Mycoplasma gallisepticum Outbreaks in North Carolina, 1999-2001: Lessons Learned from the Tail End of the Epidemic Curve

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ABSTRACT

Beginning in October 1999, North Carolina experienced unprecedented outbreaks of *Mycoplasma gallisepticum* (MG) in commercial poultry flocks resulting in over 100 farms quarantined. Our efforts focused on MG isolation, and strain identification by random amplification of polymorphic DNA (RAPD) fingerprinting; and epidemiology consisting of field investigations, reporting and feedback to stakeholders, and a case control study to identify risk factors. In 2001 (the 'tail end' of the epidemic curve), MG quarantined flocks included 1 broiler breeder and 17 progeny flocks, 4 meat turkey flocks belonging to 2 different companies, and 8 backyard flocks. MG isolates from each of the 5 commercial flocks were unique RAPD types and each of these outbreaks had some association with a backyard flock or live bird market. These were 'sporadic' cases, and did not result in widespread 'epidemic' transmission that occurred previously. Current evidence indicates that inadequate monitoring of some broiler breeder flocks, the movements and interactions of people, and lack or lapses of biosecurity were major factors contributing to the outbreaks and epidemic of MG that occurred in North Carolina from 1999 to 2001. It also appears that backyard flocks and/or live bird markets were important reservoirs of MG infections. The temporal-spatial distribution of cases indicated that sporadic cases were identified in the midst of a major epidemic. The tail end of the epidemic curve consisted of a series of sporadic cases likely identified due to heightened awareness and more active surveillance.

INTRODUCTION

Beginning in October 1999, North Carolina experienced unprecedented outbreaks of *Mycoplasma gallisepticum* (MG) in commercial poultry flocks. Thru October 2000, this epidemic involved breeder and meat flocks of chickens and turkeys resulting in 104 farms quarantined (16 broiler breeder, 40 broiler, 8 turkey breeder, 41 commercial turkey) and 11 backyard flocks **(Fig. 1)**. Investigations of this epidemic involved the North Carolina State University College of Veterinary Medicine (Poultry Health Management faculty in the Department of Population Health and Pathobiology) and College of Agriculture and Life Sciences (Cooperative Extension Service, Department of Poultry Science), North Carolina Department of Agriculture & Consumer Services, National Poultry Improvement Plan, and commercial poultry integrators. NCSU-CVM efforts focused on 1) MG isolation, and strain identification by random amplification of polymorphic DNA (RAPD) fingerprinting, and 2) epidemiology consisting of field investigations, reporting and feedback to stakeholders, and a case control study to identify risk factors.

MATERIALS AND METHODS

DNA Fingerprinting. Random amplification of polymorphic DNA (RAPD) is a PCR-based method of DNA fingerprinting that results in amplification of 'anonymous' stretches of DNA with one (or sometimes more) short arbitrary primers and subsequent visualization of the amplification products by agarose gel electrophoresis. Compared to other currently available methods of avian mycoplasma strain identification, RAPD is fast, relatively simple to perform and cost effective. However, there are disadvantages and limitations to RAPD fingerprinting that must be considered. Starting material for the test requires a pure culture of the mycoplasma isolate. RAPD tests are known to have problems with reproducibility because they are sensitive to alterations in PCR conditions. Interpretation of RAPD banding patterns can also be challenging due to the problem of reproducibility, and the possibility of co-migrating bands. The 'challenges' of reproducibility and interpretation can usually be overcome by using one or more additional primer sets to confirm apparent relationships or resolve ambiguous results. Procedures for MG culture, isolation, identification, and RAPD fingerprinting have been previously published **(1,2)**.

Epidemiology. Investigations of the 1999–2001 MG outbreaks and epidemic involved the North Carolina State University College of Veterinary Medicine (Poultry Health Management faculty) and College of Agriculture and Life Sciences (Cooperative Extension Service, Department of Poultry Science), North Carolina Department of Agriculture & Consumer Services, National Poultry Improvement Plan, and commercial poultry integrators. NCSU-CVM epidemiologic efforts were focused on field investigations, 'real time' reporting of results and feedback to stakeholders, and a case control study to identify risk factors. In addition to cases (MG-positive flocks), MG-negative flocks of similar age and type of production located within 2 miles of cases, were visited for comparison.

RESULTS AND DISCUSSION

A primary objective was to perform RAPD analyses on MG isolates from affected flocks **(Fig. 2, 3)** in North Carolina. Of 91 mycoplasma cultures from 1999-2000 that we examined, 69% were pure MG, 23% contained MG and *M. synoviae*, and 7% contained MG and some other *Mycoplasma* species **(Fig. 4)**. Of the MG isolates from 1999-2000 that we fingerprinted by RAPD, we identified four different 'RAPD types': A, B, E, and F. These RAPD types were defined by fingerprint banding patterns that were distinct from one another using two primer sets. We also found that these RAPD types or field strains had fingerprints that were different from the MG vaccine strains (F, ts11 and 6/85) and the 'House finch' strain, which indicated that these strains were not involved in this epidemic **(Fig. 5)**.

MG in North Carolina Oct 1999 - Oct 2000

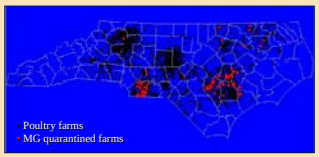

Figure 1. Distribution of poultry farms in North Carolina, and farms quarantined for MG from Oct 1999 to Oct 2000 - 104 poultry farms (16 broiler breeder, 40 broiler, 8 turkey breeder, 40 turkey) and 11 backyard flocks quarantined.

Figure 2. Clinical signs of MG infection in broiler breeder chickens, typical of the disease seen in the North Carolina 1999-2001 outbreaks.

Figure 3. Clinical signs of MG infection in meat turkeys, typical of the disease seen in the North Carolina 1999-2001 outbreaks.

Mycoplasma Isolates 1999-2000

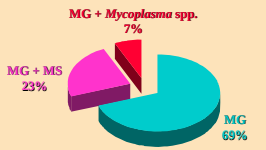

Figure 4. Of 91 mycoplasma cultures examined by species-specific direct immunofluorescence, 69% were 'pure' MG, 23% contained MG and *M. synoviae*, and 7% contained MG and some other *Mycoplasma* species.

RAPD fingerprints of MG vaccines, house finch & poultry isolates

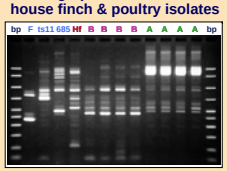

Figure 5. DNA fingerprints (RAPD types) of field strains (A and B in this example) had banding patterns that were different from the MG vaccine strains (F, ts-11, 6/85) and the 'house finch' (Hf) strain, which indicated that these strains were not involved in this epidemic.

Figure 6. Geographic information system (GIS) map shows portions of a 3 county area - poultry farms are blue diamonds named in red (obscured for confidentiality). The circles show 2 and 6 mile radius zones around an MG-positive farm. *Produced by NC Dept. of Agriculture & Consumer Services.*

MG in North Carolina Oct 1999 - Oct 2000

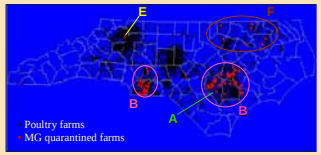

Figure 7. Distribution of poultry farms in North Carolina, and farms quarantined for MG from Oct 1999 to Oct 2000. The ability to assign RAPD types (A, B, E, F) to MG isolates enabled us to learn more about the epidemiology of the outbreaks.

MG Positive Farms – Epidemic Curve

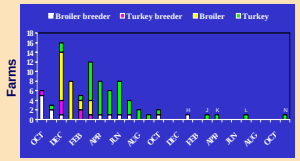

Figure 8. Epidemic curve of MG-positive commercial poultry farms showing occurrence of sporadic cases in 2001, each of which was due to a different RAPD type (H, J, K, L, N).

MG in North Carolina 2001

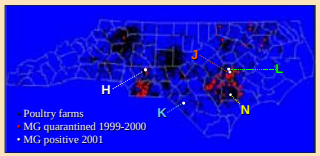

Figure 9. Distribution of poultry farms in North Carolina, farms quarantined for MG (1999 to 2000), and MG positive commercial farms in 2001. In 2001, each MG positive farm had a different RAPD type (H, J, K, L, N) and had some association with a backyard flock or live bird market.

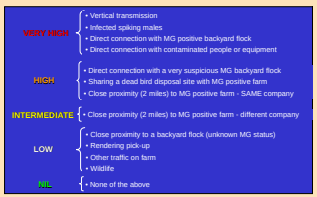

Table 1. Summary of risk factors resulting from univariate analyses (Fisher Exact Test) on data collected from 34 MG-positive and 17 MG-negative flocks.

RESULTS AND DISCUSSION cont.

Geographic information system (GIS) maps **(Fig. 6)** produced by the North Carolina Department of Agriculture & Consumer Services were invaluable for precisely identifying farm locations, establishing quarantine and/or testing zones, and contributing to 'real time' reporting of information to stakeholders.

The ability to assign RAPD type or strain identities to MG-positive flocks enabled us to learn more about the epidemiology of the outbreaks **(Fig. 7)**. For example, a large cluster of MG-positive flocks in eastern North Carolina involved RAPD types A and B. RAPD type A was isolated from a single backyard flock and multiple houses of a nearby turkey breeder farm. Type B was isolated from 5 broiler breeder farms, infected by the practice of 'spiking' males. However, before infection of these flocks was recognized progeny were placed to sites near other poultry farms. MG RAPD type B became widespread in major foci of infections in eastern and western North Carolina **(Fig. 7)**. RAPD type E was isolated from a single broiler breeder farm in the northwestern part of the state and the suspected origin was a backyard flock owned by the son of the breeder flock grower. MG RAPD type F was identified in northeastern North Carolina and involved a cluster of farms consisting of a broiler breeder flock and multiple flocks of progeny.

In 2001 **(Fig. 8)**, MG-positive farms included 1 broiler breeder, and 4 meat turkey flocks belonging to 2 different companies. MG isolates from each of the 5 commercial flocks were unique RAPD types **(Fig. 9)** and each of these 'sporadic case' outbreaks had some association with a backyard flock or live bird market. We did not see the type of widespread transmission that occurred in 1999-2000 with RAPD type B **(Fig. 7, 8)**. The last MG isolate from commercial poultry was made from meat turkeys in December 2001.

Field investigations revealed that in some cases in the Fall of 1999, MG monitoring of broiler breeder flocks was not adequate due to sub-optimal intervals between tests, and between sampling and reporting results. These circumstances opened a window of opportunity for vertical transmission of MG from broiler breeders to progeny, and horizontal transmission to other poultry farms. Since March 2000, the vast majority of confirmed MG cases involved commercial turkey flocks **(Fig. 8)**.

Univariate analyses (Fisher Exact Test) on data collected from 34 MG-positive and 17 MG-negative farms indicated that relationships among farm workers and biosecurity were primary factors that contributed to this epidemic. Approximately 63% of growers from MG-positive farms did not require coveralls for visitors compared to 7% of the MG-negative growers (p=0.0009). 25% of MG-positive growers also did not require rubber or plastic boots while all of the MG-negative growers required at least one type of foot cover for farm visits (p=0.04). A summary of risk factors and levels is presented in **Table 1**.

CONCLUSIONS

Current evidence indicates that inadequate monitoring of some broiler breeder flocks, the movements and interactions of people, and lack or lapses of biosecurity were major factors contributing to outbreaks of MG in North Carolina. It also appears that backyard flocks and/or live bird markets were important reservoirs of MG infections. The temporal-spatial distribution of cases indicated that sporadic cases were identified in the midst of a major epidemic. The tail end of the epidemic curve consisted of a series of sporadic cases likely identified due to heightened awareness and more active surveillance.

Keys to successful control and stamping out of the 1999-2001 North Carolina MG outbreaks included:

- ❑ the ability to make strain identifications by RAPD fingerprinting
- ❑ GIS for mapping of farm locations, quarantine/testing zones, reporting
- ❑ regional response/cooperation among stakeholders
- ❑ electronic communications and 'real time' reporting of results
- ❑ adequate resources – personnel & funds.

ACKNOWLEDGEMENTS

This research was supported in part the by the State of North Carolina and grants from the USDA Fund for Rural America (Project No. 01004 – Emerging Infectious Diseases of Poultry), and the US Poultry and Egg Association.

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