

Comparison of DNA Fingerprinting and Serotyping for Identification of Avian *Pasteurella multocida* Isolates

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The DNA fingerprint profiles and serotypes of 63 avian *Pasteurella multocida* field isolates, 13 attenuated vaccine isolates (propagated from vaccines manufactured by five companies), and 16 somatic reference strains were compared. DNA fingerprinting established the relationship of isolates that could not be distinguished by serotyping. Of the 76 isolates, 28 DNA fingerprint profiles and 12 somatic types were recognized. One isolate was nonreactive with 16 reference somatic and 5 reference capsule-type antisera. Thirty-one field isolates and seven vaccine isolates were identified as capsule type A. Twenty-nine field isolates and six vaccine isolates were nonencapsulated. Three field isolates were capsule type F. Isolates of capsule types B, D, and E were not found. One field isolate, identified as somatic type 7, had a DNA fingerprint identical to that of the somatic reference type 6 profile. Twelve field isolates had profiles identical to the somatic reference type 3 strain profile; 11 of these were identified as somatic type 3,4, and 1 was identified as somatic type 3. The DNA fingerprint profiles of 50 field isolates and 13 attenuated vaccine isolates did not match profiles of the 16 somatic type reference strains. Twenty-five DNA fingerprint profiles were recognized from 30 of these field isolates. The DNA fingerprint profiles of 20 field isolates and 13 attenuated vaccine isolates were identical. Three somatic types (3; 3,4; and 4,16) were identified from the field isolates, and two somatic types (3 and 3,4) were identified from the attenuated vaccine isolates. DNA fingerprinting is useful for accurate identification and epidemiologic study of *P. multocida* isolates.

Pasteurella multocida is serotyped by passive hemagglutination (2, 12) and gel diffusion precipitin (4) tests. Many serotyping surveys of *P. multocida* isolates from avian sources have been reported (1, 5, 7, 9, 14). Five capsule serogroups (A, B, D, E, and F) are recognized. Nonencapsulated and capsule group A *P. multocida* are most common in the United States, although avian isolates of capsule groups B, D, and F are found on occasion (8, 10).

Sixteen individual major somatic types (1 through 16) of *P. multocida* are recognized, but isolates that have multiple somatic antigens are routinely encountered (e.g., A:3,4, A:3,4,7, A:4,7, and A:3,4,12). Although the specificity of the somatic antigen is due to lipopolysaccharide, the nature of cross-reaction with more than one typing serum is not fully understood. At present, isolates that react to multiple antisera are considered distinct serotypes. Somatic typing for epidemiologic purposes is further complicated when attenuated vaccines are used for disease control. The use of either of two attenuated vaccines is frequently reported, and these generally consist of the Clemson University (CU) or M-9 strain. These vaccines are sometimes suspected of producing disease and usually react as somatic type 3,4. Because the 3,4 somatic type of *P. multocida* is frequently isolated from clinical cases involving vaccinated poultry, differentiation between field isolates and vaccine strains is an important diagnostic problem. Serotyping of *P. multocida* determines the antigenic composition but is an ambiguous identification technique compared with the precision of molecular techniques.

DNA fingerprint profiles have been used primarily to distinguish somatic type 3,4 clinical isolates from vaccine

strains (6, 15). The restriction endonucleases used in those surveys produced complex profiles that were often difficult to interpret. These difficulties are due principally to the large number of fragment bands in close proximity. Despite these problems, the technique is highly reproducible and does not depend on the specificities of antisera or sensitivities of serotyping methods.

DNA fingerprint profiles of *P. multocida* have been studied by ribotyping. This technique uses a cDNA probe to detect DNA restriction site heterogeneity and has been used primarily for differentiating avian isolates of the 3,4 somatic type (15, 16). One advantage of ribotyping is that fewer bands are recognized within a profile. However, ribotyping requires more equipment and expense than DNA fingerprinting for isolate characterization. Using previously reported methods (17), DNA fingerprinting can be completed in less time than ribotyping or serotyping.

Ideally, a restriction endonuclease and separation technique should yield a DNA fingerprint profile with fragment bands that are distinct and well separated. Wilson et al. (17) described methods of DNA isolation and fingerprinting for *P. multocida* that address these criteria. On the basis of these procedures, a system for characterization of *P. multocida* that involved serotyping and fingerprinting was proposed.

In this study, the DNA fingerprint profiles and serotypes of field and vaccine isolates were compared with those of 16 somatic reference strains. DNA fingerprinting was used to characterize isolates, regardless of capsular or somatic type. DNA fingerprinting confirmed the relationship of nonencapsulated and encapsulated isolates that could not be distinguished by current serotyping procedures. Fingerprint profiles were used to differentiate isolates of a given serotype or to confirm the relatedness of distinctly different serotypes.

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MATERIALS AND METHODS

Bacteria. Ninety-two *P. multocida* cultures were evaluated in this study. Sixty-three avian *P. multocida* field isolates were obtained from the Arkansas Livestock and Poultry Diagnostic Laboratory; 13 vaccine isolates were propagated from attenuated vaccines; and 16 reference somatic serotype strains were from the culture collection at the National Veterinary Services Laboratories. For the serotypes of the 16 reference somatic strains, see Table 1. For the serotypes of the 13 vaccine isolates propagated from vaccines produced by five different companies, see Table 2. Companies are arbitrarily coded C1 to C5, and all isolates are designated by strain. For the serotypes of the 63 avian field isolates, see Tables 3 through 6.

Biochemical characterization of field isolates. The 63 field isolates were obtained either from birds submitted to the Arkansas Livestock and Poultry Diagnostic Laboratories or from isolates recovered by private laboratories and referred to the Arkansas Livestock and Poultry Diagnostic Laboratories for somatic typing. Isolates initially recovered by the Arkansas Livestock and Poultry Diagnostic Laboratories were isolated on tryptic soy agar (Difco Laboratories) containing 5% sheep blood. All field isolates were propagated on tryptic soy agar. Biochemical characterizations were performed by using the API-20E or API-NFT (Analytab Products) identification system. After identification and characterization, the isolates were subjected to somatic typing by the techniques of Heddeleston et al. (4). Cultures were forwarded to the National Veterinary Services Laboratories to be identified by capsular and somatic type and DNA fingerprint profile.

Media and cultures. For serotyping and DNA fingerprinting, all isolates were grown on media described previously (17).

Serotyping. Capsule typing was done by the methods of Rimler and Brogden (12), and somatic typing was completed by using the methods of Heddeleston et al. (4). All somatic type results reported in this study are those of the National Veterinary Services Laboratories.

DNA isolation and digestion, electrophoresis, and photography. The DNA isolation, endonuclease digestion with *Hha*I (Bethesda Research Laboratories, Inc.), electrophoresis, and photographic techniques used to characterize all isolates and reference strains were done by methods described previously (17).

RESULTS

DNA fingerprint profiles of 16 somatic reference serotypes. The 16 somatic reference strains were previously fingerprinted (17) with *Hha*I and had distinct profiles, designated *Hha*I profiles 001 to 016. The serotypes and profile designations of the somatic reference strains are listed in Table 1.

DNA fingerprint profiles and serotypes of 13 attenuated vaccine isolates. One DNA fingerprint profile (Fig. 1, lane 10), temporarily designated (TD) 045, was identified from 13 attenuated *P. multocida* vaccine isolates (Table 2). Six vaccine isolates were somatic type 3,4; five of these were serotype A:3,4, and one was -:3,4. Seven vaccine isolates were somatic type 3; two of these were serotype A:3, and five were -:3. The type 4 antigen was not detected among seven isolates. A capsular antigen was not detected from six of the vaccine isolates. Although different serotypes were identified from 13 vaccine isolates, the DNA fingerprint

TABLE 1. Serotypes and DNA fingerprint profiles of *P. multocida* somatic reference serotype strains

Strain	Serotype ^a	DNA fingerprint profile ^b
X-73	A:1	<i>Hha</i> I 001
M-1404	B:2,5 ^c	<i>Hha</i> I 002
P-1059	A:3	<i>Hha</i> I 003
P-1662	A:4	<i>Hha</i> I 004
P-1702	A:5,2 ^d	<i>Hha</i> I 005
P-2192	-:6	<i>Hha</i> I 006
P-1997	-:7	<i>Hha</i> I 007
P-1581	-:8	<i>Hha</i> I 008
P-2095	A:9	<i>Hha</i> I 009
P-2100	A:10	<i>Hha</i> I 010
P-903	-:11	<i>Hha</i> I 011
P-1573	A:12	<i>Hha</i> I 012
P-1591	-:13	<i>Hha</i> I 013
P-2225	A:14	<i>Hha</i> I 014
P-2237	-:15	<i>Hha</i> I 015
P-2723	A:16	<i>Hha</i> I 016

^a The letter designates the capsule group; the number designates the somatic type; -, nonencapsulated strain.

^b The profile is indicated by the restriction endonuclease and designated profile number.

^c Somatic serotype 2 reference strain.

^d Somatic serotype 5 reference strain.

profile was consistent not only within a strain but also between strains.

DNA fingerprint profiles and serotypes of 63 field isolates. Twenty field isolates (Table 3) had DNA fingerprint profiles (TD 045) identical to profiles of 13 attenuated vaccine isolates. Sixteen field isolates were somatic type 3,4; nine of these were serotype A:3,4, and seven were -:3,4. Three of these field isolates were somatic type 3; two were serotype A:3, and one was -:3. One field isolate was serotype A:4,16. The type 4 somatic antigen was not detected among three

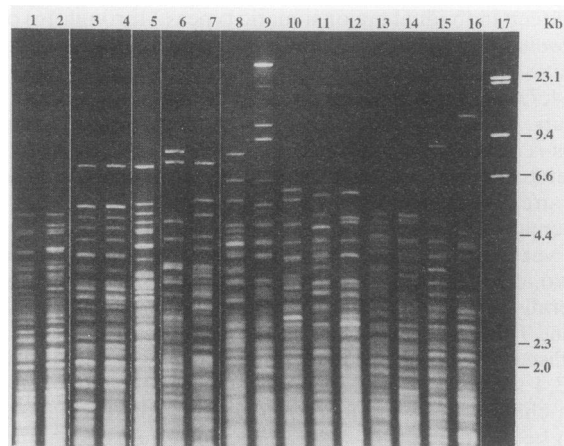


FIG. 1. Agarose gel DNA fingerprint profiles with *Hha*I of field isolates identified as somatic type 3 or 3,4. Lanes: 1, DNA fingerprint common to 12 field isolates and identical to the reference type 3 profile; 2 through 9, DNA profiles (TD 037 through TD 044, respectively) of field isolates serologically identified as somatic type 3; 10, DNA fingerprint profile (TD 045) observed from 20 field isolates and 13 attenuated vaccine isolates; 11 through 16, DNA fingerprint profiles (TD 046 through TD 051, respectively) of field isolates serologically identified as somatic type 3,4; 17, DNA from bacteriophage lambda digested with *Hind*III.

TABLE 2. Serotypes and DNA fingerprint profiles of avian *P. multocida* attenuated vaccine isolates

Strain	Serotype	DNA fingerprint profile ^a	Company
M-9	-:3	TD 045	C1
X ^b	-:3	TD 045	C3
CU	-:3	TD 045	C3
M-9	-:3	TD 045	C5
M-9	-:3	TD 045	C1
X ^b	A:3	TD 045	C5
CU	A:3	TD 045	C2
CU	A:3,4	TD 045	C2
CU	A:3,4	TD 045	C4
CU	A:3,4	TD 045	C5
CU	A:3,4	TD 045	C3
CU	A:3,4	TD 045	C5
M-9	-:3,4	TD 045	C3

^a TD denotes temporary designation of fingerprint profiles.

^b X denotes a strain identification other than CU or M-9.

isolates, and capsular antigen was not detected among eight of the isolates listed in Table 3. Expression of capsular and somatic antigens is a phenotypic trait. Comparison of isolates of profile TD 045 (Tables 2 and 3) reveals five distinct serotypes (-:3, -:3,4, A:3, A:3,4, and A:4,16).

Twelve field isolates (Table 4) had DNA fingerprint profiles identical to the somatic type 3 reference strain profile (Fig. 1, lane 1), designated *HhaI* 003. Three distinct serotypes (-:3, -:3,4, and A:3,4) were identified among these isolates. These three serotypes were also identified among isolates of profile TD 045 (Tables 2 and 3). In contrast, profile TD 045 (Fig. 1, lane 10) is distinctly different from profile *HhaI* 003 (Fig. 1, lane 1). This suggests that a given serotype is not specific to a given fingerprint profile. This is supported by DNA fingerprint profiles and serotypes of isolates listed in Table 5.

TABLE 3. Serotypes and animal origin of avian *P. multocida* field isolates that have identical profiles to those attenuated vaccines

Strain	Serotype ^a	DNA fingerprint profile ^b	Animal
270	A:4,16	TD 045	Turkey
672-92	-:3	TD 045	Turkey
3557-2-91	A:3	TD 045	Turkey
3905-91	A:3	TD 045	Turkey
547	A:3,4	TD 045	Chicken
880	A:3,4	TD 045	Chicken
1537	A:3,4	TD 045	Chicken
91-3205	A:3,4	TD 045	Chicken
91-3206	A:3,4	TD 045	Chicken
91-3287-7	A:3,4	TD 045	Chicken
4318-91	A:3,4	TD 045	Chicken
5098-91	A:3,4	TD 045	Chicken
3557-1-91	A:3,4	TD 045	Turkey
957-92	-:3,4	TD 045	Chicken
4047-91	-:3,4	TD 045	Turkey
5375-91	-:3,4	TD 045	Chicken
424-92	-:3,4	TD 045	Turkey
425-92	-:3,4	TD 045	Turkey
664-92	-:3,4	TD 045	Chicken
91-3287-8	-:3,4	TD 045	Chicken

^a See Table 1, footnote a.

^b TD denotes temporary designation of DNA fingerprint profiles generated with *HhaI*.

TABLE 4. Serotypes and animal origin of avian *P. multocida* field isolates that have profiles identical to the somatic type 3 profile

Strain	Serotype ^a	DNA fingerprint profile ^b	Animal
4332-91	-:3	<i>HhaI</i> 003	Chicken
080	-:3,4	<i>HhaI</i> 003	Chicken
1536	-:3,4	<i>HhaI</i> 003	Chicken
3015	-:3,4	<i>HhaI</i> 003	Chicken
971-92	-:3,4	<i>HhaI</i> 003	Chicken
611-92	-:3,4	<i>HhaI</i> 003	Chicken
612-92	-:3,4	<i>HhaI</i> 003	Chicken
615-92	-:3,4	<i>HhaI</i> 003	Chicken
616-92	-:3,4	<i>HhaI</i> 003	Chicken
613-92	-:3,4	<i>HhaI</i> 003	Chicken
3787-91	A:3,4	<i>HhaI</i> 003	Chicken
614-92	A:3,4	<i>HhaI</i> 003	Chicken

^a See Table 1, footnote a.

^b DNA fingerprint profile identical to the somatic type 3 reference profile.

The DNA fingerprint profiles of 18 field isolates (Fig. 1, lanes 2 through 9 and lanes 11 through 16), identified as somatic type 3,4 or type 3, were not identical to profile TD 045 (Fig. 1, lane 10) or *HhaI* 003 (Fig. 1, lane 1). Five serotypes (A:3, -:3, -:3,4, A:3,4, and F:3,4) and 14 DNA fingerprint profiles (designated TD 037 to 044 and TD 046 to 051) were identified from the isolates listed in Table 5. Three isolates were serotype F:3,4 and had DNA fingerprint profile TD 048. None of the nonencapsulated or capsule group A isolates were identified as profile TD 048. Although all isolates of profile TD 048 in this study were F:3,4, the data suggest that a group F isolate of somatic type 3,4, lacking capsular F antigen, could potentially be identical to profile TD 048.

Twelve profiles were identified from 13 isolates. One isolate (5173-91) was nonreactive to capsular and somatic antisera; 12 were somatic types other than 3 or 3,4 (Table 6).

TABLE 5. Animal origin and fingerprint profiles of avian *P. multocida* field isolates identified as somatic type 3 or 3,4 which are not identical to 13 attenuated vaccine and reference type 3 profiles

Strain	Serotype ^a	DNA fingerprint profile ^b	Animal
713	A:3	TD 041	Chicken
2805	A:3	TD 039	Turkey
1460	A:3	TD 044	Turkey
1253	-:3	TD 040	Chicken
1553	-:3	TD 042	Turkey
636 (1127)	-:3	TD 038	Turkey
91-3472	-:3	TD 043	Chicken
91-3279	-:3,4	TD 047	Turkey
1562	-:3,4	TD 050	Chicken
3775-91	-:3,4	TD 049	Chicken
617-92	A:3,4	TD 049	Chicken
1443	A:3,4	TD 037	Chicken
2701	A:3,4	TD 046	Turkey
2806	A:3,4	TD 051	Chicken
2807	-:3,4	TD 051	Chicken
618-92	F:3,4	TD 048	Turkey
619-92	F:3,4	TD 048	Turkey
620-92	F:3,4	TD 048	Turkey

^a See Table 1, footnote a.

^b See Table 3, footnote b.

TABLE 6. Animal origin and DNA fingerprint profiles of avian *P. multocida* field isolates that have somatic types other than 3 and 3,4

Strain	Serotype ^a	DNA fingerprint profile ^b	Animal
636 (1349)	A:1	TD 052	Turkey
3746-91	A:1	TD 053	Turkey
91-3249	-:1	TD 054	Chicken
1270	-:5,9,16	TD 055	Turkey
1577	A:5,9,16	TD 055	Turkey
881	-:11,15	TD 056	Turkey
2704	-:14,15	TD 057	Turkey
5361-91	-:14	TD 058	Chicken
426-92	-:4,7,12	TD 059	Turkey
5173-91	-:N ^c	TD 060	Pheasant
4070-91	-:7,10	TD 061	Turkey
4263-91	-:7,8	TD 062	Turkey
587-92	-:7	<i>HhaI</i> 006 ^d	Chicken

^a See Table 1, footnote a.

^b See Table 3, footnote b.

^c N, no somatic type identified.

^d DNA fingerprint profile identical to the somatic type 6 reference profile.

Three DNA fingerprint profiles (Fig. 2) were recognized from three field isolates, identified as somatic type 1; two of these were serotype A:1, and one was -:1. The profiles of the somatic type 1 field isolates (Fig. 2) did not match profiles of 16 reference somatic type strains. Field isolate 587-92, identified as -:7, had a DNA fingerprint profile (Fig. 3, lane 9) identical to the reference somatic type 6 profile. Eight DNA fingerprint profiles (Fig. 3, lanes 1 through 8) were recognized from nine field isolates, but none were identical to the profiles of 16 somatic type reference strains.

Twenty-eight DNA fingerprint profiles were recognized from 63 avian field isolates. The data (Tables 2 through 6) suggest that a given serotype is not specific to a given DNA fingerprint profile.

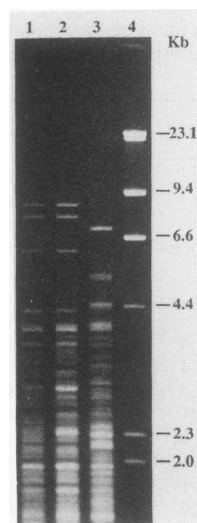


FIG. 2. Agarose gel DNA fingerprint profiles with *HhaI* of three field isolates identified as somatic type 1. Lanes 1 through 3 contain DNA profiles designated TD 052 through TD 054, respectively. Lane 4 contains DNA from bacteriophage lambda digested with *HindIII*.

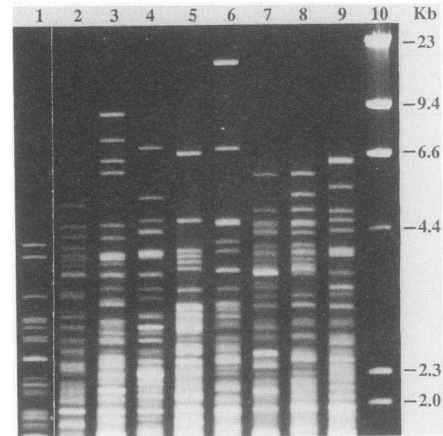


FIG. 3. Agarose gel DNA fingerprint profiles with *HhaI* of field isolates which were serologically identified as somatic types other than 3 or 3,4. Lanes: 1 through 8, DNA profiles designated TD 055 through TD 062, respectively; 9, DNA of field isolate 587-92, which is identical to profile *HhaI* 006; 10, DNA from bacteriophage lambda digested with *HindIII*.

DISCUSSION

There are many problems with the current serotyping system of *P. multocida*. Identification of a *P. multocida* serotype depends on detection of both capsular and somatic antigens. For example, an isolate identified as -:3,4 could belong to any of five capsular groups. Detection of somatic antigen is based on somatic antisera prepared and evaluated with reference somatic strains. Inability to identify somatic antigens complicates identification.

Isolates that express multiple somatic antigens (e.g., A:3,4, A:3,4,7, and A:3,4,12) can mislead diagnosticians and research scientists. Isolates identified as serotypes A:3 and A:3,4 on the basis of serotyping are considered distinct serotypes. Characterization of *P. multocida* by current serotyping techniques produces ambiguous identifications.

Serological identification depends on many factors. Production of antisera for capsular and somatic identification is difficult (11, 13). Absorption of somatic antisera to eliminate heterologous cross-reactivity is neither recommended nor used in the gel diffusion precipitin test. Absorption results in an antigen-leaching effect, which may produce precipitins that complicate interpretation of the test (11). Variation among somatic antisera as a result of different immunologic responses between animals causes a problem of standardization. Discrepancies in serological identification between laboratories is due primarily to differences in the antisera and methods used.

Development of a *P. multocida* somatic typing system similar to the *Salmonella* somatic typing scheme (3) is not desirable because the role of minor antigens is not documented in the *P. multocida* somatic typing system. Within somatic serogroup D1 of the *Salmonella* serotyping scheme, the role of minor somatic antigens is evident. *Salmonella pullorum*, a member of *Salmonella* serogroup D1, is identified as a standard, intermediate, or variant strain on the basis of the minor somatic antigens 12² and 12³ (3). This raises the question whether minor antigens play an important role in identification of *P. multocida*. Development of a *P. multocida* somatic typing system similar to the *Salmonella* somatic typing system would be tedious and would not solve

the occasional problem of nonencapsulated *P. multocida* isolates.

The problems encountered in the *P. multocida* serotyping system do not affect DNA fingerprinting. DNA fingerprinting demonstrated similarities or differences in isolates that could not be distinguished by serotyping, and it permitted precise identification of *P. multocida*.

Kim and Nagaraja (6) reported that strains CU and M-9 can be differentiated by DNA fingerprinting with *Bgl*III and sodium dodecyl sulfate-polyacrylamide gel gradient electrophoresis. In contrast, Snipes et al. (15) reported no differences between CU and M-9 fingerprint profiles when using *Sma*I and agarose gel electrophoresis. They confirmed this with ribotyping (15).

In this study, fingerprint differences between the CU and M-9 strains used in attenuated vaccines or between vaccine strains from different companies could not be demonstrated. Differences of serotype were recognized in the 13 vaccine and 20 field isolates, but all of these had identical profiles with *Hha*I. We could not verify whether the field isolates were related to the attenuated vaccines. Although a field isolate may have a DNA fingerprint profile identical to that of the 13 attenuated vaccine isolates, the question of relationship could be answered by development of a recombinant vaccine with a gene deletion.

The interpretation of DNA fingerprint and ribotype profiles is complicated by the use of different endonucleases used by researchers to evaluate *P. multocida* isolates. The use of *Hha*I for preliminary evaluation of *P. multocida* could provide standardization of experimental results. The use of other endonucleases for further characterization of isolates need not be discouraged. *Hha*I generates profiles of *P. multocida* DNA that are easier to distinguish than profiles generated by many other restriction endonucleases (17). Occasionally, DNA fingerprint profiles can be distinguished at a glance; however, in many cases, differences in profiles are minor and analysis of multiple profiles with the unaided eye is time-consuming.

Computerized restriction fragment length polymorphism equipment for analysis of fingerprint profiles is expensive; however, isolates could be grouped, and each DNA fingerprint could be given a numerical designation based on the restriction endonuclease, such as *Hha*I 003. A descriptive identification epithet (DIE) code has been proposed (17) to provide isolate identification on the basis of capsule and somatic types and fingerprint profile produced by a given restriction endonuclease, for example, A:3/*Hha*I 003. Development of a DIE code system will require time. Temporary designation of fingerprint profiles is necessary to complete an identification system. Although complex, the DIE code is most useful for its information. Field isolates of various DIE codes could be grouped and assayed by immunoblot techniques for use in vaccines. Avian isolates of a particular DIE code or of similar DIE codes could be tested for antigenicity and immunogenicity for control of fowl cholera.

ACKNOWLEDGMENT

We thank J. M. Lockhart for technical assistance.

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