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# Who is at risk of occupational Q fever: new insights from a multiprofessional cross sectional study



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| Keywords: | shepherd, veterinarian, obstetrician, cattle farmer, Coxiella burnetii |
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# Who is at risk of occupational Q fever: new insights from a multiprofessional cross sectional study

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Keywords: shepherd, veterinarian, obstetrician, cattle farmer, *Coxiella burnetii*

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## ABSTRACT

**Objectives**: Q fever is caused by the bacterium *Coxiella burnetii*. The disease has re-emerged as a significant public health issue as has been demonstrated by several outbreaks. Most of the outbreaks involved sheep and goats where *C. burnetii* can be found in high numbers in the amniotic fluid, placenta and foetal membranes of infected animals. The extracellular form of the bacterium is highly resistant to environmental stresses and human infection typically occurs by inhalation of contaminated dust or aerosols. Q fever is recognized as an occupational hazard for individuals who are in regular close contact with animal birth products. Obstetricians are sometimes infected from human birth products. Tight hygiene regulations are therefore applied during the delivery of women with known Q fever. Despite this knowledge there are no systematic investigations of Q fever prevalence in occupational risk groups.

**Design**: We carried out a cross-sectional study.

**Setting**: The study included shepherds, cattle farmers, veterinarians and obstetricians from Thuringia.

**Participants:** 77 shepherds, 74 veterinarians, 14 cattle farmer, 17 office employees and 68 obstetricians participated. The control group consisted of 92 blood donors.

**Primary outcome measure:** The primary outcome measure was *Coxiella burnetii* phase II specific IgG. The used assay was evaluated for this purpose in a previous study.

**Results**: We analysed 250 blood samples revealing the highest seroprevalences in individuals with frequent animal contact (64-77%). There were no significant differences between cattle farmers, veterinarians and shepherds. The seroprevalence in people working in administration was lower but still significantly greater than the control. No obstetricians or midwifes tested positive.

**Conclusions**: Shepherds, veterinarians and cattle farmers have a high risk of *C. burnetii* infection. However, our study clearly proves that there was no increased risk for people working in an obstetric department.

**Strengths and limitations of this study**

- Undertook a cross sectional study using a serological test with proven excellent performance for this purpose
- Investigation of different professional groups with reliable sample sizes.
- First investigation of people working in an obstetrical department.
- The study was limited to a single centre.
- Random sampling may lead to a bias toward an increased Q fever seroprevalence.

## INTRODUCTION

Q fever is caused by the bacterium *Coxiella burnetii*. The disease has re-emerged as a significant public health issue as has been demonstrated by several outbreaks. These outbreaks have affected up to 4000 people. The symptoms of human Q fever are non-specific and the acute disease presents as febrile illness, a flu-like syndrome or pneumonia. But the acute disease may pass by asymptomatically. Both symptomatic and asymptomatic infections can become chronic, causing endocarditis or vasculitis associated with a high mortality rate (1). Most outbreaks are associated with sheep and goats where *C. burnetii* can be found in high numbers in the amniotic fluid, placenta and foetal membranes of infected animals (2). The extracellular survival form of the bacterium (small cell variant) is highly resistant to environmental stresses such as desiccation (3). This means it can persist in the environment for weeks. Human infection then typically occurs by inhalation of contaminated dust or aerosols. Infection with *C. burnetii* is an occupational hazard for those who are regularly in close contact with animal birth products. Such groups include farmers, veterinarians, and workers in zoos or abattoirs (4).

*Coxiella burnetii* can also be found in the birth products of women with Q fever during pregnancy and can cause perinatal infections of obstetricians (5). In consequence, strict hygiene rules and regulations are applied in Germany during delivery by women infected with *C. burnetii*.

We investigated the question, who is at risk of occupational Q fever, determining the seroprevalence of *C. burnetii* antibodies in people of different occupational groups. We performed the study with an assay validated for seroprevalence testing.

## METHODS

We conducted a cross-sectional study between 2009 and 2016 that included several at risk groups. We recruited shepherds at several educational meetings of the occupational union for shepherds between 2009 and 2010, cattle farmers at a congress for cattle farmers in 2010, and veterinaries at a number of educational meetings on veterinary medicine and animal health in 2016. To recruit participants working in an obstetrical department, we attended educational meetings of the occupational union of midwifes and offered C. burnetii –specific antibody testing for all staff members of the Department of Obstetrics and Gynecology, University Hospital Jena between January and August 2016. Information about occupational history and contact with sheep, goats and cattle was collected by interview. A blood sample was taken from every interviewee and the sera were stored at -80°C until antibody testing. Sera of 92 blood donors were tested as a control-group. The study was approved by the Ethical committee of the University Hospital Jena (reference number 2525-04/09, 4615-11/15). Patients or the public were not involved in the conceptualization or carrying out of this research.

We analysed the sera for *C. burnetii*-Phase II-specific IgG antibodies with the Panbio-ELISA. The Panbio-ELISA assay was conducted manually according to the manufacturer's instructions. Optical densities were read at 450nm with a reference wave length of 620nm (Sunrise, Tecan).

Calculation was performed using the Statistical Package for Social Sciences (SPSS version 21, Chicago, IL, USA). The seroprevalences of the different groups were compared using the chi-squared test.

**RESULTS**

A total of 250 people participated in our study (table 1). The sex ratio of the different occupational groups ranged from 99% females in the obstetrician group to 90% males in the shepherd group.

The highest seroprevalences were found for people with frequent animal contact (64-77%). There were no significant differences between cattle farmers, veterinarians and shepherds. The seroprevalence of people working in administration was lower than in those with animal contract but still significant greater than the control group. None of the obstetrician group was positive for past Q fever infection.

Table 1. Prevalence of *Coxiella burnetii*-Phase II-specific IgG in different occupational settings

| Group | n | female:male | age (range) [years] | prevalence* [%] | p-value |
|---|---|---|---|---|---|
| Blood donors | 92 | 3:1 | 35 (18-67) | 2.2 | reference |
| Shepherds | 77 | 1:9 | 45 (19-70) | 76.6 | < 0.001 |
| Cattle farmers | 14 | 1:2 | 52 (32-65) | 70.3 | < 0.001 |
| Veterinarians | 74 | 1:1 | 45 (24-75) | 64.3 | < 0.001 |
| Office employees | 17 | 3:1 | 46 (26-56) | 41.2 | < 0.001 |
| Obstetricians | 68 | 99:1 | 44 (24-64) | 0.0 | 0.221 |

*seroprevalence for *C. burnetii*-Phase II-specific IgG

## DISCUSSION

This is the first study investigating different occupational groups for past Q fever using an assay validated for seroprevalence studies. But, as we have already shown previously, it is essential to choose the appropriate test (6). Our evaluation of three different commercially

available ELISA for detection of past *C. burnetii*- infection revealed sensitivies of 42, 51 and 100% with specificity ranging from 94 to 100% (6). Based on our evaluation data we chose the Panbio-ELISA (sensitivity 100%; specificity 94%) for our study.

Cohort composition was based on random sampling performed in different occupational groups and settings. A potential bias cannot be ruled out as people with Q fever contact in the past may have an increased interest in being tested. However, this bias should be comparable in all cohorts investigated.

We found a very high seroprevalence, 70%, in people with close occupational contact with animals. The seroprevelance was even raised, 41%, for people working in the administration despite their only having sporadic contact with animals. These remarkably high seroprevalences are reliable considering the enormous number of *C. burnetii* in some placentae ($10^9$/g ) or milk ($10^5$/ml ) (2) together with the high infectivity of the bacterium (it has been estimated that a single organism can cause disease), the enhanced resistance to environmental stresses (it survives on wool for 7-10 months (3)) and a flock level prevalence of 28% (7).

Seroprevalences in shepherds generally range from 29 to 59% (8, 9) and in veterinarians from 10 to 75% (10, 11). However, these data are not strictly comparable because they result from very different assays, in-house tests and even from tests using different cut off values. The only other study using Panbio-ELISA (12) revealed similar results to those in our study with 78% for veterinarians and 54% for cattle farmer in Sicily.

Keeping in mind that more outbreaks are related to sheep than to cattle, it is interesting that there was no difference between people handling sheep and those handling cattle. This indicates that people working with cattle are exposed to *C. burnetii* to a similar degree as those working with sheep. It also indicates that *C. burnetii* strains from cattle are as infective

for humans as those in sheep. Thus, it is likely that differences in animal husbandry are responsible for the higher number of outbreaks associated with sheep than with cattle.

There is, as yet, little data on Q fever as an occupational risk for obstetricians. The only prevalence study available is from the 1970s in Bulgaria. It used a complement fixation test and revealed 37% positivity for obstetricians compared to 8% positivity in blood donors (13). The value for obstetricians in this study is much higher than what we found. This discrepancy probably arises from the high hygiene standards of modern obstetrics. The development of the infective and highly resistant form of *C. burnetii* (small cell variant) is promoted by desiccation. But obstetrical departments are frequently cleaned and disinfected and waste is rapidly disposed of so reducing the risk of the small cell variant spreading.

Based on the tropism of the bacteria for trophoblasts and a case report of the perinatal infection of an obstetrician with pneumonia (5) a strict hygiene scenario was recommended in Germany for the delivery of women infected with *C. burnetii* . We hope that our data leads to a reevaluation of the necessity of such strict and inconvenient protecting procedures during delivery.

In conclusion, shepherds, veterinarians and cattle farmers and even people with sporadic animal contact like employees in veterinary offices have a high risk of *C. burnetii* infection. Physicians should therefore consider *C. burnetii* infection as a differential diagnosis for acute febrile illness as well as for endocarditis and vasculitis in these occupational groups. In contrast, our study clearly proves that there is no increased risk for people working in an obstetric department. The already high hygienic standards in obstetrical departments are sufficient to keep under control the occupational risk for Q fever.

**Contributor ship statement** TG and KB conceived the study. TG, KK, UM, KB designed the study. KK, UM, BH performed the experiments. TG, KK, KB analyzed the data. UM, KM provided resources. TG, KK, BH and KB wrote the manuscript. A previous version of the

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**Data sharing statement** Extra data is available by emailing katharina.boden@dianovis.de

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**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 5 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 5 |
| | | (*c*) Explain how missing data were addressed | No missing data |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (*e*) Describe any sensitivity analyses | 6 |
| **Results** | | | |

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 6 |
| | | (b) Give reasons for non-participation at each stage | 7 |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6 |
| | | (b) Indicate number of participants with missing data for each variable of interest | No missing data |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 6 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 7 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 7 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 8 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 8 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# Who is at risk of occupational Q fever: new insights from a multi-profession cross-sectional study

Tanja Groten[1], Karola Kuenzer[2], Udo Moog[3], Beate Hermann[4], Katrin Maier[5], Katharina Boden[6]

Keywords: shepherd, veterinarian, obstetrician, cattle farmer, *Coxiella burnetii*

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## ABSTRACT

**Objectives**: Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. It is recognized as an occupational hazard for individuals who are in regular close contact with animal birth products. Data from the literature are not comparable because different serological assays perform very differently in detecting past infections. It is therefore essential to choose the right assay for obtaining reliable data of Q fever seroprevalence. Obstetricians are another profession potentially at risk of Q fever because they can be infected from birth products of women with Q fever during pregnancy. There is little data, however, for Q fever in this occupational group.

Our study therefore had two purposes. The first was to obtain comparable seroprevalence data for different occupational groups by using a single immunoassay, an ELISA with proven excellent sensitivity and specificity for detecting past infections. The second purpose was to obtain data for obstetricians.

**Design**: We carried out a cross-sectional study.

**Setting**: The study included shepherds, cattle farmers, veterinarians and obstetricians from Thuringia.

**Participants:** 77 shepherds, 74 veterinarians, 14 cattle farmers, 17 office employees and 68 obstetricians participated. The control group consisted of 92 blood donors.

**Primary outcome measure:** The primary outcome measure was *Coxiella burnetii* phase II specific IgG. The assay used was evaluated for this purpose in a previous study.

**Results**: Of the 250 blood samples we analysed, the very highest seroprevalences (64-77%) occurred in individuals with frequent animal contact. There were no significant differences between shepherds, cattle farmers, and veterinarians. The seroprevalence in people working in administration was lower but still significantly greater than the control. No obstetricians or midwives tested positive.

**Conclusions**: Shepherds, cattle farmers and veterinarians have a high risk of *C. burnetii* infection. However, our study clearly proves that there was no increased risk for people working in an obstetric department.

**Strengths and limitations of this study**

- Undertook a cross sectional study using a serological test with proven excellent performance for this purpose
- Investigated different professional groups with reliable sample sizes.
- First investigation of people working in an obstetric department.
- The study was limited to a single centre.
- Non-random sampling may lead to a bias towards high Q fever seroprevalence.

## INTRODUCTION

Q fever is caused by the bacterium *Coxiella burnetii*. The disease has re-emerged as a significant public health issue in Europe as has been demonstrated by several outbreaks (1-4). The largest of these affected up to 4000 people in the Netherlands between 2007 and 2009 (1). The symptoms of human Q fever are non-specific and the acute disease presents as febrile illness, a flu-like syndrome or pneumonia (5, 6). But the acute disease may pass by asymptomatically. Both symptomatic and asymptomatic infections can become chronic, causing endocarditis or vasculitis associated with a high mortality rate (7). Most outbreaks are associated with sheep, cattle and goats (8-10) where *C. burnetii* can be found in high numbers in the amniotic fluid, placenta and foetal membranes of infected animals (11). The extracellular survival form of the bacterium (small cell variant) is highly resistant to environmental stresses such as desiccation (12). This means it can persist in the environment for weeks (13). Human infection then typically occurs by inhalation of contaminated dust or aerosols (14). Infection with *C. burnetii* is an occupational hazard for those who are regularly in close contact with animal birth products. Such groups include farmers, veterinarians, and workers in zoos or abattoirs (15).

*Coxiella burnetii* can also be found in the birth products of women with Q fever during pregnancy and can cause perinatal infections of obstetricians (16).

A meta-analysis of *C. burnetii* seroprevalence among abattoir workers revealed significant heterogeneity among serological tests (17). Different tests also gave very different results for the same people in our evaluation of commercial tests for the detection of past infection (18). Excellent performance was only found for the Panbio-ELISA (PanbioDiagnostics, Korea) (18). We therefore used this assay for a descriptive study of seroprevalence of *C. burnetii* antibodies in people from occupational groups with close animal contact and in people working in an obstetric department.

## METHODS

We conducted a cross-sectional study between 2009 and 2016 that included several at-risk groups. We initially focused on shepherds and veterinarians but offered testing to all interested people. Between 2009 and 2010 we recruited shepherds and veterinarians at several educational meetings of the occupational union for shepherds. And in 2010 we recruited among veterinarians, and also among cattle farmers and office employees, attending a congress for cattle farmers in 2010 (41 of 165 congress participants participated, 25%). In all we were able to include 84 out of about 400 professional shepherds in Thuringia in 2009 and 2010 (21%). The group of office employees consisted of animal welfare inspectors (Tierschutzkontrolleur) and veterinarians, all of whom had sporadic animal contact. In 2011, resampling of blood from people infected with *Coxiella burnetii* during an outbreak six years previously allowed us to evaluate serological assays for the detection of past infection (18). This outbreak with 331 cases occurred in a densely inhabited area of Jena, a town in Thuringia (2). As a result of the validation we found only one assay suitable for seroprevalence studies. We retested all the sera sampled and enlarged the group of veterinarians to obtain a reliable sample size. We increased the number of veterinarians by including some of those attending a number of educational meetings on veterinary medicine and animal health in 2016. To examine the question of the risk for obstetricians we added this occupational group to our study. We did this by attending educational meetings of the occupational union of midwives (proportion of participants is not available). In addition, between January and August 2016, we offered *C. burnetii*–specific antibody testing for all staff of the Department of Obstetrics and Gynaecology, University Hospital Jena (34 out of 50 employees took part, 68%). After obtaining written informed consent, we interviewed people in the different occupational groups. We recorded information about occupational history and contact with sheep, goats and cattle. We specifically asked the participants how long they had

been in their occupation and how much they were exposed to ruminants. As most of the shepherd grew up with intense animal contact, we chose the length of sheep contact instead of working years for this special group. A blood sample was taken from every interviewee and the sera were stored at -80°C until antibody testing. Sera of 92 blood donors were tested as a control-group. The blood donor group consisted of 22 women and 70 men with an average age of 35 (range 18-67). All members of this group lived in urban areas. The study was approved by the Ethical Committee of the University Hospital Jena (reference number 2525-04/09, 4615-11/15).

We analysed the sera for *C. burnetii*-Phase II-specific IgG antibodies with the Panbio-ELISA. The Panbio-ELISA assay was conducted manually according to the instructions of the manufacturer. The optical densities were read at 450nm with a reference wavelength of 620nm (Sunrise, Tecan).

For all calculations we used the Statistical Package for Social Sciences (SPSS version 21, Chicago, IL, USA). The seroprevalences of the different groups were compared using the chi-squared test.

Patient and Public Involvement

Patients or the public were not involved in the conceptualization or carrying out of this research. Study participants received their personal results and recommendations by letter.

## RESULTS

A total of 250 people participated in our study (table 1). The sex ratio of the different occupational groups ranged from 99% females in the obstetrician group to 90% males in the shepherd group.

Table 1. Prevalence of *Coxiella burnetii*-Phase II-specific IgG in different occupational settings

| Group | n | female:male | age (range) [years] | Time of exposition* (range) | proportion** [%] | p-value |
|---|---|---|---|---|---|---|
| Blood donors | 92 | 2:7 | 35 (18-67) | | 2/92 [2.2] | reference |
| Shepherds | 77 | 1:9 | 45 (19-70) | 28 (0-62) | 59/77 [76.6] | < 0.001 |
| Cattle farmers | 14 | 1:2 | 52 (32-65) | 31 (12-54) | 9/14 [70.3] | < 0.001 |
| Veterinarians | 74 | 1:1 | 45 (24-75) | 19 (0-50) | 52/74 [64.3] | < 0.001 |
| Office employees | 17 | 3:1 | 46 (26-56) | 23 (2-36) | 7/17 [41.2] | < 0.001 |
| Obstetricians | 68 | 99:1 | 44 (24-64) | 22 (0.5-45) | 0/68 [0.0] | 0.221 |

* occupational time, except for shepherds where the whole duration of sheep contact was used; **seroprevalence for *C. burnetii*-Phase II-specific IgG

The highest seroprevalences were found for people with frequent animal contact (64-77%). There were no significant differences between cattle farmers, practising veterinarians and shepherds. The seroprevalence of people working in administration was lower than in those with frequent animal contact but still significantly greater than the control group. None of the obstetrician group was positive for past Q fever infection.

The time of exposure to sheep in shepherds ranged widely from 0 to 62 years (average 28 years). Even the duration of work with animals in the group of veterinarians ranged from 0 to 50 years (average 19 years). However, infection rates in these groups were high even after only a few years of exposure (Figure 1) although the sample size of the different durations of exposure is very small.

**DISCUSSION**

This is the first seroprevalence study investigating different occupational groups using an assay validated for detecting past Q fever. Most assays are designed as tools for diagnosing clinical disease but they give differing results in other contexts. Our evaluation of three different commercially available ELISA for detection of past *C. burnetii* infection revealed ROC curves that discriminated well between infected and uninfected individuals. The AUC ranged from 0.97 to 1.0 (18). However, most antibody levels during the convalescent phase fall under the cut off titre, in accordance with the study by Blaauw (19). This phenomenon leads to sensitivities of 42 (Virion/Serion, Germany), 51 (IBL International, Germany) and 100% (Panbio Diagnostics, Korea) with specificity of 94-100% six years after infection (18). Based on our evaluation data we chose the Panbio-ELISA (sensitivity 100%; specificity 94%) for our study (18).

Cohort composition was based on sampling performed in different occupational groups and settings. A potential bias cannot be ruled out as people aware of their Q fever contact in the past may have more interest in being tested than those without such awareness. However, this bias is likely to be similar for all the occupational groups investigated.

We found very high seroprevalence, 70%, in people with close occupational contact with animals. Seroprevalence was also quite high, 41%, in the group of office employees even though they had only sporadic animal contact. However, half these people were non-practising veterinarians who had studied veterinary medicine. Because such students are at risk of Q fever (20), this finding must be investigated in more detail with a larger sample size. However, the remarkably high seroprevalences are reliable given the characteristics of the disease. There are enormous numbers of *C. burnetii* in some placentae ($10^9$/g) or milk ($10^5$/ml) (11), the bacterium is highly infective (it has been estimated that a single organism is able to cause disease), it is highly resistance to environmental stresses (it survives on wool for 7-10 months (12)) and has a flock level prevalence of 28% (21).

Seroprevalences in shepherds are generally 29-59% (22, 23) and in veterinarians 10-75% (24, 25). However, these wide ranges are, in part, illusory because the values result from very different assays, in-house tests and even from tests using different cut off values. In large seroprevalence studies in the Netherlands 18.7% of veterinary medicine students were antibody positive as were 66.7% of dairy and 51.5 of non-dairy sheep farm residents and 87.2% of cattle farmers (20, 23, 26). But the situation in the Netherlands differs from that in Germany as the general seropositivity in the population increased during the large outbreak in 2007. It was 2.3% in 2006-2007 but by 2009 was 25.1% in the epicentre of the outbreak and 12.2% in blood donors in the most Q fever-affected areas (27, 28, 29). Most of these Dutch studies used immunofluorescence (IFAT). IFAT is regarded as a reference method but several cut-off titres are used and so standardization is required if they are to be used in seroprevalence studies (19). The only other study using Panbio-ELISA produced results similar to ours with 78% for veterinarians and 54% for cattle farmers in Sicily (30).

Keeping in mind that more outbreaks are related to sheep than to cattle, it is interesting that there was no difference in our study between people handling sheep and those handling cattle. But, to rule out significant bias, a reinvestigation of the group of cattle farmers with an enlarged sample size is needed. Our findings are in accordance with the finding of Marrie that slaughtering cattle is a significant risk factor for positive antibody titres (31). We did not include the interesting group of slaughterhouse workers in our study as there is no professional slaughterhouse for sheep in Thuringia. About 95% of sheep are slaughtered outside Thuringia. But a recent metanalysis demonstrates that this group has very high seroprevalences, of 30-70%, (17).

We found much lower seroprevalence in obstetricians than did the only published study available. This study from the 1970s in Bulgaria used a complement fixation test and revealed 37% positivity for obstetricians compared to 8% positivity in blood donors (32). The

discrepancy probably arises from the high hygiene standards of modern obstetrics. The development of the infective and highly resistant form of *C. burnetii* (small cell variant) is promoted by desiccation. But obstetrical departments are frequently cleaned and disinfected and waste is rapidly disposed of so reducing the risk of the small cell variant spreading. However, the data for obstetricians should be repeated in another area with a larger sample size.

In conclusion, shepherds, veterinarians and cattle farmers, and even people with sporadic animal contact like employees in veterinary offices, have a high risk of *C. burnetii* infection. Physicians should therefore consider *C. burnetii* infection as a differential diagnosis for acute febrile illness as well as for endocarditis and vasculitis in these occupational groups. In contrast, our study clearly proves that there is no increased risk for people working in an obstetric department. The already high hygienic standards in obstetrical departments are sufficient to keep under control the occupational risk for Q fever.

Figure 1: Antibody-positivity in relation to duration of exposure

*shepherds: 0-5 years: n=5, 6-10: n=5, 11-20: n=6, 21-30: n=16, 31-40: n=11, >40: n=16

veterinarians: 0-5 years: n=13, 6-10: n=7, 11-20: n=9, 21-30: n=9, 31-40: n=9, >40: n=4

**Contributorship statement** TG and KB conceived the study. TG, KK, UM, KB designed the study. KK, UM, BH performed the experiments. TG, KK, KB analysed the data. UM, KM provided resources. TG, KK, BH and KB wrote the manuscript. Previous versions of the manuscript were edited for English by Dr A. J. Davis (English Experience Language Services, Jena, Germany).

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**Data sharing statement** Extra data is available by emailing karola.kuenzer@med.uni-jena.de

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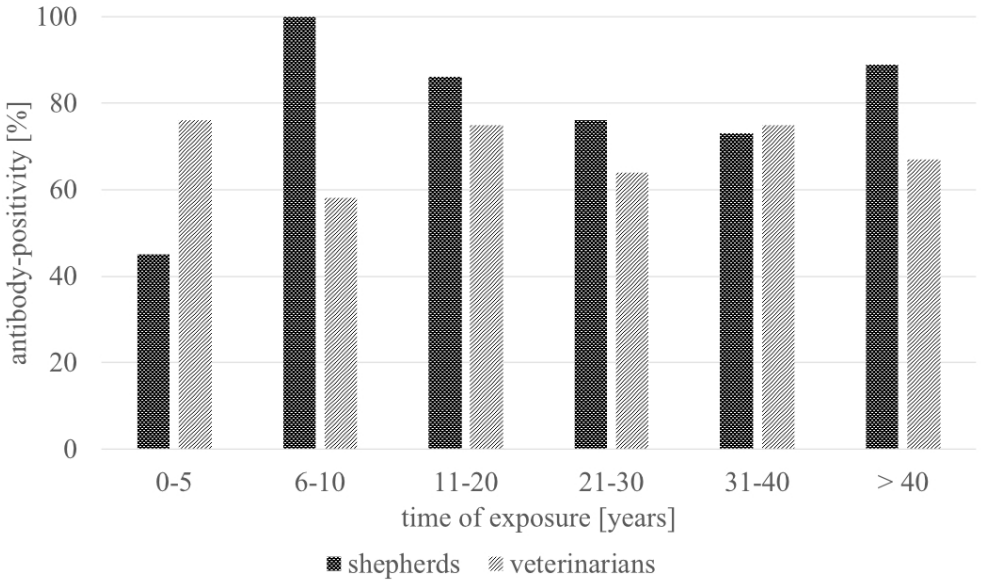


Figure 1: Antibody-positivity in relation to duration of exposure
*shepherds: 0-5 years: n=5, 6-10: n=5, 11-20: n=6, 21-30: n=16, 31-40: n=11, >40: n=16
veterinarians: 0-5 years: n=13, 6-10: n=7, 11-20: n=9, 21-30: n=9, 31-40: n=9, >40: n=4

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**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 5 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 5 |
| | | (*c*) Explain how missing data were addressed | No missing data |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (*e*) Describe any sensitivity analyses | 6 |
| **Results** | | | |

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 6 |
| | | (b) Give reasons for non-participation at each stage | 7 |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6 |
| | | (b) Indicate number of participants with missing data for each variable of interest | No missing data |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 6 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 7 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 7 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 8 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 8 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# Who is at risk of occupational Q fever: new insights from a multi-profession cross-sectional study



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# Who is at risk of occupational Q fever: new insights from a multi-profession cross-sectional study

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Keywords: shepherd, veterinarian, obstetrician, cattle farmer, *Coxiella burnetii*

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running head: Q fever as occupational disease

word count: 2091

## ABSTRACT

**Objectives**: Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. It is recognized as an occupational hazard for individuals who are in regular contact with animal birth products. Data from the literature are not comparable because different serological assays perform very differently in detecting past infections. It is therefore essential to choose the right assay for obtaining reliable data of seroprevalence. Obstetricians are another profession potentially at risk of Q fever. They can be infected from birth products of women with Q fever during pregnancy. There is little data, however, for Q fever in this occupational group.
Our study therefore had two purposes. The first was to obtain reliable seroprevalence data for occupational groups in regular contact with animal birth products by using an assay with proven excellent sensitivity and specificity for detecting past infections. The second purpose was to obtain primary data for obstetricians.

**Design**: We carried out a cross-sectional study.

**Setting**: The study included shepherds, cattle farmers, veterinarians and obstetricians from Thuringia.

**Participants:** 77 shepherds, 74 veterinarians, 14 cattle farmers, 17 office employees and 68 obstetricians participated. The control group consisted of 92 blood donors.

**Primary outcome measure:** The primary outcome measure was *Coxiella burnetii* phase II specific IgG. The assay used was evaluated for this purpose in a previous study.

**Results**: Of the 250 blood samples we analysed, the very highest seroprevalences (64-77%) occurred in individuals with frequent animal contact. There were no significant differences between shepherds, cattle farmers, and veterinarians. The seroprevalence in people working in administration was lower but still significantly greater than the control. No obstetricians or midwives tested positive.

**Conclusions**: Shepherds, cattle farmers and veterinarians have a high risk of *C. burnetii* infection. However, our study clearly proves that there was no increased risk for people working in an obstetric department.

**Strengths and limitations of this study**

- Undertook a cross sectional study using a serological test with proven excellent performance for this purpose
- Investigated different professional groups with reliable sample sizes.
- First investigation of people working in an obstetric department.
- The study was limited to a single centre.
- Non-random sampling may lead to a bias towards high Q fever seroprevalence.

## INTRODUCTION

Q fever is caused by the bacterium *Coxiella burnetii*. The disease has re-emerged as a significant public health issue in Europe as has been demonstrated by several outbreaks (1-4). The largest of these affected up to 4000 people in the Netherlands between 2007 and 2009 (1). The symptoms of human Q fever are non-specific and the acute disease presents as febrile illness, a flu-like syndrome or pneumonia (5, 6). But the acute disease may pass by asymptomatically. Both symptomatic and asymptomatic infections can become chronic, causing endocarditis or vasculitis associated with a high mortality rate (7). Most outbreaks are associated with sheep, cattle and goats (8-10) where *C. burnetii* can be found in high numbers in the amniotic fluid, placenta and foetal membranes of infected animals (11). The extracellular survival form of the bacterium (small cell variant) is highly resistant to environmental stresses such as desiccation (12). This means it can persist in the environment for weeks (13). Human infection then typically occurs by inhalation of contaminated dust or aerosols (14). Infection with *C. burnetii* is an occupational hazard for those who are regularly in close contact with animal birth products. Such groups include farmers, veterinarians, and workers in zoos or abattoirs (15).

*Coxiella burnetii* can also be found in the birth products of women with Q fever during pregnancy and can cause perinatal infections of obstetricians (16). But there are uncertainties about the real risk.

A meta-analysis of *C. burnetii* seroprevalence among abattoir workers revealed significant heterogeneity among serological tests (17). Different tests also gave very different results for the same people in our evaluation of commercial tests for the detection of past infection (18). Excellent performance was only found for the Panbio-ELISA (PanbioDiagnostics, Korea) (18). We therefore used this assay to get reliable seroprevalence data for occupational groups with close animal contact and to obtain primary data for people working in an obstetric department.

## METHODS

We conducted a cross-sectional study between 2009 and 2016 that included several at-risk groups. We initially focused on shepherds and veterinarians but offered testing to all interested people. Between 2009 and 2010 we recruited shepherds and veterinarians at several educational meetings of the occupational union for shepherds. And in 2010 we recruited among veterinarians, and also among cattle farmers and office employees, attending a congress for cattle farmers in 2010 (41 of 165 congress participants participated, 25%). In all we were able to include 84 out of about 400 professional shepherds in Thuringia in 2009 and 2010 (21%). The group of office employees consisted of animal welfare inspectors (Tierschutzkontrolleur) and veterinarians, all of whom had sporadic animal contact. In 2011, resampling of blood from people infected with *Coxiella burnetii* during an outbreak six years previously allowed us to evaluate serological assays for the detection of past infection (18). This outbreak with 331 cases occurred in a densely inhabited area of Jena, a town in Thuringia (2). As a result of the validation we found only one assay suitable for seroprevalence studies. We retested all the sera sampled and enlarged the group of veterinarians to obtain a reliable sample size. We increased the number of veterinarians by including some of those attending a number of educational meetings on veterinary medicine and animal health in 2016. To examine the question of the risk for obstetricians we added this occupational group to our study. We did this by attending educational meetings of the occupational union of midwives (proportion of participants is not available). In addition, between January and August 2016, we offered *C. burnetii*–specific antibody testing for all staff of the Department of Obstetrics and Gynaecology, University Hospital Jena (34 out of 50 employees took part, 68%). After obtaining written informed consent, we interviewed people in the different occupational groups. We recorded information about occupational history and contact with sheep, goats and cattle. We specifically asked the participants how long they had

been in their occupation and how much they were exposed to ruminants. As most of the shepherd grew up with intense animal contact, we chose the length of sheep contact instead of working years for this special group. A blood sample was taken from every interviewee and the sera were stored at -80°C until antibody testing. Sera of 92 blood donors were tested as a control-group. The blood donor group consisted of 22 women and 70 men with an average age of 35 (range 18-67). All members of this group lived in urban areas. The study was approved by the Ethical Committee of the University Hospital Jena (reference number 2525-04/09, 4615-11/15).

We analysed the sera for *C. burnetii*-Phase II-specific IgG antibodies with the Panbio-ELISA. The Panbio-ELISA assay was conducted manually according to the instructions of the manufacturer. The optical densities were read at 450nm with a reference wavelength of 620nm (Sunrise, Tecan).

For all calculations we used the Statistical Package for Social Sciences (SPSS version 21, Chicago, IL, USA). The seroprevalences of the different groups were compared using the chi-squared test.

Patient and Public Involvement

Patients or the public were not involved in the conceptualization or carrying out of this research. Study participants received their personal results and recommendations by letter.

## RESULTS

A total of 250 people participated in our study (table 1). The sex ratio of the different occupational groups ranged from 99% females in the obstetrician group to 90% males in the shepherd group.

Table 1. Prevalence of *Coxiella burnetii*-Phase II-specific IgG in different occupational settings

| Group | n | female:male | age (range) [years] | Time of exposition* (range) [years] | proportion** [%] | p-value |
|---|---|---|---|---|---|---|
| Blood donors | 92 | 2:7 | 35 (18-67) | | 2/92 [2.2] | reference |
| Shepherds | 77 | 1:9 | 45 (19-70) | 28 (0-62) | 59/77 [76.6] | < 0.001 |
| Cattle farmers | 14 | 1:2 | 52 (32-65) | 31 (12-54) | 9/14 [70.3] | < 0.001 |
| Veterinarians | 74 | 1:1 | 45 (24-75) | 19 (0-50) | 52/74 [64.3] | < 0.001 |
| Office employees | 17 | 3:1 | 46 (26-56) | 23 (2-36) | 7/17 [41.2] | < 0.001 |
| Obstetricians | 68 | 99:1 | 44 (24-64) | 22 (0.5-45) | 0/68 [0.0] | 0.221 |

* occupational time, except for shepherds where the whole duration of sheep contact was used; **seroprevalence for *C. burnetii*-Phase II-specific IgG

The highest seroprevalences were found for people with frequent animal contact (64-77%). There were no significant differences between cattle farmers, practising veterinarians and shepherds. The seroprevalence of people working in administration was lower than in those with frequent animal contact but still significantly greater than the control group. None of the obstetrician group was positive for past Q fever infection.

The time of exposure to sheep in shepherds ranged widely from 0 to 62 years (average 28 years). Even the duration of work with animals in the group of veterinarians ranged from 0 to 50 years (average 19 years). However, infection rates in these groups were high even after only a few years of exposure (Figure 1) although the sample size of the different durations of exposure is very small.

**DISCUSSION**

This is the first seroprevalence study investigating different occupational groups using an assay validated for detecting past Q fever. Most assays are designed as tools for diagnosing clinical disease but they give differing results in other contexts. Our evaluation of three different commercially available ELISA for detection of past *C. burnetii* infection revealed ROC curves that discriminated well between infected and uninfected individuals. The AUC ranged from 0.97 to 1.0 (18). However, most antibody levels during the convalescent phase fall under the cut off titre, in accordance with the study by Blaauw (19). This phenomenon leads to sensitivities of 42 (Virion/Serion, Germany), 51 (IBL International, Germany) and 100% (Panbio Diagnostics, Korea) with specificity of 94-100% six years after infection (18). Based on our evaluation data we chose the Panbio-ELISA (sensitivity 100%; specificity 94%) for our study (18).

Cohort composition was based on sampling performed in different occupational groups and settings. The first specimens collected during 2009 and 2010 were tested with the Virion/Serion-ELISA and revealed questionable results. Several people with close contact to animals with Q fever history showed negative results, despite the high infectivity of *C. burnetii*. Knowing that the used assay was only evaluated for acute disease, we supposed an insufficient test performance for detecting past infection. Potentially antibody levels could have fallen under the cut off. We evaluated different assays for this purpose and found the Panbio-ELISA of excellent performance for seroprevalence detection (18). We retested our samples and enlarged our study group until 2016. From the epidemiological point of view the rather long period of recruitment may bias our results. However, the actual stable living and working conditions of our study group especially the shepherds minimise the risk of bias. A potential bias due to non-random sampling cannot be ruled out as people aware of their Q fever contact in the past may have more interest in being tested than those without such

awareness. However, this bias is likely to be similar for all the occupational groups investigated. Another limitation might be the restriction to one single centre.

We found very high seroprevalence, 70%, in people with close occupational contact with animals. Seroprevalence was also quite high, 41%, in the group of office employees even though they had only sporadic animal contact. However, half these people were non-practising veterinarians who had studied veterinary medicine. Because such students are at risk of Q fever (20), this finding must be investigated in more detail with a larger sample size. However, the remarkably high seroprevalences are reliable given the characteristics of the disease. There are enormous numbers of *C. burnetii* in some placentae ($10^9$/g) or milk ($10^5$/ml) (11), the bacterium is highly infective (it has been estimated that a single organism is able to cause disease), it is highly resistance to environmental stresses (it survives on wool for 7-10 months (12)) and has a flock level prevalence of 28% (21).

Seroprevalences in shepherds are generally 29-59% (22, 23) and in veterinarians 10-75% (24, 25). However, these wide ranges are, in part, illusory because the values result from very different assays, in-house tests and even from tests using different cut off values. In large seroprevalence studies in the Netherlands 18.7% of veterinary medicine students were antibody positive as were 66.7% of dairy and 51.5 of non-dairy sheep farm residents and 87.2% of cattle farmers (20, 23, 26). But the situation in the Netherlands differs from that in Germany as the general seropositivity in the population increased during the large outbreak in 2007. It was 2.3% in 2006-2007 but by 2009 was 25.1% in the epicentre of the outbreak and 12.2% in blood donors in the most Q fever-affected areas (27, 28, 29). Most of these Dutch studies used immunofluorescence (IFAT). IFAT is regarded as a reference method but several cut-off titres are used and so standardization is required if they are to be used in seroprevalence studies (19). The only other study using Panbio-ELISA produced results similar to ours with 78% for veterinarians and 54% for cattle farmers in Sicily (30).

Keeping in mind that more outbreaks are related to sheep than to cattle, it is interesting that there was no difference in our study between people handling sheep and those handling cattle. But, to rule out significant bias, a reinvestigation of the group of cattle farmers with an enlarged sample size is needed. Our findings are in accordance with the finding of Marrie that slaughtering cattle is a significant risk factor for positive antibody titres (31). We did not include the interesting group of slaughterhouse workers in our study as there is no professional slaughterhouse for sheep in Thuringia. About 95% of sheep are slaughtered outside Thuringia. But a recent metanalysis demonstrates that this group has very high seroprevalences, of 30-70%, (17).

We found much lower seroprevalence in obstetricians than did the only published study available. This study from the 1970s in Bulgaria used a complement fixation test and revealed 37% positivity for obstetricians compared to 8% positivity in blood donors (32). The discrepancy probably arises from the high hygiene standards of modern obstetrics. The development of the infective and highly resistant form of *C. burnetii* (small cell variant) is promoted by desiccation. But obstetrical departments are frequently cleaned and disinfected and waste is rapidly disposed of so reducing the risk of the small cell variant spreading. However, the data for obstetricians should be repeated in another area with a larger sample size.

In conclusion, shepherds, veterinarians and cattle farmers, and even people with sporadic animal contact like employees in veterinary offices, have a high risk of *C. burnetii* infection. Physicians should therefore consider *C. burnetii* infection as a differential diagnosis for acute febrile illness as well as for endocarditis and vasculitis in these occupational groups. In contrast, our study clearly proves that there is no increased risk for people working in an obstetric department. The already high hygienic standards in obstetrical departments are sufficient to keep under control the occupational risk for Q fever.

Figure 1: Antibody-positivity in relation to duration of exposure

*shepherds: 0-5 years: n=5, 6-10: n=5, 11-20: n=6, 21-30: n=16, 31-40: n=11, >40: n=16

veterinarians: 0-5 years: n=13, 6-10: n=7, 11-20: n=9, 21-30: n=9, 31-40: n=9, >40: n=4

**Contributorship statement** TG and KB conceived the study. TG, KK, UM, KB designed the study. KK, UM, BH performed the experiments. TG, KK, KB analysed the data. UM, KM provided resources. TG, KK, BH and KB wrote the manuscript.

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**Competing interests** The authors declare that they have no competing interests.

**Data sharing statement** Extra data is available by emailing karola.kuenzer@med.uni-jena.de

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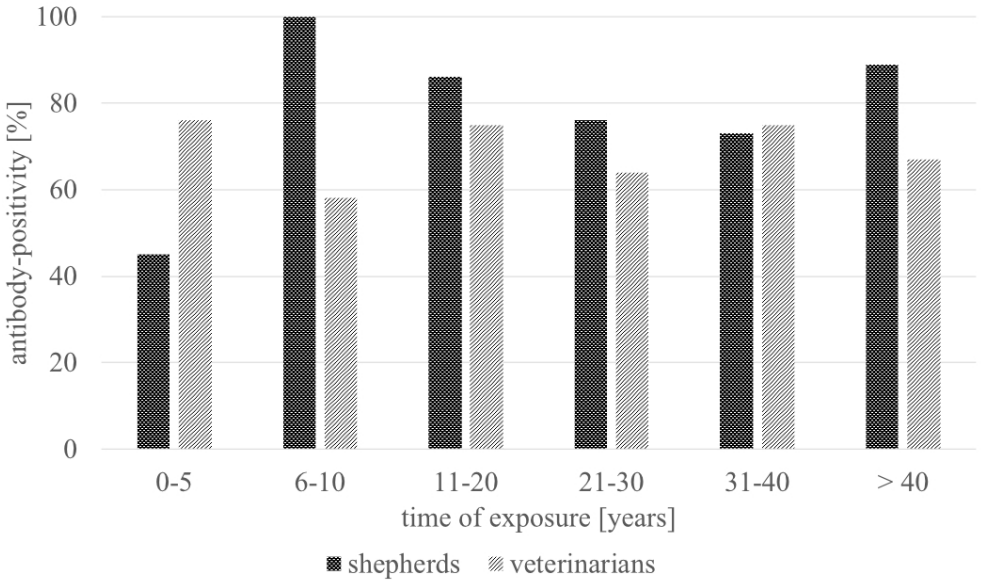


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90x90mm (300 x 300 DPI)

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 5 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 5 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 5 |
| | | (*c*) Explain how missing data were addressed | No missing data |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (*e*) Describe any sensitivity analyses | 6 |
| **Results** | | | |

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 6 |
| | | (b) Give reasons for non-participation at each stage | 7 |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6 |
| | | (b) Indicate number of participants with missing data for each variable of interest | No missing data |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 6 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 7 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 7 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 8 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 8 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.