JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2011, p. 383–384 Vol. 49, No. 1
0095-1137/11/$12.00 doi:10.1128/JCM.01491-10

Identification by Genotyping of a Commercial Antigen Preparation as the Source of a Laboratory Contamination with *Coxiella burnetii* and as an Unexpected Rich Source of Control DNA▽

Jeroen J. H. C. Tilburg,[1] Alphons M. Horrevorts,[1] Marcel F. Peeters,[2] Corné H. W. Klaassen,[1]* and John W. A. Rossen[2]

Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, Netherlands,[1] *and Laboratory of Medical Microbiology and Immunology, St. Elisabeth Hospital, Tilburg, Netherlands*[2]

Received 22 July 2010/Returned for modification 1 October 2010/Accepted 18 October 2010

By performing genotyping, a laboratory contamination involving Q fever was traced back to the antigen preparation used in a commercially available complement fixation test. It was established that such antigen preparations contain relatively high loads of DNA/RNA, making them potential sources of contamination but also convenient preparations for control material.

Q fever is a zoonosis caused by the ubiquitous pathogen *Coxiella burnetii* (1). Laboratory diagnosis of Q fever is classically performed by serological methods, such as the complement fixation test (CFT) or immunofluorescence assay (IFA) (2). In addition, real-time PCR-based methods are increasingly being used to overcome the diagnostic gap in the acute phase of the disease when a serological response is still absent (4). Multiple-locus variable-number tandem-repeat analysis (MLVA) was used to study the distribution of *C. burnetii* genotypes of an ongoing Dutch Q fever outbreak (3).

In five serum samples analyzed from one of the diagnostic laboratories, a completely different genotypic group was found. This suggests that the ongoing outbreak has a multifocal origin. However, this genotype was identical to that of the *C. burnetii* Nine Mile strain (RSA493), a genotype that has never been observed before in the Netherlands. Moreover, these samples had relatively high DNA loads as determined by a real-time PCR targeting the *C. burnetii*-specific IS*1111a* element (5), and these were among the highest values observed. We therefore suspected that these five serum samples had somehow been contaminated with Nine Mile DNA. In the involved laboratory, diagnosis of Q fever is performed by a CFT by using commercially available antigen preparations (Institut Virion/Serion GmbH, Würzburg, Germany) and by real-time PCR. According to the kit insert, these antigen preparations contain "antigens isolated from infected cells." We expected that one of the components of the CFT kit might also contain DNA from the *C. burnetii* Nine Mile strain. DNA was extracted from the control antigen preparations in this kit and analyzed by real-time PCR and MLVA genotyping. A very low threshold cycle (C_T) value (14.4) confirmed the presence of very high DNA loads in the antigen preparation. MLVA genotyping confirmed the identity of the Nine Mile strain. Our results confirm the commercial antigen preparation as the source of contamination of the serum samples.

Lack of a serological response after 8 weeks of follow-up in the involved five patients confirmed that the original positive PCR results were indeed most likely the result of a laboratory contamination. Moreover, in another 11 samples, a possible false-positive PCR result was generated, as no serological response was found in these patients either. Although the lack of a serological response might also be explained by other factors, a laboratory contamination could not be excluded for these samples. However, it could not be confirmed since these other samples contained insufficient DNA to allow genotyping.

The most likely way in which contamination might have taken place is by the formation of aerosols. The CFT procedure includes incubation and subsequent centrifugation of 96-well plates that are sealed but perforated. These steps were performed close to the workstation where patient sera are added to the plates. Standard laboratory guidelines recommended for the prevention of nucleic acid contamination in molecular testing procedures are operational in the laboratory, but these do not consider the presence of large amounts of DNA in commercial antigen preparations. Samples that appeared to be contaminated had been present in the room where the CFT was performed before they were transferred to the molecular facilities. This diagnostic workflow was not because of logistic problems but mostly because a PCR was requested as an additional diagnostic test in the same sample after the results of the CFT had become available. This part of the workflow has now been adapted to avoid similar future contamination problems. If a PCR test is requested after the blood sample has been in the CFT room, a new blood sample is requested and both the original and new blood sample are tested. If only the original sample is positive, a definitive test result is given only after the infection has been confirmed by serological methods.

To demonstrate that problems similar to those reported here may be realistic with other microorganisms, we also checked several other commercially available antigen preparations from the same supplier for the presence of target-specific

* Corresponding author. Mailing address: Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Weg door Jonkerbos 100, 6532 SZ Nijmegen, Netherlands. Phone: 31 24 3657514. Fax: 31 24 3657516. E-mail: c.klaassen@cwz.nl.

▽ Published ahead of print on 27 October 2010.

TABLE 1. Relative DNA/RNA loads of several commercially available antigen preparations as determined by target-specific (reverse transcription) real-time PCR

Target	C_T value
Adenovirus	21.1
Echovirus	34.5
Coxsackie B virus	23.7
Herpes simplex virus 1	25.4
Herpes simplex virus 2	24.8
Influenza A virus	31.8
Influenza B virus	32.5
Mycoplasma pneumoniae	37.3
Parainfluenza 1 virus	Negative
Parainfluenza 2 virus	29.1
Parainfluenza 3 virus	Negative
Coxiella burnetii	14.4
Coxiella burnetii phase1	24.9
Respiratory syncytial virus	25.5
Varicella-zoster virus	21.4

nucleic acids. The results show that almost all tested antigen preparations still contain considerable amounts of target nucleic acids (either DNA or RNA) (Table 1). The presence of such large DNA or RNA quantities in these antigen preparations poses a serious risk for laboratory contamination. On the other hand, the unexpected advantage of our findings is that such preparations can be used as convenient control material for molecular diagnostic test procedures.

The high loads of target nucleic acid in commercially available antigen preparations indicate that molecular diagnostic methods should be performed in a location physically separated from where serological methods are being used. In addition, it should preferably be performed on an aliquot of a sample that has not already been used for serological testing.

REFERENCES

1. **Angelakis, E., and D. Raoult.** 2010. Q fever. Vet. Microbiol. **140:**297–309.
2. **Fournier, P. E., T. J. Marrie, and D. Raoult.** 1998. Diagnosis of Q fever. J. Clin. Microbiol. **36:**1823–1834.
3. **Klaassen, C. H. W., M. H. Nabuurs-Franssen, J. J. H. C. Tilburg, M. A. W. M. Hamans, and A. M. Horrevorts.** 2009. Multigenotype Q fever outbreaks, The Netherlands. Emerg. Infect. Dis. **15:**613–614.
4. **Schneeberger, P. M., M. H. A. Hermans, E. J. van Hannen, J. J. A. Schellekens, A. C. A. P. Leenders, and P. C. Wever.** 2010. Real-time PCR on serum samples is indispensable for early diagnosis of acute Q fever. Clin. Vaccine Immunol. **17:**286–290.
5. **Tilburg, J. J. H. C., W. J. G. Melchers, A. M. Pettersson, J. W. A. Rossen, M. H. A. Hermans, E. J. van Hannen, M. H. Nabuurs-Franssen, M. C. de Vries, A. M. Horrevorts, and C. H. W. Klaassen.** 2010. Interlaboratory evaluation of different extraction and real-time PCR methods for detection of *Coxiella burnetii* DNA in serum. J. Clin. Microbiol. **48:**3923–3927.