacterial infections in general. There were no time-related changes in trace elements in non-infected mice, i.e. the lowest *P*-value was 0.16. The Cu/Zn ratio in serum for each group is presented in Fig. 6. The concentration of Cu in the heart was not changed by the *C. pneumoniae* infection. However, on day 2, the level of Cu in the liver was increased (25%, $P < 0.05$), but on days 5 and 8 it had normalized. The concentration of Cu in serum increased progressively on days 2 (7%, ns), 5 (30%, ns), and 8 (51%, $P < 0.05$).

The concentration of Zn in the heart was not changed by the *C. pneumoniae* infection. Although not significant, there was a clear tendency of an increased (>40%) concentration of Zn in the liver on days 2 and 5. Zinc levels in serum did not show any significant changes during the infection. However, an increase of the Cu/Zn molar ratio (98%, $P < 0.05$), which is a well-known marker of infection, could be observed at the peak of disease, i.e. on day 5.

Fe tended to be increased in the heart on all sampling days. In the liver, Fe increased progressively through the course of the disease to reach a level of 26% ($P < 0.05$) above that of controls on day 8. These changes in liver Fe contrast to the Fe concentrations in serum, where Fe decreased progressively through the course of the disease to reach a level of 63% ($P < 0.05$) below that of the controls on day 8.

Other studied trace elements showed no consistent or clear pattern of changes. However, noteworthy findings were changes in Co in the liver that significantly increased on days 2 and 5, Se that decreased in the heart during the infection and the decreased concentration of As in the liver.

**Table 1** Concentrations (ng/g wet weight) of selected elements in the heart of infected and sham-inoculated control mice on days 2, 5, and 8 ($n = 6$ in all groups except day 5 where $n = 5$)

| Element | Element concentration (ng/g wet weight) in heart | | | |
|---|---|---|---|---|
| | Controls | Day 2 | Day 5 | Day 8 |
| Magnesium (Mg)[a] | 243 (10) | 255 (15) | 238 (11) | 231 (19) |
| Aluminium (Al) | 63 (36) | 67 (30) | 27 (10) | 54 (22) |
| Calcium (Ca)[a] | 36.5 (3.4) | 36.0 (3.5) | 37.7 (6.6) | 37.2 (3.0) |
| Vanadium (V) | 0.2 (0.1) | 0.1 (0.1) | 0.2 (0.1) | 0.2 (0.1) |
| Manganese (Mn) | 309 (16) | 331 (12) | 283 (11) | 278 (31) |
| Iron (Fe)[a] | 87.5 (5.5) | 99.9 (16.3) | 96.0 (18.4) | 95.7 (12.9) |
| Cobalt (Co) | 18.7 (1.6) | 20.0 (0.9) | 19.9 (2.3) | 18.8 (2.1) |
| Copper (Cu)[a] | 6.5 (0.3) | 6.9 (0.3) | 6.4 (0.2) | 6.1 (0.6) |
| Zinc (Zn)[a] | 18.0 (0.8) | 18.9 (0.9) | 17.4 (1.0) | 18.2 (2.1) |
| Arsenic (As) | 18.1 (2.9) | 20.0 (6.5) | 16.5 (5.7) | 13.9 (4.5) |
| Selenium (Se) | 603 (138) | 815 (168) | 555 (147) | 360 (80) |
| Cadmium (Cd) | 2.5 (0.7) | 2.6 (0.6) | 2.2 (0.4) | 2.5 (0.4) |
| Mercury (Hg) | 0.4 (0.1) | 0.3 (0.1) | 0.4 (0.1) | 0.4 (0.2) |

[a] Concentrations in μg/g wet weight

Data expressed as mean (standard deviation)

Asterisks (*) denote a significant difference (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between non-infected and infected mice

**Table 2** Concentrations (ng/g wet weight) of selected elements in the liver of infected and sham-inoculated control mice on days 2, 5, and 8 ($n = 6$ in all groups except day 5 where $n = 5$)

| Element | Element concentration (ng/g wet weight) in liver | | | |
|---|---|---|---|---|
| | Controls | Day 2 | Day 5 | Day 8 |
| Magnesium (Mg)[a] | 225 (15) | 256 (15)* | 240 (14) | 226 (16) |
| Aluminium (Al) | 26 (9) | 35 (19) | 30 (18) | 30 (11) |
| Calcium (Ca)[a] | 34.7 (5.0) | 39.6 (3.7) | 40.9 (5.7) | 33.3 (2.8) |
| Vanadium (V) | 0.9 (0.2) | 0.7 (0.1) | 0.8 (0.1) | 1.0 (0.2) |
| Manganese (Mn) | 518 (73) | 570 (109) | 427 (80) | 454 (38) |
| Iron (Fe)[a] | 136 (22) | 162 (19) | 184 (37) | 172 (35)* |
| Cobalt (Co) | 44.8 (4.5) | 58.8 (5.6)* | 67.5 (17.6)* | 60.2 (11.4) |
| Copper (Cu)[a] | 4.0 (0.5) | 5.0 (0.3)* | 4.6 (0.6) | 3.9 (0.3) |
| Zinc (Zn)[a] | 30.8 (8.3) | 43.7 (8.8) | 44.0 (15.8) | 29.1 (3.1) |
| Arsenic (As) | 9.1 (3.8) | 8.4 (6.3) | 4.4 (2.2) | 4.8 (3.9) |
| Selenium (Se)[a] | 2.0 (0.3) | 2.4 (0.2) | 2.0 (0.2) | 1.8 (0.1) |
| Cadmium (Cd) | 12.0 (3.1) | 15.1 (1.7) | 15.2 (2.6) | 13.1 (3.0) |
| Mercury (Hg) | 1.7 (0.1) | 1.8 (0.2) | 1.7 (0.1) | 1.6 (0.1) |

[a] Concentrations in μg/g wet weight

Data expressed as mean (standard deviation)

Asterisks (*) denote a significant difference (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between non-infected and infected mice

**Table 3** Concentrations (ng/g wet weight) of selected elements in serum of infected and sham-inoculated control mice on days 2, 5, and 8

| Element | Element concentration (ng/g wet weight) in serum | | | |
|---|---|---|---|---|
| | Controls ($n$ = 6) | Day 2 ($n$ = 4) | Day 5 ($n$ = 3) | Day 8 ($n$ = 6) |
| Magnesium (Mg)[a] | 25.5 (2.0) | 22.3 (1.5) | 20.6 (1.8) | 26.5 (4.2) |
| Aluminium (Al) | 94 (31) | 68 (24) | 69 (46) | 92 (27) |
| Calcium (Ca)[a] | 86.0 (7.8) | 87.0 (10.3) | 92.9 (2.4) | 94.5 (5.4) |
| Vanadium (V) | 0.9 (0.2) | 0.7 (0.1) | 0.8 (0.1) | 1.0 (0.2) |
| Manganese (Mn) | 14.2 (3.5) | 13.3 (2.1) | 12.7 (2.3) | 12.2 (2.4) |
| Iron (Fe)[a] | 14.8 (5.7) | 9.1 (5.2) | 5.4 (1.8) | 5.5 (3.3)* |
| Cobalt (Co) | 3.2 (1.1) | 2.8 (0.2) | 4.7 (1.6) | 3.6 (1.4) |
| Copper (Cu) | 632 (84) | 675 (135) | 820 (272) | 953 (170)* |
| Zinc (Zn) | 983 (157) | 947 (100) | 645 (168) | 987 (97) |
| Arsenic (As) | 8.4 (2.9) | 9.7 (6.0) | 12.6 (2.4) | 7.9 (3.4) |
| Selenium (Se) | 581 (186) | 725 (48) | 671 (80) | 611 (52) |
| Cadmium (Cd) | 2.7 (0.4) | 2.0 (0.3) | 1.5 (0.4) | 2.3 (0.7) |
| Mercury (Hg) | 0.7 (0.4) | 0.3 (0.1) | 0.3 (0.1) | 1.2 (0.2) |

[a] Concentrations in μg/g wet weight

Data expressed as mean (standard deviation)

Asterisks (*) denote a significant difference (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) between non-infected and infected mice

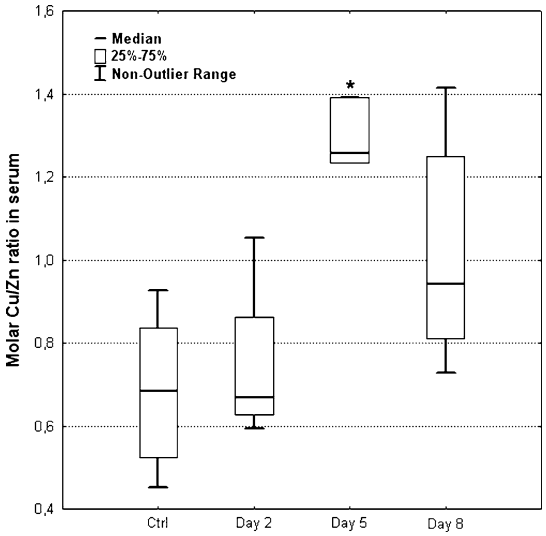


**Fig. 6** Copper/Zinc ratio in serum on days 2 ($n$ = 4), 5 ($n$ = 3), and 8 ($n$ = 6) in infected and control mice ($n$ = 6)

## Discussion

In the present study tissue and serum samples were first taken on day 2 when pneumonia is known to be fully developed in this mouse model of *C. pneumoniae* infection (Yang et al. 1993). Additional samples were subsequently taken on day 5 and, finally, on day 8 when the clinical signs of acute infection had resolved. Thus, the peak of the infection seemed to occur on days 4 to 5 regarding the development of clinical symptoms, changes in body weight, and the markedly elevated Cu/Zn ratio in serum. At some time during the course of the disease, all infected mice, except for one mouse, showed bacterial colonization in the lungs, liver, or heart. In addition, the bacterial load in the heart seemed to peak on day 2 and in the liver on day 5, although the number of mice being positive for *C. pneumoniae* in the liver was highest on day 8, with all mice being positive. Expected changes in Fe, Cu, and Zn occurred in serum. However, a new and noteworthy finding was the concomitant increase of Fe in the liver and a similar tendency of an increase of Fe in the heart. It was also worthy to note the different responses in this bacterial infection compared with viral infection regarding changes in essential and non-essential trace elements in infected organs (Ilback et al. 2003b)

During the course of most infections, trace elements, both essential and non-essential, are redistributed between organs involved by the disease process (Ilback et al. 2004). For instance, there is a release of the major Cu-containing plasma protein

ceruloplasmin from the liver, concomitant to an uptake of Zn in the liver, which is typical to infectious diseases regardless of aetiology (Beisel 2004). The Cu-containing ceruloplasmin is an important acute-phase reactant in generalized infection (Beisel 2004) and the Cu level in plasma is known to increase in infections that are caused by various microorganisms (Friman et al. 1982; Ilback et al. 1983; Funseth et al. 2000; Ilback et al. 2003a; Beisel 2004). In addition, ceruloplasmin is an inflammatory marker and its serum concentrations have been reported to be high in atherosclerotic patients (Manttari et al. 1994). Thus, these well-established serum markers of disease responded also in this *C. pneumoniae* infection with an expected increase in serum Cu/Zn ratio on day 5. We have observed in viral infections that the peak of this increase is highly related to the peak of disease (Funseth et al. 2000; Ilback et al. 2003a; Beisel 2004). In studies using coxsackievirus infection in the mouse we have found that the increase in serum Cu is associated with a decrease of Cu in the liver, possibly because of mobilization of Cu from the liver into target organs affected by the disease (Ilback et al. 2003b; Ilback et al. 2004). Thus, the present result of an increase of Cu in the liver during the course of bacterial infection is a different response compared with that observed in viral infections.

In coxsackievirus infection there is an early increase of Fe in the serum and a concomitant increase in the liver (Funseth et al. 2000; Ilback et al. 2003a). However, in the present bacterial infection Fe tended to increase in the heart and in the liver, even though serum levels of Fe were progressively decreased during the course of the disease. The protein hepcidin is produced in the liver and regulates serum Fe levels in infection and inflammation by regulating the absorption of Fe from the intestine (Ganz and Nemeth 2006). Hepcidin has been reported to be induced in bacterial infections, which may explain the present results (Motley et al. 2004). This is in contrast to viral infections where the gene expression of hepcidin only shows a minor increase during the course of the infection (Ilback et al. 2007).

Fe is believed to be essential for the growth of *C. pneumoniae* because Fe restriction in cell cultures inhibits the growth of the bacteria (Al-Younes et al. 2001; Freidank et al. 2001). We have previously found a 20-fold increase in Fe concentration in sclerotic aortic heart valves in patients compared with aortic heart valves in healthy individuals (Nystrom-Rosander et al. 2003). A significant number of these sclerotic heart valves were positive for *C. pneumoniae* nucleic acid. The Cu/Zn ratio was also significantly higher in sera from these patients compared with blood donor sera (Nystrom-Rosander et al. 2004). Measurements of Fe and Cu in atherosclerotic plaques have shown significantly higher concentrations of these elements as compared with the levels found in arterial samples from healthy individuals (Stadler et al. 2004). Endothelial cell activation by *C. pneumoniae* infection has also been shown to be enhanced by Fe supplementation when compared with *C. pneumoniae* infection in cells not supplemented with Fe (Kartikasari et al. 2006). Moreover, this Fe-induced activation could be reversed by Fe chelation.

In conclusion, we demonstrated an elevated Cu/Zn ratio in serum in *C. pneumoniae*-infected mice, a well-known marker of infectious diseases and an indicator of increased risk of cardiovascular disease. Moreover, the concentration of Cu was increased in the liver, and Fe was elevated in the liver and the heart though the concentration of Fe concomitantly decreased in serum. Although changes in Fe in the heart were not significant, the pattern of Fe changes in all studied compartments indicates that Fe may be associated with the growth of *C. pneumoniae*. Thus, *C. pneumoniae* infection seems to cause a change in the trace element balance in infected organs that may be pivotal for bacterial growth and development of adverse cardiovascular effects.

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