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# Restriction Endonuclease Analysis and Ribotyping Differentiate *Pasteurella haemolytica* Serotype A1 Isolates from Cattle within a Feedlot†

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***Pasteurella haemolytica* serotype A1 isolates were collected from cattle within a feedlot during an outbreak of bovine respiratory disease. Genetic heterogeneity among the isolates was examined by restriction endonuclease analysis (REA), ribotyping, and analysis of plasmid content. The susceptibilities of isolates to several antibiotics were also examined. Five different REA patterns and three different ribotypes were observed among the isolates. Fifty percent of the isolates had an identical REA type, ribotype, and plasmid profile. Examination of the plasmid content of the isolates revealed that most (73%) carry a single plasmid which encodes β-lactamase, 13.5% carry two plasmids, and 13.5% carry no plasmid. The data reveal the presence of genetic differences among isolates of *P. haemolytica* A1, associated with shipping fever pneumonia within a closed feedlot, and suggest that a combination of REA, ribotyping, plasmid analysis, and antibiotic susceptibility determination will be useful in analyzing the molecular epidemiology of this disease.**

Bovine respiratory disease complex is one of the most costly diseases affecting beef cattle, especially those recently introduced into feedlots. Cattle management practices enhance physiological and emotional stress, predisposing animals to viral and bacterial infections. This combination of factors contributes significantly to the bovine respiratory disease complex (7, 31). The end result is often colonization of the lungs with *Pasteurella haemolytica* serotype A1, which may lead to the development of the often fatal fibrinous pleuropneumonia in beef cattle known as pneumonic pasteurellosis or shipping fever pneumonia (reviewed in references 13 and 14).

*P. haemolytica* A1, although prevalent in the nasopharynx of healthy cattle, is often difficult to isolate. In cattle that develop pneumonic pasteurellosis, the strain of *P. haemolytica* A1 which causes the pneumonia may be that carried by the animal when it was healthy, or it may be another strain acquired from a different animal.

Although methods to distinguish the different serotypes of *P. haemolytica* exist (10), little is known regarding differences among strains within a single serotype. Several studies have examined differences in plasmid profiles of *P. haemolytica* isolates among a single serotype (4, 5, 9, 23). However, the presence of plasmids in *P. haemolytica* A1 strains, particularly the 4.2-kbp plasmid encoding β-lactamase (18), is so widespread that this trait alone offers little in distinguishing among A1 isolates.

Examinations of restriction fragment length polymorphisms of genes coding for rRNA, or ribotyping (16, 30), and restriction endonuclease analyses (REA) of chromosomal DNA have proven to be powerful techniques for studying the molecular epidemiology of strains from within a single bacterial species (1–3, 17, 26, 28, 29, 32).

In this study, we adapted the technique of ribotyping for analysis and comparison of different *P. haemolytica* A1 isolates. In addition, we used ribotyping and REA to compare *P. haemolytica* A1 strains isolated from cattle within a feedlot during an outbreak of shipping fever pneumonia and *P. haemolytica* A1 strains isolated from different locations within individual animals during that outbreak. We have also combined these techniques with examinations of plasmid content and antimicrobial susceptibility to further refine our ability to distinguish between *P. haemolytica* A1 isolates.

## MATERIALS AND METHODS

**Feedlot.** Upon arrival, calves (~150 kg) were placed into an isolated section of a feedlot in Booker, Tex. Calves received no antibiotic treatment until symptoms of respiratory disease were displayed.

**Bacterial strains, isolation, identification, serotyping, growth, and antibiotic MICs.** Bacteria were isolated from tracheae of calves with symptoms of respiratory disease before treatment with antibiotics. Swabs were taken aseptically from the first 6 to 12 inches (15.24 to 30.48 cm) of the trachea. A closed-end uterine catheter containing a sterile cotton swab was inserted into the trachea. The cap was opened, and the swab was extended for sampling of the trachea. After sampling, the swab was pulled back into the catheter before withdrawal. Transtracheal washes were taken from near the first bronchial division by washing with 10 ml of phosphate-buffered saline from a syringe attached to flexible tubing inserted through a speculum. Swabs and recovered saline were plated on brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.) agar plates supplemented with 5% bovine blood. Isolates identified as *P. haemolytica*, by the Oklahoma Animal Disease Diagnostic Laboratory, were grown on BHI agar with no antibiotics overnight at 37°C with 5% $CO_2$. Individual colonies of each isolate were serotyped by the rapid plate agglutination method (15). Antibiotic MICs were determined with commercially prepared microtitration trays (Radiometer/Sensititre of America, Westlake, Ohio) as previously described

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(12). The MIC was identified as the concentration of antibiotic in the first well of a dilution series without detectable macroscopic growth of bacteria.

**DNA preparation.** *P. haemolytica* strains were grown in 40 ml of BHI broth overnight at 37°C on a rotary shaker (125 rpm). Total cell DNA was prepared as described previously (25). To ensure removal of all protein, phenol-chloroform extraction was repeated until no interface was present. DNA was precipitated with isopropanol, resuspended in 400 μl of TE (10 mM Tris, 1 mM EDTA [pH 8]), reprecipitated with ammonium acetate, and resuspended in 400 μl of TE. DNA concentration and purity were determined spectrophotometrically. Plasmids were purified from 3 ml of overnight BHI broth cultures with the Magic Mini-Prep DNA purification system (Promega Corp., Madison, Wis.) or were purified from 75-ml cultures with the Qiagen system (Qiagen Inc., Chatsworth, Calif.).

**Restriction endonuclease digestion and Southern transfer.** For ribotyping and REA, total DNA (5 μg) was digested with restriction endonucleases (Promega) according to the manufacturer's instructions. DNA fragments were separated by agarose gel electrophoresis (0.4 and 0.7% agarose, 20-by-24-cm gel) at 50 V for 20 h in TAE buffer (40 mM Tris-acetate, 1 mM EDTA [pH 8]). Gels were stained for photography with ethidium bromide. DNA was transferred to Duralose UV membranes (Stratagene Cloning Systems, La Jolla, Calif.) with the Posiblot apparatus (Stratagene) according to the manufacturer's instructions and was bound to the membranes by UV cross-linking.

**RNA probe and hybridizations.** *Escherichia coli* (MRE600) rRNA (16S + 23S) (Boehringer Mannheim Corp., Indianapolis, Ind.) was dephosphorylated with calf intestinal alkaline phosphatase (US Biochemicals Corp., Cleveland, Ohio). For probing, 6 pmol was 5′-end labeled with [$\gamma$-$^{32}$P]ATP with T4 polynucleotide kinase (US Biochemicals) according to the manufacturer's instructions. Hybridizations were performed as previously described (20) in sealed bags with (0.5 to 1) × $10^6$ cpm of purified probe per ml of hybridization solution. Blots were washed twice for 15 min each at 55°C in 3× SSPE (1× SSPE is 20 mM $NaH_2PO_4 \cdot H_2O$, 180 mM NaCl, 8 mM NaOH, 1 mM EDTA [pH 7.0])–2× Denhardt's solution (25)–0.1% NaPPi (sodium pyrophosphate)–0.1% sodium dodecyl sulfate (SDS) twice for 60 min each at 55°C in 1× SSPE–0.1% NaPPi–0.1% SDS, and twice for 15 min each at 55°C in 0.1× SSPE–0.1% NaPPi–0.1% SDS. Membranes were exposed to X-ray film overnight.

REA and ribotyping experiments were done a minimum of five times each to ensure that results were not affected by partial digestions. For some isolates, two or three separate DNA preparations were examined to verify the reproducibility of results.

## RESULTS

**Serotyping.** Thirty-one *P. haemolytica* isolates were cultured from 27 calves. Of these, 22 were serotyped as A1, 3 were serotyped as A2, 1 was serotyped as A5, 1 was serotyped as A9, and 4 were not typeable. Among the A1 strains, 14 were isolated by the tracheal swab method and 7 were isolated by the tracheal wash method. The method of isolation for one A1 strain was not recorded.

**REA.** DNA from each *P. haemolytica* A1 isolate and from type strains of *P. haemolytica* serotypes A2, A6, and A9 was digested with numerous restriction endonucleases (*Bgl*II, *Cla*I, *Eco*RV, *Hae*III, *Hin*dIII, *Nhe*I, *Nru*I, *Nsp*V, *Pvu*II, *Sal*I, *Xho*I). Several enzymes (*Eco*RV, *Hae*II, *Hin*cII,

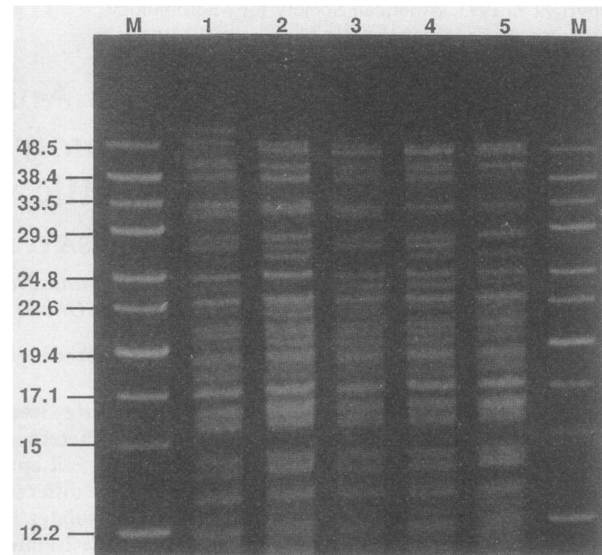


FIG. 1. Representative REA types of *P. haemolytica* A1 feedlot isolates. Shown is an ethidium bromide-stained agarose gel (0.4%) of *Hin*cII-digested total DNA from 93-13(B), REA type 3 (lane 1); 4-18, REA type 4 (lane 2); 46-18(1), REA type 2 (lane 3); 37-14, REA type 1 (lane 4); and 34-17, REA type 5 (lane 5). Lanes M carry a high-molecular-weight DNA ladder (Life Technologies Inc., Gaithersburg, Md.).

*Pvu*II) easily distinguished DNA from strains of different serotypes (data not shown).

Visible and distinct REA patterns among the serotype A1 isolates were most apparent upon observation of high-molecular-weight fragments (>19 kbp) in *Hin*cII-digested DNA (Fig. 1). Among the serotype A1 isolates, five different REA types were observed, which differed in the banding patterns of restriction fragments at sizes of 22, 24, 26.5 to 28, 31 to 33.5, 42, and >48 kbp (Fig. 1). Analysis of agarose gels containing *Hin*cII-digested DNA from representatives of each REA type and from other A1 isolates allowed for the easy assignment of a particular REA type to each isolate. The REA type designated type 1 (Fig. 1, lane 4) was the most common among these isolates (14 isolates [Table 1]).

**Ribotyping.** Upon digestion of *P. haemolytica* DNA, the restriction endonucleases mentioned above produced several fragments that bound the probe. As with REA, these were useful for distinguishing different ribotypes (RTs) among *P. haemolytica* strains of different serotypes (data not shown).

Among the serotype A1 isolates, only *Hin*cII-digested DNA distinguished different RTs. Three distinct RTs can be seen (Fig. 2). RTs of all isolates displayed bands of hybridization with sizes of approximately 2.2, 4.1, 5.5, 6.7, 8.5, 23, 33.5, and 48 kbp. An additional 17.5-kbp band was present in RT II isolates. The hybridization pattern of RT III isolates differed from that of RT I isolates by the intensity of the largest band (48 kbp). In RT I isolates, the two largest bands were of nearly equal intensity, while in RT III isolates, the 48-kbp band was much less intense than the 33.5-kbp band. RT I and RT III patterns were consistent among DNA preparations from the same isolate and among different restriction enzyme digests of a DNA sample, and the patterns were not affected by the location of the sample on the gel or the concentration of the rRNA probe (data not shown). The reproducibility of the RT I and RT III hybrid-

TABLE 1. Characteristics of *P. haemolytica* A1 feedlot isolates

| Isolate[a] | Method | RT | REA type | No. of different plasmids/cell | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ampicillin | Penicillin G | Sulfachlorpyridazine | Oxytetracycline | Erythromycin |
| 2-17W | Wash | I | 1 | 1 | ND[b] | ND | ND | ND | ND |
| 2-17S | Swab | I | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 77-13 | Swab | I | 1 | 1 | >8 | >4 | 25 | >16 | >8 |
| 42-19 | Wash | I | 1 | 1 | ND | ND | ND | ND | ND |
| 60-13 | Wash | I | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 37-14 | Swab | I | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 16-14 | Swab | I | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 28-13 | Swab | I | 1 | 1 | >8 | >4 | >100 | >16 | 4 |
| 32-17 | Wash | I | 1 | 1 | >8 | >4 | <12.5 | >16 | 4 |
| 82-13 | Wash | I | 1 | 1 | >8 | >4 | 50 | >16 | 4 |
| 88-13 | Unknown | I | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 67-13 | Swab | III | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 46-18(2) | Swab | III | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 105-13 | Swab | III | 1 | 1 | >8 | >4 | 25 | >16 | 4 |
| 41-13 | Swab | II | 2 | 1 | >8 | >4 | 25 | >16 | 4 |
| 46-18(1) | Swab | II | 2 | 1 | >8 | >4 | 25 | >16 | 4 |
| 93-13(A) | Wash | II | 3 | 2 | >8 | >4 | 25 | >16 | 8 |
| 93-13(B) | Wash | II | 3 | 2 | >8 | >4 | 25 | >16 | 4 |
| 4-18 | Swab | III | 4 | 2 | >8 | >4 | 50 | >16 | 4 |
| 36-17 | Swab | III | 5 | 0 | <0.25 | 0.5 | 25 | 1 | 4 |
| 34-17 | Swab | III | 5 | 0 | <0.25 | 0.25 | 25 | 1 | 4 |
| 51-19 | Swab | III | 5 | 0 | <0.25 | 0.5 | 50 | 1 | 4 |

[a] 2-17W and 2-17S are from the same calf, 46-18(1) and 46-18(2) are from the same calf, and 93-13(A) and 93-13(B) are from the same calf.
[b] ND, not determined.

ization patterns suggests that these are distinct. Fifty percent of the isolates were of RT I. Four isolates displayed RT II, and seven displayed RT III (Table 1).

Differences in the REA patterns can be seen among isolates of a given RT (Fig. 1 and 2, Table 1). For example, RT II strains 93-13(A) and 93-13(B), both exhibit REA pattern 3, but the RT II strains 46-18(1) and 41-13 both exhibit REA pattern 2. Similarly, the RT III strains 4-18 (REA type 4) and 67-13 (REA type 1) differ from the other RT III isolates 36-17, 34-17, and 51-19 (REA type 5).

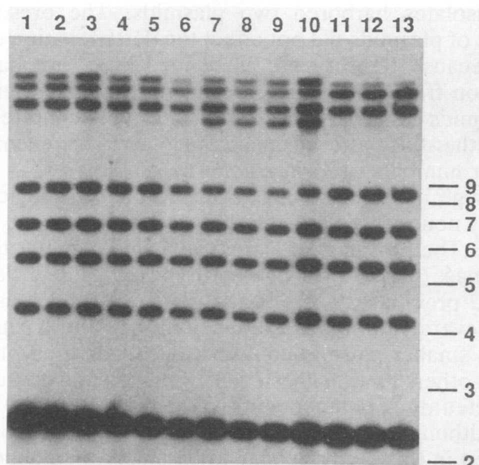


FIG. 2. Representative Southern blot showing RTs of several *P. haemolytica* A1 isolates. Lanes: 1, 2-17W; 2, 2-17S; 3, 77-13; 4, 42-19; 5, 60-13; 6, 46-18(2); 7, 46-18(1); 8, 93-13(A); 9, 93-13(B); 10, 41-13; 11, 4-18; 12, 67-13; 13, 36-17. Isolates in lanes 1 through 5 are RT I, those in lanes 7 through 10 are RT II, and those in lanes 6 and 11 through 13 are RT III.

Three pairs of isolates were separately cultured from individual calves. Both 2-17 isolates, one from a tracheal swab and one from a tracheal wash, have RT I (Fig. 2). The 93-13 isolates, both cultured from washes, have RT II (Fig. 2). However, the 46-18 isolates, each cultured from a swab, have distinct RTs {II [46-18(1)] or III [46-18(2)]} (Fig. 2).

**Plasmid analysis.** Analysis of plasmid DNA revealed that the serotype A1 isolates possessed either no plasmids, one plasmid, or two plasmids. Plasmid DNA, from isolates carrying one or two plasmids, was digested with *Ava*I and separated by agarose gel electrophoresis. Representative restriction digests of plasmid DNA from strains carrying one or two plasmids are shown in Fig. 3. The *P. haemolytica* β-lactamase plasmid is known to contain one *Ava*I site (18). The slightly smaller cryptic plasmid, which has been previously described (6), is shown here to contain two *Ava*I sites and, upon digestion with that enzyme, yields fragments of 1.45 and 2.75 kbp (Fig. 3). Sixteen isolates (73%) contain a single 4.2-kbp plasmid (Table 1), most likely the β-lactamase plasmid (18). Isolates 93-13(A), 93-13(B), and 4-18 carry the additional slightly smaller plasmid. Three isolates have no plasmids (Table 1).

All RT I isolates possess only one plasmid. RT II isolates possess either one or two plasmids. Isolates of RT III and REA pattern 5 are the only ones to lack plasmids. However, certain RT III isolates also harbor one or two plasmids (Table 1).

**Antibiotic resistance.** The MICs of trimethoprim-sulfadiazine (<0.25 μg/ml), clindamycin (>5 μg/ml), spectinomycin (24 μg/ml), ceftiofur (<0.5 μg/ml), and gentamicin (2 μg/ml) are identical for all A1 isolates. The MICs of other antibiotics are shown in Table 1. The isolates which lack plasmids display higher susceptibilities to ampicillin, penicillin G, and oxytetracycline. One isolate (32-17) is highly susceptible to sulfachlorpyridazine (<12.5 μg/ml), and another (28-13) is resistant to that compound (>100 μg/ml). Minor variations in

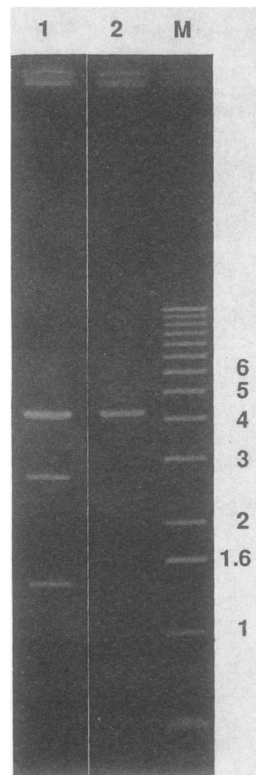


FIG. 3. Agarose gel electrophoresis of plasmid DNA from representative *P. haemolytica* A1 feedlot isolates carrying one or two plasmids. Lanes: 1, 93-13(A), two plasmids; 2, 46-18(1), one plasmid; M, 1-kbp DNA ladder (Life Technologies Inc.)

their susceptibilities to erythromycin occur among the isolates.

## DISCUSSION

Numerous studies have examined the bacterial flora of feedlot cattle with bovine respiratory disease, and *P. haemolytica* A1 is often the predominant species isolated (14). To this point, characterization of *P. haemolytica* isolates from cattle has generally been limited to the identification of different serotypes. However, one recent study examined enzyme and antimicrobial susceptibility profiles from a group of *P. haemolytica* A1 isolates (22).

In this report, we have utilized several methods, including REA, ribotyping, plasmid content analysis, and antibiotic MICs, to distinguish *P. haemolytica* A1 isolates from a single outbreak of bovine respiratory disease. We have also shown here that in addition to distinguishing isolates from different cattle, both REA and ribotyping could distinguish two isolates from different sites within the same animal.

REA defined a larger number of groups among *P. haemolytica* A1 isolates than were defined by ribotyping. In this study, five separate REA groups were recognized as opposed to three separate RTs. However, the combination of REA analysis with ribotyping appears to be a more sensitive approach than either technique alone for analyzing the molecular epidemiology of *P. haemolytica* A1 isolates. In this study, the combination of the two techniques allowed for the identification of six distinct groups of isolates.

The concentration of agarose gels and the gel electrophoresis buffer are important factors in distinguishing REA types and RTs. With REA, observable differences among the isolates were limited to the number and sizes of high-molecular-weight fragments. Higher-percentage agarose gels ($\geq$0.7%) were not capable of adequately separating these large fragments. Our ability to detect these differences was greatly enhanced by using low-percentage agarose gels (0.4%) and TAE buffer. Similarly, the ability to distinguish three separate RTs was dependent on the use of 0.4% agarose gels. However, we could distinguish two RT groups by using 0.7% agarose gels for electrophoresis.

Although others have previously utilized ribotyping to distinguish *P. haemolytica* strains of biotype T isolated from bighorn sheep (29), significant genetic differences between the A and T biotypes may account for the inability to utilize the same enzymes for ribotyping as were utilized in that study. Our results here, with *P. haemolytica* biotype A serotype 1 isolates, reveal significantly different patterns of rRNA hybridization than those previously observed with biotype T strains. Several analyses (reviewed in reference 21), including the comparison of 16S rRNA sequences (10), suggest that these groups are two distinct species and that each is more closely related to other species from the currently designated *Actinobacillus* and *Haemophilus* genera.

It has been suggested that *Haemophilus ducreyi* may be more closely related to *P. haemolytica* biotype A strains than are *P. haemolytica* biotype T strains (10). However, ribotyping patterns with *Hin*cII-digested *H. ducreyi* DNA are significantly different from those observed here with *P. haemolytica* A1 isolates (26). Additionally, with *H. ducreyi*, *Hin*dIII proved to be another useful enzyme for ribotyping (26), while in our study, this enzyme was unable to distinguish RTs among *P. haemolytica* serotype A1 isolates. Our interpretations of these results suggest that, although ribotyping may be useful for distinguishing isolates of a given species, this is probably not an indication of its usefulness for a different species from the same genus or from related genera.

Some interesting observations can be made regarding plasmid distribution among these isolates. Here, REA type 5 isolates stand alone as lacking plasmids, and all REA type 3 and 4 isolates harbored two plasmids. The presence or absence of plasmids did not affect the determination of REA type, because REA types, as defined here, are based on restriction fragments which are much larger than either of the plasmids carried by these strains. It will be interesting to see whether this pattern is maintained upon the examination of larger numbers of *P. haemolytica* A1 isolates.

Although numerous isolates carried only the β-lactamase plasmid, none of the isolates here carried only the cryptic plasmid. The presence of a single plasmid, smaller than the β-lactamase plasmid, in one serotype A1 isolate has been reported previously (24). That isolate was not resistant to any of several antibiotics examined in that study, suggesting that the smaller plasmid could be the putative cryptic plasmid. As others have suggested (11, 26), plasmid content may be affected by spontaneous loss of a plasmid in the laboratory. Although we have observed that the two plasmids identified in this study appear to be stably maintained after repeated in vitro culture of *P. haemolytica*, plasmids may be acquired or lost more readily in vivo, leading to the possibility that two isolates may differ in their plasmid content yet otherwise be identical clones.

A need exists for future, in-depth, molecular analyses of the mechanisms of antibiotic resistance in *P. haemolytica*.

Others have identified strains, such as isolate 28-13 reported here, which are resistant to several antibiotics (4, 8, 24, 33). In this study, as expected, those isolates which lack the 4.2-kbp plasmid are susceptible to penicillin G and ampicillin. The β-lactamase gene from this plasmid has been cloned and sequenced (18). Interestingly, a correlation also exists between oxytetracycline susceptibility and the absence of this plasmid. Others have observed a similar correlation with tetracycline, and it is possible that this plasmid may also encode resistance to that antibiotic (33). However, to our knowledge, definitive proof for the presence of a tetracycline resistance gene has not yet been published.

The combination of methods used here, especially REA and ribotyping, should be useful for future studies on the epidemiology of bovine pneumonic pasteurellosis. By comparing *P. haemolytica* isolates at the genetic level, it may be possible to distinguish new groups among those which have been identified by examinations of phenotypic traits, such as antibiotic susceptibility and enzyme profiles. As others have observed with *P. haemolytica* A2 isolates, phenotypic traits can change depending on the growth conditions of the organism (27). Here, our analyses of plasmid content and antimicrobial susceptibility suggest further differences between *P. haemolytica* A1 isolates of a given RT or REA type. The analysis of both genetic and phenotypic traits has proven useful in epidemiologic studies of other pathogens (1, 19).

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## REFERENCES

1. **Akhtar, N., and A. Eley.** 1992. Restriction endonuclease analysis and ribotyping differentiate genital and nongenital strains of *Bacteroides ureolyticus*. J. Clin. Microbiol. **30:**2408–2414.
2. **Altwegg, M., F. W. Hickman-Brenner, and J. J. Farmer III.** 1989. Ribosomal RNA gene restriction patterns provide increased sensitivity for typing *Salmonella typhi* strains. J. Infect. Dis. **160:**145–149.
3. **Blumberg, H. M., D. S. Stephens, C. Licitra, N. Pigott, R. Facklam, B. Swaminathan, and I. K. Wachsmuth.** 1992. Molecular epidemiology of group B streptococcal infections: use of restriction endonuclease analysis of chromosomal DNA and DNA restriction fragment length polymorphisms of ribosomal RNA genes (ribotyping). J. Infect. Dis. **166:**574–579.
4. **Boyce, J. R., and R. L. Morter.** 1986. Plasmid profile analysis of bovine isolates of *Pasteurella haemolytica*. Am. J. Vet. Res. **47:**1204–1206.
5. **Briggs, R. E., and G. H. Frank.** 1984. Plasmid patterns of *Pasteurella haemolytica* isolated from cattle, p. 517–518. *In* R. Loan (ed.), Bovine respiratory disease: a symposium. Texas A&M University Press, College Station.
6. **Briggs, R. E., and G. H. Frank.** 1991. Isolation of a cryptic plasmid from *Pasteurella haemolytica* by electroporation, abstr. P92, p. 80. Abstr. 72nd Annu. Meet. Conf. Res. Workers Anim. Dis. 1991.
7. **Bryson, D. G.** 1985. Calf pneumonia, p. 237–257. *In* R. Breeze (ed.), Veterinary clinics of North America: food animal practice, vol. 1. W. B. Saunders Co., Philadelphia.
8. **Chang, W. H., and G. R. Carter.** 1976. Multiple drug resistance in *Pasteurella multocida* and *Pasteurella haemolytica* from cattle and swine. J. Am. Vet. Med. Assoc. **169:**710–712.
9. **Chang, Y.-F., H. W. Renshaw, and R. Young.** 1987. Pneumonic pasteurellosis: examination of typeable and untypeable *Pasteurella haemolytica* strains for leukotoxin production, plasmid content, and antimicrobial sensitivity. Am. J. Vet. Res. **48:**378–384.
10. **Dewhirst, F. E., B. J. Paster, I. Olsen, and G. J. Fraser.** 1992. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as determined by comparison of 16S rRNA sequences. J. Bacteriol. **174:**2002–2013.
11. **Eisenstein, B. I.** 1990. New molecular techniques for microbial epidemiology and the diagnosis of infectious disease. Rev. Infect. Dis. **161:**595–602.
12. **Fales, W. H., and G. E. Burrows.** 1983. A new *in vitro* susceptibility testing system for veterinary medical diagnostic laboratories using a commercially prepared stabilized and dried antimicrobial minimal inhibitory concentration system, p. 77–86, Proc. 26th Annu. Meet. Am. Assoc. Vet. Lab. Diagn.
13. **Frank, G. H.** 1986. The role of *Pasteurella haemolytica* in the bovine respiratory disease complex. Vet. Med. **81:**838–846.
14. **Frank, G. H.** 1989. Pasteurellosis of cattle, p. 197–222. *In* C. Adlam and J. M. Rutter (ed.), *Pasteurella* and pasteurellosis. Academic Press Limited, London.
15. **Frank, G. H., and G. E. Wessman.** 1978. Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. J. Clin. Microbiol. **7:**142–145.
16. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur Microbiol. **137B:**165–175.
17. **Izard, N. C., H. Hächler, M. Grehn, and F. H. Kayser.** 1992. Ribotyping of coagulase-negative staphylococci with special emphasis on intraspecific typing of *Staphylococcus epidermidis*. J. Clin. Microbiol. **30:**817–823.
18. **Livrelli, V., J. Peduzzi, and B. Joly.** 1991. Sequence and molecular characterization of the ROB-1 β-lactamase gene from *Pasteurella haemolytica*. Antimicrob. Agents Chemother. **35:**242–251.
19. **Martinetti Lucchini, G., and M. Altwegg.** 1992. rRNA gene restriction patterns as taxonomic tools for the genus *Aeromonas*. Int. J. Syst. Bacteriol. **42:**384–389.
20. **Murphy, G. L., and W. S. Dallas.** 1991. Analysis of two genes encoding heat-labile toxins and located on a single Ent plasmid from *Escherichia coli*. Gene **103:**37–43.
21. **Mutters, R., W. Mannheim, and M. Bisgaard.** 1989. Taxonomy of the group, p. 3–34. *In* C. Adlam and J. M. Rutter (ed.), *Pasteurella* and pasteurellosis. Academic Press Limited, London.
22. **Purdy, C. W., C. M. Scanlan, R. W. Loan, and G. S. Foster.** 1993. Identification of *Pasteurella haemolytica* A1 isolates from market-stressed feeder calves by use of enzyme and antimicrobial susceptibility profiles. Am. J. Vet. Res. **54:**92–98.
23. **Richards, A. B., L. W. Sneed, and H. W. Renshaw.** 1984. Isolation and partial characterization of plasmids and bacteriophages from isolates of *Pasteurella haemolytica*, p. 519–520. *In* R. Loan (ed.), Bovine respiratory disease: a symposium. Texas A&M University Press, College Station.
24. **Rossmanith, S. E. R., G. R. Wilt, and G. Wu.** 1991. Characterization and comparison of antimicrobial susceptibilities and outer membrane protein and plasmid DNA profiles of *Pasteurella haemolytica* and certain members of the genus *Pasteurella*. Am. J. Vet. Res. **52:**2016–2022.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Sarafian, S. K., T. C. Woods, J. S. Knapp, B. Swaminathan, and S. A. Morse.** 1991. Molecular characterization of *Haemophilus ducreyi* by ribosomal DNA fingerprinting. J. Clin. Microbiol. **29:**1949–1954.
27. **Scanlan, C. M., R. W. Loan, and C. W. Purdy.** 1992. *In vitro* studies on inducible and quantitative enzyme activities of bovine *Pasteurella haemolytica* serovar 2 respiratory tract isolates, abstr. 52, p. 11. Abstr. 73rd Annu. Meet. Conf. Res. Workers in Anim. Dis.
28. **Snipes, K. P., D. C. Hirsh, R. W. Kasten, L. M. Hansen, D. W.**

**Hird, T. E. Carpenter, and R. H. McCapes.** 1989. Use of an rRNA probe and restriction endonuclease analysis to fingerprint *Pasteurella multocida* isolated from turkeys and wildlife. J. Clin. Microbiol. **27:**1847–1853.

29. **Snipes, K. P., R. W. Kasten, M. A. Wild, M. W. Miller, D. A. Jessup, R. L. Silflow, W. J. Foreyt, and T. E. Carpenter.** 1992. Using ribosomal RNA gene restriction patterns in distinguishing isolates of *Pasteurella haemolytica* from bighorn sheep (*Ovis canadensis*). J. Wild. Dis. **28:**347–354.
30. **Stull, T., J. J. LiPuma, and T. D. Edlind.** 1988. A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. J. Infect. Dis. **157:**280–286.
31. **Yates, W. D. G.** 1982. A review of infectious bovine rhinotracheitis, shipping fever pneumonia, and viral bacterial synergism in respiratory disease of cattle. Can. J. Comp. Med. **46:**225–263.
32. **Zhao, G., C. Pijoan, M. P. Murtaugh, and T. W. Molitor.** 1992. Use of restriction endonuclease analysis and ribotyping to study epidemiology of *Pasteurella multocida* in closed swine herds. Infect. Immun. **60:**1401–1405.
33. **Zimmerman, M. L., and D. C. Hirsh.** 1980. Demonstration of an R plasmid in a strain of *Pasteurella haemolytica* isolated from feedlot cattle. Am. J. Vet. Res. **41:**166–169.