

SYMPOSIUM I:
Respiratory Zoonoses

ZOONOTIC RESPIRATORY DISEASES CAUSED BY VIRUSES

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Respiratory diseases of men caused by infectious agents originating from animals rank among the potentially most devastating infectious diseases in humans. The pandemic influenza of 1918/1919 was apparently caused by an avian influenza virus which directly transmitted to humans without further reassortment. It resulted in an estimated 20-50 million deaths in the human population exceeding the toll exacted by the first world war. The current epidemic of highly pathogenic avian influenza of subtype H5N1 in the wild bird and poultry population on three continents is threatening since the virus has the capacity to infect humans by close contact to infected wild birds or poultry. Although notified infections of humans by H5N1 are still rare overall, the death rate is significant and human deaths by H5N1 HPAI infection have been recorded from eight countries in Asia and Africa. Thus, due to its wide distribution and potential high pathogenicity for humans, HPAI H5N1 is regarded by many as a candidate for a new pandemic human influenza virus. Worldwide efforts are undertaken to protect poultry from H5N1 infection which also limits exposure of humans. The appearance of HPAI H5N1 in Central Europe this spring has led to an increase in awareness and surveillance also in Germany which is paralleled by increased research funded by national and international institutions. Efforts focus on epidemiology, improvement of diagnostics and vaccines as well as a better understanding of the molecular requirements for AIV pathogenicity in birds and other species.

However, animals are the source of other zoonotic viral infections which infect humans via the respiratory tract and cause either respiratory symptoms or infection of other organs. In particular bats are an important reservoir for zoonotic viruses which include coronaviruses, among them the notorious SARS virus as well as paramyxoviruses, e.g. Nipah and Hendra viruses, and rhabdoviruses such as bat lyssaviruses. Rodents harbour hantaviruses which can also cause serious disease in humans. A recent increase in the detection of hantavirus infections in humans in certain parts of Germany has been observed. Unfortunately, our knowledge of wildlife as a reservoir for potentially zoonotic infectious agents is still rather rudimentary and an intense screening of wildlife populations for viral infectious agents is necessary to gain a more complete picture. To achieve this goal, a special research program on zoonoses has been established by the German government which focuses in particular on bringing together veterinary and human health specialists. This cooperation is essential in the understanding and subsequent control of zoonotic infections in humans.

CHLAMYDIAE AS AGENTS OF RESPIRATORY INFECTIONS

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Chlamydiae are a group of obligately intracellular bacteria that can generate a variety of clinical diseases ranging from acute self-limiting infection, as in the case of human urogenital disease, neonatal conjunctivitis and pneumonia, to chronic inflammation, as in trachoma, pelvic inflammatory disease, reactive arthritis, chronic obstructive pulmonary disease (COPD) and cardiovascular disease. Apart from this, chlamydial infections often take a mild or subclinical course.

Historically, the first scientific account of human chlamydiosis dates back to 1879, when J. Ritter published a report on an outbreak of severe "typhoid pneumonia" near Zurich (Switzerland) involving seven persons who had previous contact with sick "exotic birds". Although there were recurring and even larger outbreaks in Berne, Paris (termed "psittacosis") and Berlin during the following decades, and epidemics following imports of South American psittacine birds in Europe and North America in 1929, it took researchers until 1930 to isolate the causative agent, which was initially believed to be a virus or rickettsia. With the advent of cell culture techniques and electron microscopy it became evident that the agents of psittacosis and other chlamydioses were bacteria, so that the genus *Chlamydia* was defined by L.A. Page in 1966.

The remarkable variety of clinical manifestations of chlamydial diseases should, at least in part, be a consequence of the distinctive features of the causative agents, particularly their unique biphasic developmental cycle. In the course of a replication cycle, infectious, but metabolically inactive elementary bodies (EBs) evolve into non-infectious, but metabolically active reticulate bodies (RBs). The latter reside in a vacuole-like inclusion of the host cell and undergo binary fission before transforming back into elementary bodies to start a fresh cycle. This life cycle enables the pathogen to pursue distinctive survival strategies in the host, which are giving rise to the evasion of host defence, as well as chronic and persistent courses of infection.

Chlamydial respiratory disorders in humans comprise psittacosis or "atypical pneumonia" (caused by *Chlamydophila psittaci*), pneumonia, asthma and COPD (associated with *Chlamydophila pneumoniae*) and neonatal pneumonia (caused by *Chlamydia trachomatis*). Although psittacosis has become a rare disease nowadays, there are reports of severe cases in Europe and North America virtually every year which demonstrate that this zoonotic infection deserves more attention. Typical signs include an initial flu-like phase with headache, fever and weariness. Symptoms of an atypical pneumonia (dry cough) usually appear towards the end of the first week. Severe and life-threatening cases are often due to the absence of proper laboratory diagnosis precluding the application of effective antibiotic treatment.

In a recent outbreak of psittacosis in Germany, 18 persons in contact with a poorly managed poultry flock became infected, of which 7 had to be hospitalised. One of the major conclusions from the experience of this event is that rapid species-specific detection of the causative agent is a crucial prerequisite for the elucidation of the epidemiological chain and the identification of persons directly affected and at risk. Moreover, close and unbureaucratic cooperation between veterinary and human health authorities, as well as veterinary and human diagnostic laboratories, is indispensable for rapid and efficient control of this zoonotic infection.

HUMAN Q FEVER CASES

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Q fever is a zoonosis with a worldwide distribution. The causative agent *Coxiella (C.) burnetii* is a small, intracellular bacterium. The reservoir is large and includes domestic animals like cattle, sheep and goat. These ruminants represent the most frequent source of human Q fever (Tab. 1). In ruminants *C. burnetii* causes abortions. Due to the multiplication of the agent in the trophoblasts of the placental villi large amounts of coxiellae are shed during parturition with the placentae and birth fluids. The route of acquisition of *C. burnetii* infections is by aerosol. Clinical findings of human Q fever are severe fever, chills, and headaches. The infection may be present with acute or chronic clinical manifestations. However, many cases of Q fever are asymptomatic (Maurin and Raoult, 1999).

Over the last 10 years in Germany outbreaks and sporadic cases of Q fever have involved 46 – 416 people per year. Persons at greatest risks are those who have contact with farm animals and include farmers, veterinarians and abattoir workers.

Year	Place	Number of infected people	Source
1997	Gießen, Hessen	47	sheep
1997	Stuttgart Baden-Württemberg	13	fallow-deer
1998	Freiburg/ Breisgau Baden-Württemberg	101	sheep?
2003	Bad Sassendorf Nordrhein-Westfalen	299	sheep
2003	Baden-Württemberg	9	cattle
2005	Jena Thüringen	312	sheep

Tab. 1: Ruminants as source of Q fever cases

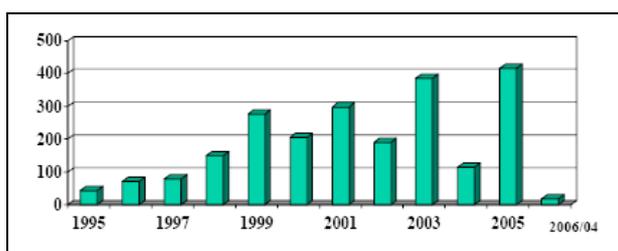


Fig. 1: Cases of human Q fever in Germany (data: Robert Koch-Institut)

In 2005 312 human Q fever cases from an outbreak in Jena/Thüringen were reported. The outbreak was associated with a flock of 500 ewes which pastured near a suburban colony. Between June 2nd and June 18th 35 lambs were born. On June 19th the flock was put to grass on a pasture-land which was located far from the settlement. No clinical symptoms or abortions were observed during this period.

In humans the first cases with symptoms of Q fever were reported in the week from June 13th to June 19th. On June 27th the veterinary board was informed by the sanitary board because of the increase of cases of atypical pneumonia. The number of cases of Q fever in this period are shown in Fig. 1. A flock of sheep were suspected as source of infection. Clinical investigation of the flock showed not evidence for an infection with *C. burnetii*. On 30th the first case of human Q fever was confirmed by serological positive results. Therefore the sheep were investigated for antibodies using the ELISA test: 23 of 50 sheep were positive for antibodies against *C. burnetii*. 51 Vaginal swabs and material from

abortions (14 samples) was taken and investigated for the Q fever agent by PCR and cell culture. The Q fever agent could be demonstrated in 1 vaginal swab and 4 samples of abortion material. Epidemiological investigation showed many risk factors for a Q fever epidemic. The high rate of serological positive ewes (46%) and the fact that the Q fever agent could be demonstrated in different samples may be a hint that *C. burnetii* was shed during the lambing. Also the weather at the time of the outbreak in Jena was typical for Q fever situations: cold days changed with days of high temperature (up to 30°C). There were only a few days with rain but days with development of dust. These factors may be reasons for a possible contamination of the suburban colony and its 11,000 inhabitants.

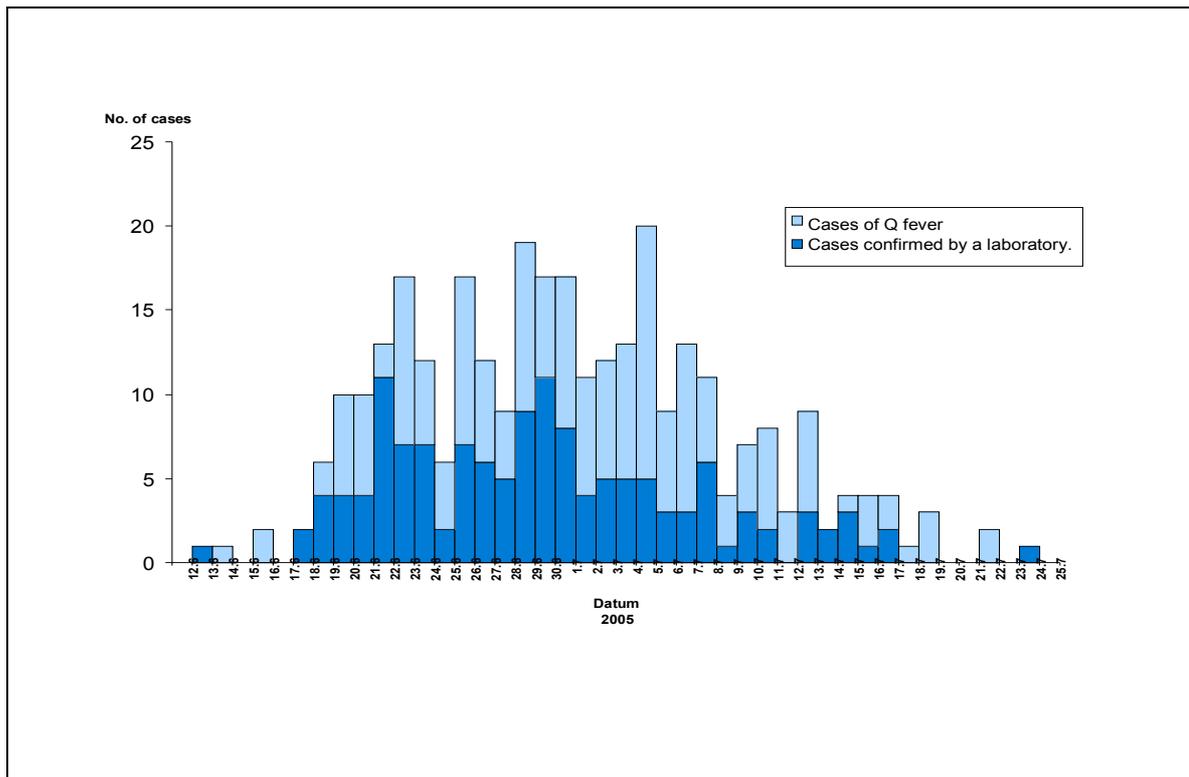


Fig. 2: Cases of human Q fever in Jena from June 13th to July 24th

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EPIDEMIOLOGICAL INVESTIGATION OF *COXIELLA BURNETII* IN SHEEP IN LOWER SAXONY, GERMANY

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INTRODUCTION

Q fever is a mainly airborne zoonosis induced by the highly contagious, obligate intracellular bacteria *Coxiella (C.) burnetii*. In animals, Q fever affects livestock and is associated with reproductive disorders, abortion, still-birth, delivery of weak and unviable newborns, placentitis, endometritis, infertility and pneumonia. Q fever in humans is characterized by acute and chronic courses. Acute Q fever usually presents a flu-like, self-limiting disease accompanied by myalgia, enteritis and severe headache, but often complications such as pneumonia or hepatitis may occur. In chronic cases, endocarditis is the main severe complication in patients with valvulopathies. Granulomatous hepatitis, vasculitis, osteomyelitis, post-Q fever fatigue syndrome and premature delivery or abortion has also been reported. Based on epidemiological evidences, the main route of infection in humans is inhalation of contaminated aerosol or dust containing bacteria shed by infected animals especially sheep with placenta or vaginal secretions, milk, and faeces. However, oral transmission is also discussed and the consumption of contaminated raw milk and dairy-products represents a potential source of human infection (Maurin and Raoult 2002, Kloppert et al. 2004, Woldehiwet 2004, Arricau-Bouvery and Rodolakis 2005, Arricau-Bouvery et al. 2006).

More than fifty outbreaks of Q fever in humans were registered in Germany from 1947 to 2005. 14 outbreaks occurred during the last 5 years. Sheep were involved in about 30 of the outbreaks and in almost all outbreaks during the last 5 years. Most of these outbreaks were reported from the south- and south-west regions of Germany, but reports on outbreaks in mid and northern regions of Germany increase (Hellenbrand et al. 2001). One of the largest outbreaks in humans in Germany happened in 2003 in North-Rhine Westphalia. Nearly 300 individuals were infected with *C. burnetii* after parturition of live twin lambs during a farmers market in Bad Sassendorf. Many of them were hospitalised (Robert Koch Institut 2003). Another large outbreak occurred in July 2005 in Thuringia. During this outbreak about 340 individuals were infected with *C. burnetii* released from birth products from ewes of a flock migrating at the outskirts of Jena (Zemke 2005). It seems that the pathogen spreads to the northern parts of the country.

Systematic epidemiological information about the distribution of *C. burnetii* in sheep, especially in the northern parts of Germany, is lacking (Sting et al. 2004). Routine monitoring is not established in any European country. In most countries Q fever is not even notifiable.

In humans Q fever is an underestimated disease. Due to the unspecific clinical symptoms Q fever is often not diagnosed except during epidemics. But even under epidemic conditions Q fever is often not the first choice of differential diagnosis. So for example the Bad Sassendorf outbreak was first diagnosed as SARS. The aim of our studies was to get more information about the epidemiological situation of *C. burnetii* in sheep.

MATERIALS AND METHODS

In 2004 a total of 1732 serum samples of 1714 sheep from 95 flocks were investigated for antibodies against *C. burnetii* by means of an ELISA. Additionally, 488 milk, 65 colostrum and 7 placenta samples from 560 sheep of 55 flocks were tested for *C. burnetii*-specific DNA-sequences by PCR. A questionnaire was also answered by shepherds on the date of sampling. Flock size, number of abortions, number of losses, hygiene-measures practiced, vaccinations against abortion, investigation results concerning abortions, other species held on the farm bar sheep (especially cats), were items investigated. 88 questionnaires were answered.

In a follow-up study in one serological positive flock samples were taken during lambing season. 95 serum, 88 colostrum, 88 placenta samples were obtained from 100 ewes within 4 days post partum. In addition, 11 aborted fetuses were collected. Another 203 serum samples were collected some weeks after lambing.

RESULTS

The flocks were divided into 4 groups depending on the management system. The flocks with grazing enclosure (K, n=50) had on average 54 ewes. Their mean abortion rate (2.0 %) was the lowest within the 4 groups. In milk producing flocks (M, n=15), which on average had 30 ewes, the mean abortion rate was 3.6 %. In shepherded flocks (H, n=12) sheep were kept in stable at night, but were tended in the surroundings during day-time and did not leave their grazing area around the barn. They had 389 ewes per flock on average and a mean abortion rate of 2.6 %. The migrating flocks (W, n=11) grazed in several districts and had 666 ewes on average per flock. The mean abortion rate was 4.0 %. There was a significant difference ($P < 0.05$, t-test) between the 4 management systems in flock size, but no significant difference ($P > 0.05$) could be detected in the abortion rate, despite a linear correlation between the herd size and the abortion rate over all flocks.

47 (2.7 %) serum samples were positive and 23 (1.3 %) were inconclusive for antibodies against *C. burnetii*. Positive and inconclusive results were obtained from 9 flocks. More than one positive or inconclusive sample could be found in 3 of these 9 flocks. These 3 flocks were migrating flocks with pasture in the southern part of Lower Saxony. The intra-flock prevalence (=IFP, positive and inconclusive results summarised) in these flocks were 0.48, 0.26 and 0.05. There was no correlation between *C. burnetii* IFP and the abortion rates. But in the flock with the highest IFP, abortion rate and neonatal losses increased in spring 2004. In the remaining 2 flocks, 4 inconclusive samples were found.

C. burnetii-specific DNA sequences could be detected in 4 out of 560 samples (3 milk samples, 1 colostrum sample). The 4 positive samples were obtained from 4 different flocks. The serological investigation in all 4 flocks proved to reveal negative results.

During lambing season 2005 further investigations were performed in the positive flock with an IFP of 0.26 in 2004. In this flock with 1200 ewes the abortion rate in 2005 was less than 1 %. The lamb losses counted 5.4 %. All aborted fetuses with their placentas were negative in *C. burnetii* PCR but positive in Chlamydia PCR. 30 % of the placenta samples of normal births were *C. burnetii* PCR-positive. Additionally in 12.5 % of the colostrum samples *C. burnetii*-DNA was found by PCR. The sero-prevalence was 0.27.

CONCLUSIONS

The survey of 2004 shows on an average a low *C. burnetii* sero-prevalence in sheep in Lower Saxony. But it is noticeable that 3 flocks with positive results graze in the southern parts of the country and are migrating flocks. Due to the large radius of grazing of all 3 flocks, spreading of *C. burnetii* occurs. The flock with the highest IFP in 2004 (0.48) was

probably the source of some human Q fever cases in 2006. Investigations and control measurements are in progress in this flock. In spring 2005 *C. burnetii* could also be detected by PCR in dust sampled during shearing of another flock with high IFP in 2004 (0.27) (Schulz et al. 2005).

To avoid transmissions to humans the control measurements recommended by the Robert Koch Institut, Berlin, should be complemented by vaccination with a phase I-vaccine which seems to be able to reduce shedding (Arricau-Bouvery et al. 2005).

Investigations of aborted fetuses and their placentas are not always the best specimens, especially when co-infections with *Chlamydophila abortus* occurred in the flock. The results of the investigations in the positive flock in 2005 reveal that placentas of normal births are better specimens in flocks with mixed infections. These investigations also show that in flocks enzootically infected, clinical signs, especially an increase in abortion rate, may not occur due to Q fever. So neither the shepherd nor the veterinarian may be aware of the disease. Whether the spread of *Dermacentor marginatus* or other ticks is necessary for the spread of *C. burnetii* has to be investigated. When contaminated dust and vaginal discharge can infect humans, it is also likely that sheep can be infected by aerosols.

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GLANDERS, A RE-EMERGING DISEASE IN HORSES

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Glanders is a highly contagious infectious disease of solipeds. The causative agent of glanders is *Burkholderia (B.) mallei*, a gram-negative bacillus primarily noted for producing disease in horses, mules, and donkeys. The acute forms are more common in mules and donkeys, and death typically occurs within 3 to 4 weeks. The chronic form of the disease is more common in horses. Furthermore, infections with *B. mallei* have been reported in sheep, goats, camels, wolves, bears, cats, dogs and carnivores in zoos after having been fed with meat of infected horses (Scholz et al., 2006). Several laboratory animals are susceptible to infection, including hamsters and guinea pigs. The susceptibility of the latter species formed the basis of the Strauss reaction in the diagnosis of the disease. Swine and cattle are resistant to infection with *B. mallei*. Glanders is also considered to be a life-threatening zoonosis causing severe systemic infections in humans. The disease is introduced into horse populations by diseased or latently infected animals. Ingestion of the pathogen, present in secretions from infected animals, constitutes the major route of infection in glanders. Transmission is facilitated if the animals share feeding troughs or watering facilities (Wittig et al., 2006).

Clinical descriptions of glanders distinguish between cutaneous, nasal, and pulmonary forms of the disease, but in most outbreaks these manifestations may occur simultaneously in the same animal. Chronic infections with slow progression of the disease are more common than the acute form of glanders. The acute form typically progresses to death within about a week. The nasal form of glanders is characterised by unilateral or bilateral nasal discharge. The yellowish-green exudate is highly infectious. The nasal mucosa has nodules and ulcers. These ulcers may coalesce to form large ulcerated areas, or they may heal as stellate scars of the mucosa. In some cases the septum may even be perforated. Nasal lesions are accompanied by enlargement and induration, or sometimes rupture and suppuration, of regional lymph nodes. In the cutaneous form of glanders, multiple nodules may develop in the skin of the legs or other parts of the body. These nodules may rupture, leaving ulcers that discharge a yellow exudate to the skin surface and heal slowly. Cutaneous lymphatic vessels in the region become involved. They become distended and firm by being filled with a tenacious, purulent exudates. In the pulmonary form of glanders, lesions in the lungs develop in combination with nasal and cutaneous lesions or may be the sole manifestation of the disease (Wittig et al., 2006).

The organism spreads to man by invading the nasal, oral, and conjunctival mucous membranes, by inhalation into the lungs, and by invading abraded or lacerated skin. Aerosols from cultures have been observed to be highly infectious to laboratory workers. The human form of the disease is painful and frequently fatal. Laboratory workers and animal attendants are most at risk. Work with this organism in the laboratory requires biosafety level 3 containment practices. Since aerosol spread is efficient, and there is no available vaccine or really dependable therapy, *B. mallei* has been viewed as a potential biological weapon agent (Neubauer et al., 1997).

B. mallei may be cultured from fresh lesions or lymph nodes. The agent grows slowly on ordinary nutrient agar, but growth is accelerated with addition of 1-5% glucose and or 5% glycerol. A variety of molecular diagnostic tests are available (Scholz et al., 2006; Tomaso et al., 2006). Furthermore, several serologic tests for glanders have been developed (Neubauer et al., 2005). They are superior to mallein testing in sensitivity and specificity.

The complement fixation test (CFT) is the prescribed test for international trade with horses widely used.

Glanders has been eradicated from many countries by statutory testing, elimination of infected animals, and import restrictions. The disease is currently limited to parts of Africa, the Middle East, and Asia (specifically Turkey, Syria, Iraq, Iran, Pakistan, India, Burma, Indonesia, the Philippines, China, and Mongolia) and possibly the Balkan states, former Soviet republics, Mexico, and South America. During the last 14 years, outbreaks of glanders have been reported from India, Iran, Iraq, Pakistan, and Brazil. In 2004, the United Arab Emirates notified equine glanders to the Office International des Epizooties. (Scholz et al., 2006).

Germany has been free of glanders since 1957. However, through the globalisation and international trade of horses, the introduction of the disease from endemic areas into free populations is always possible.

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HUMAN BRUCELLOSIS – A CASE REPORT

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Background: Brucellosis is an emerging zoonosis worldwide, but still not very well known in non-endemic countries like Germany.

Case report: Three months before hospital admittance a 15 year old girl showed a lightening of the 7th and 9th thoracic vertebra. A bothsided episcleritis was found. For eight weeks she complained about perspiration at night, and moderate fever. Computertomography revealed coarsely spotted infiltrates in both lower fields of the lungs. Serology for rheumatic diseases was negative. In her history, a spontaneous fracture of the 8th thoracic vertebra at the age of seven had been diagnosed as eosinophilic granuloma. Thoracoscopic wedge resection was done for the histological verification of pulmonary changes.

Results: Microscopically a granulomatous inflammation with central necrosis was seen. Multinuclear giant cells showed not the typical arrangement of a in tuberculosis lesion. In spite of a very floride process, Ziehl-Neelsen stain revealed no acid-fast bacteria. Thus human brucellosis was supposed. Genus-specific *bcs31* real-time PCR and *Brucella*-LPS ELISA from formalin fixed tissue were done. Results for were positive for PCR but negative for ELISA. Serology (CFT) for *Brucella* was negative, too. Holiday stays in different Mediterranean countries during childhood were identified as possible times of exposition. Contaminated goat milk products e.g. cheese from unpasteurised milk is supposed to be the likely source of infection. Successful therapy was done using rifampicin, pantoprazol, doxycycline and prednison.

Conclusions: After eradication of brucellosis in the German animal population, patients are infected during stays in endemic countries or by laboratory infections. About 20 to 35 cases are reported every year. In endemic countries the disease is mainly transmisted by consumption of unpasteurized milk or cheese from infected goats or sheep. Pathology shows necrosis and granulomatous inflammation similar to tuberculosis. Negative Ziehl-Neelsen and Grocott stains, dominance of giant multinucleated cells, as well as the occurence of different lesions should raise suspect on brucellosis. In most cases focal complications, e.g. spondylitis, endocarditis, and meningoencephalitis are predominant.