

## *Afipia clevelandensis* Antibodies and Cross-Reactivity with *Brucella* spp. and *Yersinia enterocolitica* O:9

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*Afipia clevelandensis* is a recently described gram-negative bacterium whose potential pathogenic role in human disease is under investigation. Only one strain, from the pretibial lesion of a patient hospitalized with necrotizing pancreatitis for 5 months, has been isolated. Using an indirect immunofluorescence assay to detect anti-*A. clevelandensis* antibodies, we found a seroprevalence of 1.5% among 30,194 sera routinely submitted for laboratory diagnosis of rickettsial diseases. However, among the 52 patients who were clinically evaluable and who exhibited detectable antibodies against *A. clevelandensis*, 42% were eventually diagnosed as certainly or probably having brucellosis and 15% were eventually diagnosed as certainly or probably having *Yersinia enterocolitica* O:9 infection, which is the serotype most often encountered in Europe. Western immunoblotting and cross-adsorption tests showed that an 11.5-kDa proteinase K-labile band and a 21-kDa proteinase-stable band, presumably lipopolysaccharide, were responsible for cross-reactivity among *A. clevelandensis*, *Brucella abortus*, and *Y. enterocolitica* O:9. Other diagnoses included nosocomial infections and various community-acquired diseases for which the role of *A. clevelandensis* remains undefined. Physicians and clinical microbiologists should be aware of this cross-reactivity in future assessments of the role of *A. clevelandensis* in human pathology.

*Afipia* is a recently described genus of gram-negative bacteria which includes *Afipia felis*, *Afipia broomeae*, *Afipia clevelandensis*, and three unnamed genospecies (8). This genus is part of the alpha 2-subgroup of the class *Proteobacteria*, and along with *Bartonella* species (22), it is closely related to the human pathogens *Brucella* species and *Ochrobactrum anthropi* (formerly CDC group Vd bacteria) (18).

The precise role of *Afipia* species in human pathology remains largely unknown due to their rare isolation. Although *A. felis* was proposed as a putative agent of cat scratch disease (15), *Bartonella henselae* is diagnosed in most cases. *A. felis* was isolated on axenic medium from the adenomegalies of 10 patients with confirmed cat scratch disease (15), and 14 isolates were obtained with cultured cell systems (7). One additional isolate from an Israeli patient has recently been obtained on axenic medium (16). *A. felis* DNA has seldom been detected in clinical specimens (5), including codetection with *B. henselae* DNA (2), and one study failed to detect *A. felis* DNA in the enlarged lymph nodes of European patients with cat scratch disease (6). Discrepant results regarding the detection of specific antibodies in the serum of patients with cat scratch disease (15, 23) and the absence of detection in cat, however, has led to questions concerning the precise role of this species in cat scratch disease, particularly as growing evidence has been obtained for the role of *B. henselae* in this pathology (3, 4, 6). Three strains of *A. broomeae* have been cultured from human sources, including one isolate from the sputum of a patient in New Zealand in 1981, one isolate from the bone marrow of an American woman, and one isolate from the synovial fluid of an American patient with a wrist abscess. Three other *Afipia* strains, designated genospecies 1, 2, and 3 and as yet unnamed, have been described. These three isolates were obtained from

human pleural fluid in a fatal case of pulmonary obstruction in 1981 (genospecies 1), from the bronchial washing of an 80-year-old woman with pneumonia (genospecies 2), and from water (genospecies 3). Only one strain of *A. clevelandensis* has been isolated to date and that was from a pretibial area biopsy in a 69-year-old American male patient hospitalized for 5 months with necrotizing pancreatitis who developed petechial lesions and erythematous plaques of the lower extremities (17). The timing of isolation indicated that this isolate was of nosocomial origin, and this strain was first related to other *Afipia* species on the basis of its unusual cellular fatty composition, characterized by 11-methyloctadec-12-enoic acid (20). Its precise pathogenic role in human beings remains unknown, particularly as no serology was performed in the unique patient with the *A. clevelandensis* isolate.

While investigating the potential role of this bacterium in human diseases, we prospectively tested the human sera sent to our laboratory for the presumptive diagnosis of rickettsiosis for the presence of anti-*A. clevelandensis* antibodies. We encountered a significant number of patients with confirmed brucellosis or *Yersinia enterocolitica* O:9 infection who demonstrated positive serology for *A. clevelandensis*. This observation led us to study the possibility of cross-reactivity among *A. clevelandensis*, *Brucella abortus*, and *Y. enterocolitica* O:9, and the results of this study are presented herein.

### MATERIALS AND METHODS

**Bacteria.** *A. clevelandensis* (ATCC 49720<sup>T</sup>) was obtained from the American Type Culture Collection (Rockville, Md.) and was grown on 5% sheep blood agar at 30°C under ambient atmosphere. *B. abortus* was isolated in our laboratory from a patient with endocarditis and was grown on 5% sheep blood agar. *Y. enterocolitica* O:9 was obtained as a clinical isolate from a patient with enteritis and was grown on Trypticase-soy agar.

**Patients and sera.** All the sera received by our laboratory between 1 January 1993 and 31 December 1996 were tested for the presence of antibodies against *A. clevelandensis*. These sera had been sent by clinical microbiology laboratories from all over France for serological diagnosis of a number of rickettsial and bacterial diseases including spotted fever group and typhus group rickettsiosis, Q

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fever, cat scratch disease, and *Bartonella* species infection. For sera exhibiting an anti-*A. clevelandensis* immunoglobulin G (IgG) titer of  $\geq 1:200$  or an IgM titer of  $\geq 1:100$ , the clinical history of the patient was reviewed for any previous diagnosis of infectious disease, in particular those demonstrated to have been caused by *Brucella* or *Yersinia* species. Serological tests for *Brucella* and *Y. enterocolitica* O:9 antibodies were performed on sera obtained from such patients. A patient was defined as suffering brucellosis when any febrile illness was associated with the isolation of *Brucella* species from any clinical specimen or with exposure to cattle and a standard tube agglutination (STA) test titer of  $>1:160$ . A patient was defined as suffering from yersiniosis when enteritis was associated with the isolation of *Y. enterocolitica* O:9 from stool. One hundred sera collected from 100 healthy blood donors in France were included in the study as a negative control group. A panel of 32 sera obtained from 22 patients suffering past or active brucellosis who had been the subjects of a previous study (unpublished data) were screened for the presence of anti-*A. clevelandensis* IgG and IgM antibodies and for the presence of anti-*Y. enterocolitica* antibodies by the tests described above.

**Serological tests for *B. abortus* and *Y. enterocolitica*.** Serological testing for *Brucella* was performed by using a commercial STA test (Brucelloslide-Test; Biomérieux, Marcy-l'Etoile, France) and by using a microimmunofluorescence test. The STA test uses heat- and 0.5% phenol-inactivated *B. abortus* 99 (Weybridge strain) as the antigen, and the agglutination tests were performed according to the instructions of the manufacturer. Any agglutination was read as a positive result. Microimmunofluorescence was performed according to standard procedures by using the same antigen as for the STA test. Serological testing for *Y. enterocolitica* O:9 was performed by using a commercial complement fixation test (Institut Virion SA, Zürich, Switzerland) according to the instructions of the manufacturer. Any titer of  $>1:10$  was considered positive.

**Microimmunofluorescence assay for *A. clevelandensis*.** Bacterial colonies of *A. clevelandensis* were harvested and suspended in phosphate-buffered saline (PBS) to obtain a suspension of 1-mg/ml protein. Aliquots of this suspension were disposed onto the wells of microscope slides, air dried, and then fixed in cold acetone. The sera were diluted in PBS containing 3% nonfat dry milk. Initially, all sera were screened at a dilution of 1:25 and, with a goat anti-human globulin fluorescein conjugate (Biomérieux), diluted to 1:400. Sera reacting at a dilution of 1:25 were retested to determine their IgG and IgM titers. The determination of IgM titers was performed after removing IgG with the rheumatoid factor absorbent reagent (Behring AG, Marburg, Germany) by using a goat anti-human IgM fluorescein conjugate (Biomérieux) diluted to 1:200. Slides containing fixed antigen overlaid with dilutions of sera were incubated for 30 min at 37°C in a moist chamber. The slides were then washed three times, for 10 min each time, in PBS and air dried. A drop (10  $\mu$ l) of conjugate, diluted in PBS-3% nonfat dry milk with 0.025% Evans blue was then added to each slide well, and slides were reincubated for 30 min at 37°C in a moist chamber. The slides were again washed three times for 10 min each time in PBS and were then washed once for 10 min in distilled water and air dried before being mounted with buffered glycerol (Fluoprep; Biomérieux). The slides were observed with an epifluorescent microscope with a 40 $\times$  objective. Serum bled from a mouse 1 month after intraperitoneal injection with a suspension of *A. clevelandensis* was used as a positive control. This serum was revealed with an anti-mouse globulin fluorescein conjugate (Immunotech, Marseille, France).

**Western immunoblotting.** Bacteria were suspended in sterile distilled water, and the protein concentration was adjusted to 2.5 mg/ml by spectrophotometric reading at 280 nm (Protein analysis program pack; Shimadzu, Kyoto, Japan). Antigens were then prepared following one of two protocols: (i) 1 volume of antigen was mixed with one-half volume of 2 $\times$  Laemmli solubilizer (4% sodium dodecyl sulfate [SDS], 0.125 M Tris hydrochloride [pH 6.8], 25% glycerol, 10% 2-mercaptoethanol, 0.5% bromophenol blue) (19) and one-half volume of sterile distilled water and incubated at 56°C for 2 h; (ii) 1 volume of antigen was mixed with one-half volume of 2 $\times$  Laemmli solubilizer and one-quarter volume of 20-mg/ml proteinase K (Boehringer Mannheim France SA, Meylan, France) and was incubated at 56°C for 1 h; an additional quarter-volume of proteinase K was then added, and the mixture was incubated at 56°C for 1 additional h. SDS-polyacrylamide gel electrophoresis was performed on 20  $\mu$ l of each preparation by using a 12.5% acrylamide separating gel with a 4% stacking gel and the Mini-Protean II cell apparatus (Bio-Rad, Richmond, Calif.) at 100 V for 2 h. Low-range molecular weight standards (Bio-Rad) were used to estimate the molecular weight of the separated proteins. The gel was stained with Coomassie blue R-250 (Bio-Rad). For Western blot analysis, the polyacrylamide gel was prepared as described above and transferred to a nitrocellulose membrane in a transblot cell (Bio-Rad) at 50 V for 4 h in a cooled bath. The membrane was blocked with 5% nonfat dry milk in TBS (20 mM Tris-HCl [pH 7.5], 500 mM NaCl, 0.1% Merthiolate). Sera from three patients were tested, one from a patient with confirmed brucellosis (IgG, 1:100; IgM, 1:50), one from a patient with confirmed *Y. enterocolitica* infection (IgG, 1:200; IgM, 1:50), and one from a patient with positive *A. clevelandensis* serology (IgG, 1:200, IgM, 1:25) without evidence of brucellosis or *Y. enterocolitica* infection. Each serum sample was diluted (1:50) in TBS containing 3% nonfat dry milk and incubated with the membrane overnight. After three washes in TBS, the membrane was incubated for 1 h in peroxidase-conjugated goat anti-human IgG, anti-human IgM, or anti-human IgA (Immunotech). Conjugated antibodies were diluted (1:200) in 3% NFDN-TBS before use. The nitrocellulose membrane was washed again in

TABLE 1. Distribution of IgG and IgM anti-*A. clevelandensis* antibody titers among 30,194 sera prospectively tested by microimmunofluorescence assay<sup>a</sup>

Antibody titer	Number of sera positive for:	
	IgG	IgM
1:25	17	111
1:50	149	97
1:100	89	58
1:200	46	18
1:400	18	5
1:800	19	1
1:1,600	17	0
1:3,200	1	0

<sup>a</sup> Of the total number of samples tested, 29,838 had an antibody titer of 0 for IgG and 29,904 had antibody titer of 0 for IgM.

TBS, and immunologic reactions were detected colorimetrically (0.015% 4-chloro-1-naphthol-0.015% hydrogen peroxide in 16.7% methanol in TBS). The blots were analyzed using the Imager apparatus and the QGEL-1D program (Appligène, Illkirch, France).

**Adsorption tests.** A total of 900  $\mu$ l of serum from the patients with isolated *A. clevelandensis* serology, brucellosis, or *Y. enterocolitica* infection was split into three aliquots. Each aliquot was adsorbed with one of the three tested antigens by rocking the serum with the antigen (protein concentration, 2 mg/ml) at room temperature. Adsorption was tested with the adsorbed serum and its reciprocal antigen by means of the microimmunofluorescence assay described above. If reactivity was still observed, serum was reabsorbed, and this process was continued until no reactivity was detectable. Each aliquot of adsorbed sera was then tested against *A. clevelandensis*, *B. abortus*, and *Y. enterocolitica* by the techniques reported above and by Western immunoblotting.

## RESULTS

**Sera and clinical records.** A total of 30,194 sera were received and prospectively tested for the presence of antibody against *A. clevelandensis* between 1 January 1993 and 31 December 1996. The distribution of the titers is reported in Table 1. A total of 458 serum samples exhibited an IgG or IgM titer of  $\geq 1:25$  (1.5%). Among these serum samples, 154 samples collected in 102 patients exhibited an IgG titer of  $\geq 1:200$  or an IgM titer of  $\geq 1:100$ . Clinical data were not available for 50 of these patients. Review of the clinical records of the remaining 52 patients found 22 patients with a diagnosis of brucellosis, 9 patients with nosocomial infection, 8 patients with a diagnosis of *Y. enterocolitica* O:9 enteritis, 2 patients with cat scratch disease, and 11 patients with miscellaneous diagnoses. Of the 11 patients with miscellaneous diagnoses (8 females and 3 males, aged 29 to 66 years old), 2 patients presented with community-acquired pneumonia of unknown etiology, 2 patients presented with enlarged lymph nodes of unknown etiology (cat scratch disease was excluded in both by means of negative serology for *B. henselae* and *A. felis*, negative molecular detection of *B. henselae*, and histology incompatible with this diagnosis), and 2 patients presented with chronic diarrhea of unknown etiology (one of them had a previously diagnosed chronic monocytic leukemia and the other developed a 6-month neutropenia with marked monocytosis; diarrhea resolved after treatment with doxycycline in both patients). One patient presented with a community-acquired meningoencephalitis with coma of unknown etiology. A 29-year-old Spanish woman presented with relapsing culture-negative endocarditis which occurred 5 years after the implantation of a prosthetic aortic valve. The endocarditis finally resolved after administration of ceftriaxone plus doxycycline, and although microbiological documentation failed, the serum samples exhibited a 1:40 titer against *Brucella* and a 1:100 IgM titer against *A. clevelandensis*. One patient presented with an *A. clevelandensis* IgG

TABLE 2. Comparison of anti-*Brucella* IgG titers and anti-*Afpipia clevelandensis* IgG titers among 32 sera of 22 patients with confirmed past or active brucellosis

Anti- <i>Brucella</i> IgG titer	No. of sera with anti- <i>A. clevelandensis</i> IgG titers of:		
	<1:25	1:25-1:50	>1:100
>1:640	3	3	14
1:160-1:320	1	4	2
<1:160	3	2	0

titer of 1:200 associated with auto-antibodies and a final diagnosis of Gougerot-Sjögren syndrome, and one patient exhibited an *A. clevelandensis* IgG titer of 1:200 as a routine serology (this was a man who had had contact with cats but was without cat scratch disease). A 61-year-old woman with an unremarkable medical history was hospitalized for necrotizing pancreatitis of unknown etiology with left pleural effusion in March 1995. Fifteen days later, the patient presented a pancreatic abscess which resolved without debridement, and no microbiological documentation of the abscess was obtained. The patient developed no nosocomial infection during the course of her disease and eventually recovered after 40 days of hospitalization. A serum sample drawn 3 weeks after the outcome of pancreatitis disclosed an anti-*A. clevelandensis* titer of 1:200 for both IgM and IgG.

The nine patients with nosocomial infection included five males and four females, 46 to 75 years old, with medical histories of diabetes mellitus in three cases, chronic pulmonary insufficiency in three cases, and alcoholism and tobacco addiction in two cases. These patients presented with nosocomial pneumonia in six cases, nosocomial septicemia in two cases, and fever of unknown origin in one case. Microbiological documentation was available for five cases, and the organisms detected included *Pseudomonas aeruginosa* (two cases), *Pseudomonas fluorescens* (one case), *Acinetobacter* species (one case), and *Staphylococcus aureus* (one case). Two patients presented with a diagnosis of cat scratch disease confirmed by the presence of anti-*B. henselae* IgG of 1:400 (measured by micro-immunofluorescence assay) and the detection of *B. henselae* DNA in one case; these patients exhibited *A. clevelandensis* serologies of IgG = 0 and IgM = 1:100 and IgG = 1:400 and IgM = 0, respectively.

**Cross-reactivity by immunofluorescence techniques.** No antibodies against *A. clevelandensis*, *Brucella* or *Y. enterocolitica* O:9 were detected among the 100 sera collected in 100 healthy blood donors. A correlation between IgG titers against *B. abortus* and *A. clevelandensis* was found among 32 sera from patients with documented, past or active brucellosis (Table 2). Of five sera exhibiting an anti-*Brucella* IgG titer of <1:160, none exhibited an anti-*A. clevelandensis* titer of >1:100. Of 20 sera with anti-*Brucella* IgG titers of >1:640, however, 14 (70%) exhibited anti-*A. clevelandensis* titers of >1:100. Likewise, 11 of 23 sera with a positive STA test for *Brucella* and 1 of 9 sera with a negative STA test exhibited anti-*A. clevelandensis* IgM titers of >1:40.

**Western immunoblotting.** The results of Western immunoblotting studies are presented in Fig. 1. The serum of the patient with documented brucellosis reacted strongly with native *B. abortus* antigen, including a proteinase K-labile band of 11.5-kDa molecular mass. The same serum also reacted with *Y. enterocolitica* O:9 antigen, including the 11.5-kDa, proteinase K-labile band and a 21-kDa, proteinase K-stable band. This serum reacted with *A. clevelandensis* antigen, including the

11.5-kDa proteinase K-labile band, and an 18- to 22-kDa, proteinase K-stable smear. The serum of the patient with documented *Y. enterocolitica* O:9 infection reacted strongly with homologous antigen, including the 11.5-kDa band. It reacted only with this proteinase K-labile, 11.5-kDa band in *B. abortus* antigen and with a few antigenic bands in *A. clevelandensis* antigen, including the 11.5-kDa, proteinase K-labile band and a faint, 21-kDa, proteinase K-stable band. The serum of the patient with positive *A. clevelandensis* serology but no documented infection reacted with several bands in *A. clevelandensis* antigen, including the 11.5-kDa, proteinase K-labile band and a 21-kDa, proteinase K-stable band. This serum only reacted with the 11.5-kDa, proteinase K-labile band in *B. abortus* antigen and with numerous bands in *Y. enterocolitica* O:9 antigen, including the 11.5-kDa, proteinase K-labile band.

**Adsorption tests.** As featured by the study design, adsorption tests resulted in the disappearance of homologous antibodies, regardless of the antigen tested. Adsorption tests with *Brucella* as the antigenic adsorbant did not modify the titer of antibody against *Y. enterocolitica* O:9 but led to a fourfold decrease in the anti-*A. clevelandensis* antibody titer. An ad-

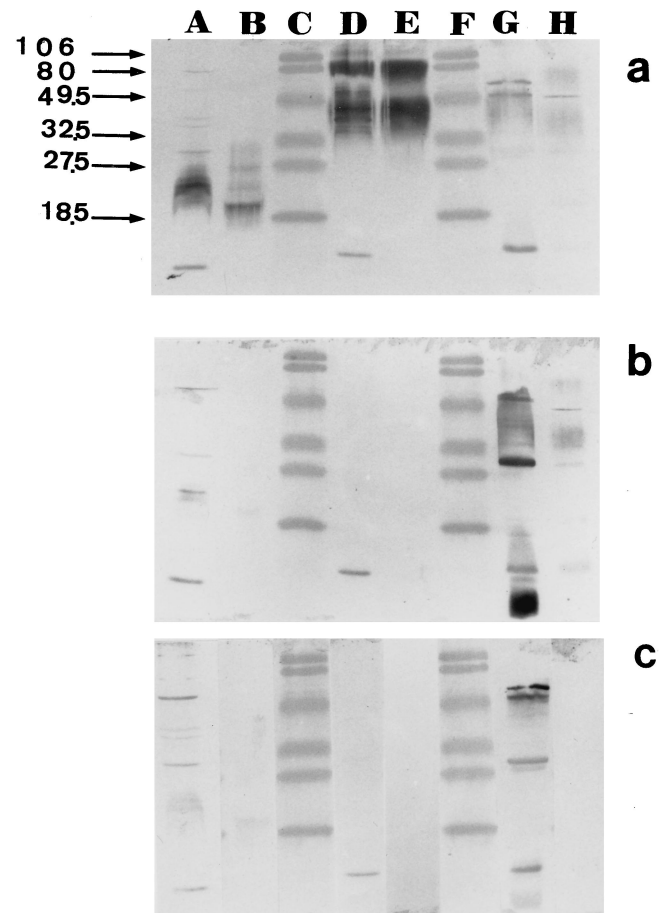


FIG. 1. Western immunoblotting profiles against *A. clevelandensis*, *B. abortus*, and *Y. enterocolitica* O:9 obtained with sera of patients with brucellosis (a) or yersiniosis (b) or which exhibited isolated positive serology for *A. clevelandensis* (c). Lane A, undigested *A. clevelandensis* antigen; lane B, proteinase K-digested *A. clevelandensis* antigen; lane C, molecular mass marker; lane D, undigested *B. abortus* antigen; lane E, proteinase K-digested *B. abortus* antigen; lane F, molecular mass marker; lane G, undigested *Y. enterocolitica* O:9 antigen; lane H, proteinase K-digested *Y. enterocolitica* O:9 antigen. Molecular masses are indicated on the left in kilodaltons.

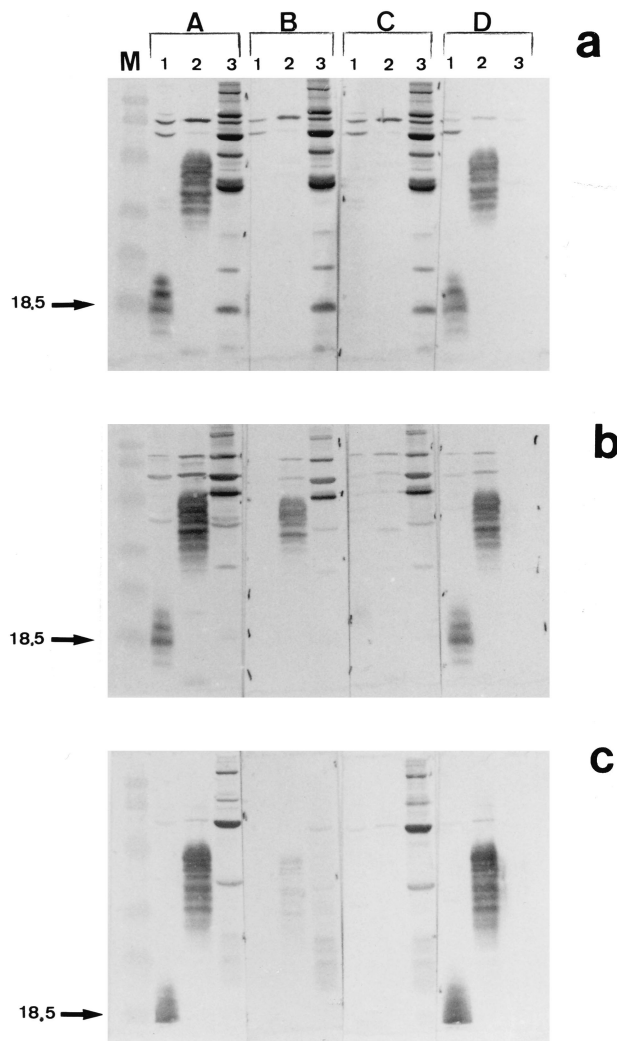


FIG. 2. Western immunoblotting profiles against *A. cleavelandensis*, *B. abortus*, and *Y. enterocolitica* O:9 obtained with sera of patients with brucellosis (a) or yersiniosis (b) or which exhibited isolated positive serology for *A. cleavelandensis* (c) after adsorption with one of the three bacterial antigens. Lanes 1, *A. cleavelandensis* antigen, lanes 2, *B. abortus* antigen, lanes 3, *Y. enterocolitica*. Column A, nonadsorbed serum; column B, adsorption with *A. cleavelandensis*; column C, adsorption with *B. abortus*; column D, adsorption with *Y. enterocolitica* O:9. M, molecular mass markers. The position of the 18.5-kDa band is indicated for each panel.

sorption test with *Y. enterocolitica* O:9 did not modify the antibody titer against *A. cleavelandensis* but led to a onefold decrease in the anti-*Brucella* antibody titer. An adsorption test with *A. cleavelandensis* as antigen did not modify the antibody titer against *Y. enterocolitica* O:9 but led to a fourfold decrease in the anti-*Brucella* antibody titer. An analysis of cross-reactions using Western immunoblotting is presented in Fig. 2. Adsorption of serum from the brucellosis patient with homologous antigen suppressed its reactivity against all but the >80-kDa bands of *B. abortus* and *A. cleavelandensis* antigens but did not alter the protein profile of *Y. enterocolitica*. Adsorption of serum from a patient with documented *Y. enterocolitica* O:9 with homologous antigen suppressed all reactivity against *Y. enterocolitica* but did not modify the profiles of *B. abortus* or *A. cleavelandensis*, except for the 11.5-kDa band which disappeared. Adsorption of serum from a patient with positive

*A. cleavelandensis* serology with homologous antigen altered the profiles of *A. cleavelandensis* and *B. abortus* and suppressed reactivity against the 11.5-kDa band of *Y. enterocolitica* O:9.

## DISCUSSION

Using a microimmunofluorescence assay, we prospectively tested 30,194 human serum samples collected over 48 months for the presence of antibodies to *A. cleavelandensis* and correlated positive results with clinical presentation and diagnosis of the patients. During the course of this study, it appeared that among the 52 patients clinically evaluable and exhibiting detectable antibodies against *A. cleavelandensis*, 42% were eventually diagnosed as certainly or probably having brucellosis and 15% were eventually diagnosed as certainly or probably having *Y. enterocolitica* O:9 infection, which is the serotype most often encountered in Europe (10). No isolation of *A. cleavelandensis* was made among these patients. Considering the group of patients with certain and probable brucellosis or *Y. enterocolitica* infection, an IgM titer of 1:200 or more had a positive predictive value of 0.6 for brucellosis, an IgG titer of 1:400 or more had a positive predictive value for either brucellosis or *Y. enterocolitica* infection of 0.9, and a IgG titer of 1:800 or more had a positive predictive value for brucellosis of 0.84. Apart from patients with brucellosis or *Yersinia* infection, we observed 20 patients with significant levels of anti-*A. cleavelandensis* antibodies who did not match the diagnostic criteria for brucellosis or yersiniosis. *Pseudomonas* species may account for some cross-reactivities in patients with nosocomial infection. Hall et al. reported the unique isolation of *A. cleavelandensis* in an American patient after months of hospitalization and undescribed antibiotic treatments (17), supporting the conclusion that *A. cleavelandensis* is thus capable of causing nosocomial infection. Antibiotic resistance reported for this species (8), presumably constitutive in the absence of detectable plasmid, is compatible with its selection either from the hospital environment or indigenous flora of this patient. Among patients with community-acquired diseases, one patient presented with necrotizing pancreatitis reminiscent of the patient who has yielded the only *A. cleavelandensis* isolate. This patient in our study had elevated IgG and IgM titers at the time of admission, but as no microbiological documentation was performed the significance of this result is unclear. Only one serologic study of *A. cleavelandensis* has been previously reported (21). In that study, which used a microagglutination test, 94 of 336 sera exhibited agglutination and 47 sera (14%) were considered positive (antibody titer of >20) by the authors. In the same study, the percent positivity for *A. felis*, *A. broomeae*, and each of the three unnamed *Afiplia* genospecies was 7, 3, and  $\leq 1$ , respectively. No correlation between positive serology and clinical disease was made. Using microimmunofluorescence, we found a lower seroprevalence of anti-*A. cleavelandensis* antibodies, i.e., 1.5% (IgG or IgM titers of  $\geq 1:25$ ). This discrepancy between the rates of seroprevalence may be due to differences in the number of sera included in these studies (336 versus 30,194) or to the serological techniques themselves (microagglutination versus microimmunofluorescence). Also, differences in seroprevalence in different geographic areas could be hypothesized since the prevalence of *Yersinia* infections is greater in northern Europe and that of brucellosis is greater in southern Europe. Both studies, however, confirm a significant seroprevalence against *A. cleavelandensis* despite this species being represented by a sole isolate and having an unknown pathologic role in humans.

Our clinical observations led us to postulate a cross-reactivity among *A. cleavelandensis*, *B. abortus*, and *Y. enterocolitica*

O:9. Cross-reactivity has been previously reported between *B. abortus* and other lipopolysaccharide-containing bacteria including *Escherichia coli* O157 (13), *Y. enterocolitica* O:9 (1), and *O. anthropi* (24), as demonstrated by agglutination, complement fixation tests, immunoblotting, and delayed-type hypersensitivity tests. Interestingly, the genera *Afipia*, *Brucella*, and *Ochrobactrum* all belong to the  $\alpha$  subdivision of the class *Proteobacteria*. The smooth-type *Brucella* species have been found to contain two distinct antigenic determinants designated A and M (14). Cross-reaction between the A lipopolysaccharide of *Brucella* species and *Y. enterocolitica* has been associated with the chemical similarity of the O-polysaccharide chains of the two species (9, 11, 12). The data presented herein confirm cross-reactivity between both bacterial species and show that a 21-kDa, proteinase K-stable antigen, presumably lipopolysaccharide, and an 11.5-kDa, proteinase K-labile antigen are responsible for the cross-reactivity among the three bacterial species. Interestingly, the 11.5-kDa protein is common to the three species.

In the search for the potential role of *A. clevelandensis* in human diseases, bacteriologists and physicians must be aware of the cross-reactivity among this bacterium, *Brucella* species, and *Y. enterocolitica* and that no serological method can reliably distinguish between these pathogens. Further assessment of the role of *A. clevelandensis* in human pathology must therefore aim for the isolation or direct characterization of the bacterium in clinical samples, particularly in patients with nosocomial infections.

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#### REFERENCES

- Ahvonon, P., E. Jansson, and K. Aho. 1969. Marked cross-agglutination between *Brucella* and a subtype of *Yersinia enterocolitica*. *Acta Pathol. Microbiol. Scand.* **75**:291-295.
- Alkan, S., M. B. Morgan, R. L. Sandin, L. C. Moscinski, and C. W. Ross. 1995. Dual role for *Afipia felis* and *Rochalimaea henselae* in cat scratch disease. *Lancet* **345**:385.
- Anderson, B., C. Kelly, R. Threlkel, and K. Edwards. 1993. Detection of *Rochalimaea henselae* in cat-scratch disease skin test antigens. *J. Infect. Dis.* **168**:1034-1036.
- Anderson, B., K. Sims, R. L. Regnery, L. Robinson, M. J. Schmidt, S. Goral, C. Hager, and K. Edwards. 1994. Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J. Clin. Microbiol.* **32**:942-948.
- Andrews, D. M., J. T. Kurnick, and K. L. Ruoff. 1992. Case 22-1992. *N. Engl. J. Med.* **327**:1600-1601. (Author's reply.)
- Bergmans, A. M., J. W. Groothedde, J. F. P. Schellekens, J. D. A. van Embden, J. M. Ossewaarde, and L. M. Schouls. 1995. Etiology of cat scratch disease: comparison of polymerase chain reaction detection of *Bartonella* (formerly *Rochalimaea*) and *Afipia felis* DNA with serology and skin tests. *J. Infect. Dis.* **171**:916-923.
- Birkness, K. A., V. G. George, E. H. White, D. S. Stephens, and F. D. Quinn. 1992. Intracellular growth of *Afipia felis*, a putative etiologic agent of cat scratch disease. *Infect. Immun.* **60**:2281-2287.
- Brenner, D. J., D. G. Hollis, C. W. Moss, C. K. English, G. S. Hall, J. Vincent, J. Radosevic, K. A. Birkness, W. F. Bibb, F. D. Quinn, B. Swaminathan, R. E. Weaver, M. W. Reeves, S. P. O'Connor, P. S. Hayes, F. C. Tenover, A. G. Steigerwalt, B. A. Perkins, M. I. Daneshvar, B. C. Hill, J. A. Washington, T. C. Woods, S. B. Hunter, T. L. Hadfield, G. W. Ajello, A. F. Kaufmann, D. J. Wear, and J. D. Wenger. 1991. Proposal of *Afipia* gen. nov., with *Afipia felis* sp. nov. (formerly the cat scratch disease bacillus), *Afipia clevelandensis* sp. nov. (formerly the Cleveland Clinic Foundation strain), *Afipia broomeae* sp. nov., and three unnamed genospecies. *J. Clin. Microbiol.* **29**:2450-2460.
- Bundle, D. R., M. A. J. Gidney, M. B. Perry, J. R. Duncan, and J. W. Cherwonogrodzky. 1984. Serological confirmation of *Brucella abortus* and *Yersinia enterocolitica* O:9 O-antigens by monoclonal antibodies. *Infect. Immun.* **46**:389-393.
- Butler, T. 1995. *Yersinia* species (including plague) p. 2070-2078. In G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), *Principles and practice of infectious diseases*. Churchill Livingstone, New York, N.Y.
- Caroff, M., D. R. Bundle, M. B. Perry, J. W. Cherwonogrodzky, and J. R. Duncan. 1984. Antigenic S-type lipopolysaccharide of *Brucella abortus* 1119-3. *Infect. Immun.* **46**:384-388.
- Caroff, M., D. R. Bundle, and M. B. Perry. 1984. Structure of the O-chain of the phenol-phase soluble cellular lipopolysaccharide of *Yersinia enterocolitica* serotype O:9. *Eur. J. Biochem.* **139**:195-200.
- Chart, H., O. A. Okubadejo, and B. Rowe. 1992. The serological relationship between *Escherichia coli* O157 and *Yersinia enterocolitica* O9 using sera from patients with brucellosis. *Epidemiol. Infect.* **108**:77-85.
- Diaz-Aparicio, E., V. Aragon, C. Marin, B. Alonso, M. Font, E. Moreno, S. Perez-Ortiz, J. M. Blasco, R. Diaz, and I. Moriyon. 1993. Comparative analysis of *Brucella* serotype A and M and *Yersinia enterocolitica* O:9 polysaccharides for serological diagnosis of brucellosis in cattle, sheep, and goats. *J. Clin. Microbiol.* **31**:3136-3141.
- English, C. K., D. J. Wear, A. M. Margileth, C. R. Lissner, G. P. Walsh. 1988. Cat-scratch disease: isolation and culture of the bacterial agent. *JAMA* **259**:1347-1352.
- Giladi, M., B. Avidor, Y. Kletter, S. Abulafia, L. N. Slater, and D. F. Welch. 1996. Cat scratch disease: the rare role of *Afipia felis*. *Clin. Infect. Dis.* **23**:922. (Abstract 333.)
- Hall, G. S., K. Pratt-Rippin, and J. A. Washington. 1991. Isolation of agent associated with cat scratch disease bacillus from pretibial biopsy. *Diagn. Microbiol. Infect. Dis.* **14**:511-513.
- Holmes, B., M. Popoff, M. Kiredjian, and K. Kersters. 1988. *Ochrobactrum anthropi* gen. nov., sp. nov. from human clinical specimens and previously known as group Vd. *Int. J. Syst. Bacteriol.* **38**:406-416.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-685.
- Moss, C. W., G. Holzer, P. L. Wallace, and D. G. Hollis. 1990. Cellular fatty acid compositions of an unidentified organism and a bacterium associated with cat scratch disease. *J. Clin. Microbiol.* **28**:1071-1074.
- Müller, H. E. 1993. Detection of antibodies to *Afipia* species by the micro-agglutination test. *Eur. J. Clin. Microbiol. Infect. Dis.* **12**:951-954.
- O'Connor, S. P., M. Dorsch, A. G. Steigerwalt, D. J. Brenner, and E. Stackebrandt. 1991. 16S rRNA sequences of *Bartonella bacilliformis* and cat scratch disease bacillus reveal phylogenetic relationships with the alpha-2 subgroup of the class *Proteobacteria*. *J. Clin. Microbiol.* **29**:2144-2150.
- Regnery, R. L., J. G. Olson, B. A. Perkins, and W. Bibb. 1992. Serologic response to *Rochalimaea henselae* antigen in suspected cat-scratch disease. *Lancet* **339**:1443-1445.
- Velasco, J., R. Diaz, M. J. Grilló, M. Barberán, C. Marín, J. M. Blasco, and I. Moriyón. 1997. Antibody and delayed-type hypersensitivity responses to *Ochrobactrum anthropi* cytosolic and outer membrane antigens in infections by smooth and rough *Brucella* spp. *Clin. Diagn. Lab. Immunol.* **4**:279-284.