Serotyping of Avian Mycoplasma Species in India

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Two-hundred and two strains of avian mycoplasma species belonging to 10 biotypes were typed serologically by employing disk growth inhibition (DGI) and indirect hemagglutination (IHA) tests. These could be placed into seven serotypes, namely A (80), B (50), C (3), E (34), L (13), P (4), and 1 and R (18). The figures in parentheses show the number of strains within each type. A close relationship was observed between DGI and IHA tests. The IHA test, however, was more sensitive and specific. It was also noticed that biochemically identical biotypes, namely E and G, and B and M were also found identical in serotyping, thus confirming the biochemical identity. In view of these facts, the strains of biotypes M and G were grouped under serotypes B and E, respectively. The antigenic relationships between the serotypes are also discussed.

A literature survey revealed that probably no or only a few sporadic attempts have been made to serotype avian mycoplasma species prevalent in India (R. C. Katoh, M.Sc thesis, Haryana Agricultural University, Hisar, India, 1970).

It was felt that unless an extensive study was undertaken covering a majority of the Indian states, it would be difficult or impossible to define the extract medicine of avian mycoplasmosis, as a few other species or serotypes in addition to Mycoplasma gallisepticum are also known to be pathogens. It was further difficult to develop any prophylactic measures or to prepare antigens for a quick spot diagnosis of mycoplasma infection among the poultry industry or to protect the birds.

Keeping in view the above facts and economics of the losses due to mycoplasma infection in the poultry industry, it was felt that attempts should be made to determine the prevalence of various serotypes or species so that effective serological methods could be developed to diagnose the infection.

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MATERIALS AND METHODS

Two-hundred and two mycoplasma strains belonging to 10 biotypes as described previously (P. J. Asnani and S. C. Agarwal, Mycoplasmal Diseases Symposium, 31 December 1972) were used for serotyping (Table 1).

Eighteen reference strains of avian mycoplasma representing 18 serotypes, namely A to R, were obtained from M. L. Frey, Iowa State University, Ames. These strains were used to prepare hyperimmune sera for serological typing of the above mycoplasma strains to be used in serotyping of the test strains.

Preparation of antigen. Antigens of each of the 18 reference strains were prepared to produce hyperimmune sera following the methods of Dierks et al. (2). Antigens of test strains for indirect hemagglutination test (IHA) were also prepared following the methods of Lind (7). Before preparing the reference strain antigens, they were tested for purity.

Production of hyperimmune serum. Antiserum against each of the 18 reference strains was prepared in white albino rabbits weighing 2.5 kg. Preimmunization serum was collected from each rabbit and tested for the possible presence of normal growth inhibiting antibodies and hemagglutinins against each of these. Only negative reactors were used for immunization. The preimmunization sera served as negative controls. The method of Taylor-Robinson et al. (9) was followed for raising the hyperimmune sera. Briefly, each rabbit received three injections each of 1 ml at 21-day intervals. The first injection was made with equal amounts of antigen and Freund complete adjuvant. The second injection contained equal amounts of antigen and Freund incomplete adjuvant; the last injection contained antigen alone. The first two injections were given intradermally, and the last injection was given intraperitoneally. Bleeding was made 21 days after the last injection.

Serological techniques. The serological techniques employed were disk growth inhibition (DGI) and IHA tests, following the methods of Stanbridge and Hayflick (8) and Lind (7), respectively. In each test, known positive and negative controls were included.
The strains identified each into homologous antisera M, and partially inhibited by the homologous and heterologous antisera completely typed presented confirmed antisera complex agglutination antisera. Asnani and S. C. (5) used for serotyping the DGI and IHA tests. A close agreement was found between the results of the DGI and IHA tests. In the DGI test, 80 strains belonging to biotype A were inhibited completely by the antiserum of serotype A only, and no heterologous inhibition was noticed by other antisera used. These results were confirmed by the IHA test. Similarly, identity between serotypes B and M was observed in both tests, and this was true also for serotypes E and G. These observations agree with the results of others (2, 3, 5, 10) who used a variety of different serological techniques, namely the growth inhibition test (3, 5, 6), growth inhibition and tube agglutination tests (2, 5), and tube agglutination test (10). We also agree with Dierks et al. (2) and Kelton and Van Roekel (5) as far as results of the growth inhibition tests are concerned, with respect to serotypes B and M, and E and G. In view of these findings, it may be concluded that biotypes B and M, and E and G, as grouped on the basis of their biochemical properties (P. J. Asnani and S. C. Agarwal, Mycoplasmal Diseases Symposium, 31 December 1972), behaved identically serologically. Serotype B is identical to M, and serotype E is identical to G, and in consequence, only serotypes B and E, or M. gallinarum and M. iners should be retained. Similar suggestions were made by Dierks et al. (2). It is further noticed that strains under serotypes C and P did not show any heterologous inhibition with the exception that serotype P was inhibited partially by antiserum D, whereas this serotype did not show any heterologous agglutination by any other antiserum used in the test. Isolates of serotype L did not show any heterologous reaction, neither in the DGI nor IHA tests, and thus also appear to be distinct antigenically. We therefore agree with Kelton and Van Roekel (5), Yoder and Hofstad (10), and Dierks et al. (2) in considering serotype B as identical with M, and serotype E identical with G. Our results from the DGI and IHA tests showed that serotypes I and R, if not

### RESULTS

**Growth inhibition test.** A total of 202 strains belonging to 10 biotypes were identified by the DGI test. All of these strains could be grouped into 10 serological types. The biotypes and respective number of strains identified for each are as follows: A (80), B (24), C (3), E (23), G (11), I (2), L (13), M (26), P (4), and R (16). The strains identified as serotypes A, C, and L were completely inhibited by their respective antisera and did not show heterologous inhibition. The strains placed under serotypes B and M, and E and G were either completely or partially inhibited by the homologous and heterologous antisera, and partially with serotype D antiserum. Similarly, serotypes I and R were completely inhibited with homologous antisera and partially with either one or more antisera of a complex group (I, J, K, N, Q, and R).

**Indirect hemagglutination test.** The identification of all 202 strains by the DGI test was confirmed by the IHA test. The results are presented in Table 2, which shows that strains typed as serotypes A, L, and P were distinct, showing agglutination with their homologous antisera only. The strains placed under serotypes B, C, E, and I and R also reacted with antisera against M, O, G, R, and I, respectively, and vice versa. Serotype I and R also showed agglutination with antisera of serotypes of a complex group (I, J, K, N, Q, and R). However, the IHA titers obtained with heterologous antisera were either identical with homologous antisera or low compared with the homologous titers.

### DISCUSSION

In the present study 202 strains grouped into 10 biotypes (P. J. Asnani and S. C. Agarwal, Mycoplasmal Diseases Symposium, 31 December 1971) on the basis of their biological properties were serotyped by using the DGI and IHA tests.

### Table 1. Biotypes of avian mycoplasma species used for serotyping

| State origin                                      | Biotypes |
|---------------------------------------------------|----------|
| Chandigarh (Union Territory)                      | A: 27    |
| Punjab                                            | B: 10    |
| Haryana                                          | C: 1     |
| Uttar Pradesh                                    | E: 6     |
| Madhya Pradesh                                   | G: 2     |
| Delhi                                             | I: 4     |
| West Bengal                                       | L: 6     |
| Total                                             | P: 26    |

| Species   | A | B | C | E | G | I | L | M | P | R |
|-----------|---|---|---|---|---|---|---|---|---|---|
| Biotypes  | 27| 10| 1 | 9 | 6 | 2 | 4 | 6 | 2 | 6 |

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TABLE 2. Serological identification of avian mycoplasma isolates by indirect hemagglutination test

| No. of strains falling in each category | Reference antisera |
|----------------------------------------|--------------------|
|                                        | A  | B  | C  | D  | E  | F  | G  | H  | I  |
|                                        |    | 160-5,120* (80) | 160-5,120 (26) | 160-640 (3) | 160-2,560 (23) | 80-1,280 (23) | 640-1,280 (11) | 160-320 (2) | 20-320 (14) |
| 80                                     |    |    |    |    |    |    |    |    |    |
| 26                                     |    |    |    |    |    |    |    |    |    |
| 3                                      |    |    |    |    |    | 80-640 (24) |    |    |    |
| 23                                     |    |    |    |    |    |    | 80-640 (11) |    |    |
| 11                                     |    |    |    |    | 80-160 (3) |    |    |    |    |
| 2                                       |    |    |    | 80-640 (24) |    |    |    |    |
| 13                                       |    |    |    |    |    |    |    |    | 80-160 (2) |
| 24                                       |    |    |    |    |    |    |    |    | 80-160 (13) |
| 16                                       |    |    |    |    |    |    |    | 320-1,280 (4) | 160-5,120 (16) |

* Antibody titer reciprocal of serum dilution; figures in parentheses indicate the number of strains giving agglutination test.

identical, were closely related antigenically to one or more serotypes of a complex group (I, J, K, N, Q, and R). Similar were the observations of others (1, 2) who employed metabolic inhibition and growth inhibition tests. Thus, we also agree with Fabricant (4) and Barber and Fabricant (1) that all six serotypes of Dierks et al. (2) in this complex group are closely related antigenically if not identical, although these were identical biochemically (P. J. Asnani, and S. C. Agarwall, Mycoplasmal Diseases Symposium, 31 December 1972). These findings were further supported by our study.

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