Immunoblot Characterization of *Porphyromonas* Species from Infected Dog and Cat Bite Wounds in Humans. M. E. Mulligan, D. M. Citron, R. Y. Y. Kwok, and E. J. C. Goldstein. From the Veterans Affairs Medical Center, Long Beach, Long Beach; the University of California, Irvine, College of Medicine, Irvine; the R. M. Alden Research Laboratory, Santa Monica; and the UCLA School of Medicine, Los Angeles, California

The organisms that cause infections of dog and cat bite wounds in humans are varied and typically reflect the normal flora of the biting animal. Many of these organisms are not well characterized. *Porphyromonas* species are common members of the oral flora of cats and dogs and are therefore likely pathogens in bite wound infections [1]. *Porphyromonas* species are fastidious, slow-growing, pigmented, asaccharolytic gram-negative rods and often cannot be fully identified by means of routine methods [2]. We studied isolates that had been recovered from infected wounds and had been characterized by using a variety of biochemical tests and AP (arbitrarily primed)-PCR fingerprinting [3]. Our purpose was to determine whether immunoblotting would be a useful method for characterizing these organisms and to compare results with those obtained by use of biochemical tests and AP-PCR fingerprinting.

*Figure 1.* Examples of immunoblot patterns for *Porphyromonas* species. Lane 1, *P. gingivalis* (clinical isolate); lanes 2–4, *P. cansulci* (clinical isolates); lane 5, *P. salivosa* (clinical isolate); lane 6, *P. circumdentaria* (NCTC [National Collection of Type Cultures] 12469); lane 7, *P. canoris* (NCTC 12835); lane 8, *P. canginalis* (NCTC 12856); lane 9, *P. cansulci* (NCTC 12858) (numbers indicate molecular weight standards in kD).

*Porphyromonas* species were isolated at the R. M. Alden Research Laboratory (Santa Monica, CA), where specimens from 18 emergency departments at various locations in the United States were sent as part of a multicenter study of infected dog and cat bite wounds in humans [3]. We studied eight type strains in addition to 29 clinical isolates. These type strains were *P. gingivalis* ATCC (American Type Culture Collection) 33277, *P. salivosa* NCTC (National Collection of Type Cultures) 11632, *P. macacae* ATCC 33141, *P. canoris* NCTC 12835, *P. canginalis* NCTC 12856, *P. cansulci* NCTC 12858, *P. circumdentaria* NCTC 12469, and *P. levii* ATCC 29147.

The enzyme tests were performed with the RapID ANA II (Innovative Diagnostic Systems, Norcross, GA), and AnIDENT panels and API-ZYM strips (bioMérieux, St. Louis).

We performed AP-PCR fingerprinting by using a nonspecific primer derived from DNA intergenic spacer (T3B 5'AGGTCG-determine whether immunoblotting would be a useful method for characterizing these organisms and to compare results with those obtained by use of biochemical tests and AP-PCR fingerprinting.

Immunoblotting was performed with use of pooled human sera as the antibody source, as previously described [4]. This method has been used for typing and identifying both facultative [4] and anaerobic bacteria [5]. Performance and interpretation of immunoblots were conducted by individuals blinded to results of other laboratory studies and to clinical data regarding the isolates. Isolates that differed by at least four major bands were considered to belong to different species, whereas isolates with three or fewer different bands were considered to represent different types within a species. Isolates of *P. macacae* and *P. salivosa* could not be separated into two distinct species on the basis of these criteria, although there were five different types among these isolates on
the basis of the immunoblots. The AP-PCR profiles of all isolates identified as \textit{P. macacae} and \textit{P. salivosa} were identical.

There were 26 isolates of \textit{Porphyromonas} species recovered from 54 specimens obtained from infected cat bite wounds and 14 isolates recovered from 48 specimens obtained from infected dog bite wounds; the biochemical test results for these isolates have been reported [3]. Although most of these veterinary species of \textit{Porphyromonas} could be grouped on the basis of biochemical testing, \textit{P. cansulci} and \textit{P. circumdentaria} are biochemically inactive and could not be characterized with use of biochemical tests. None of the biochemical systems was able to identify all species. In addition, no differences in pigmentation were noted in the present study, for which five different test media were used. We used AP-PCR profiles successfully to separate these species. Twenty-nine of the clinical isolates were selected for immunoblot typing so as to represent all distinct isolates identified by biochemical testing and AP-PCR.

Figure 1 shows the immunoblot patterns for representative organisms. Isolates were identified by immunoblots as \textit{P. salivosa} \((n = 10, \text{four subgroups})\), \textit{P. canoris} \((n = 6, \text{two subgroups})\), \textit{P. cansulci} \((n = 6, \text{two subgroups})\), \textit{P. cangingivalis} \((n = 4, \text{three subgroups})\), \textit{P. circumdentaria} \((n = 3, \text{homogenous})\), \textit{P. macacae} \((n = 1)\), and \textit{P. levii} \((n = 1)\); the last two isolates were type strains. Immunoblots were distinctly different for the different species, but \textit{P. canoris} and \textit{P. cangingivalis} were most closely related. Isolates identified as \textit{P. cansulci} by AP-PCR were readily differentiated from organisms identified as \textit{P. circumdentaria} by immunoblots. There was complete concordance between the results obtained with AP-PCR and the immunoblots.

Many fastidious organisms that are part of the normal oral flora are difficult to identify or characterize with use of traditional methods. Veterinary isolates are usually not represented in the data bases of commercial systems. We could not fully characterize the \textit{Porphyromonas} species recovered from infected cat and dog bite wounds in humans by using conventional biochemical systems. AP-PCR fingerprinting and immunoblotting both yielded distinct profiles for all type strains and clinical isolates, and the results with these two systems were completely concordant. Although immunoblots do not produce bands as vivid as those produced by AP-PCR and therefore require time and expertise for interpretation, immunoblotting is relatively rapid and inexpensive and offers another technique for identifying unusual bacteria.

References