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Detection of Antibodies to *Mycoplasma gallisepticum* in Egg Yolk versus Serum Samples†

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Serum (n = 1,636) and egg yolk (n = 802) samples collected from hens on four commercial egg farms in Florida were tested for the presence of specific antibodies to *Mycoplasma gallisepticum* in a commercially available enzyme-linked immunosorbent assay. No significant differences were noted between serum and egg yolk samples with respect to distribution of positive, suspect, and negative test results or for the mean sample/positive control ratio values of positive, suspect, and negative test results. A linear relationship between the distribution of positive and negative results and the age of the birds was observed for results obtained with both serum and egg yolk samples. On the basis of the results of this study, egg yolk samples can be used in lieu of serum samples to screen flocks for antibodies to *Mycoplasma gallisepticum*.

Respiratory mycoplasmosis in poultry is a major worldwide economic problem (2, 7, 13). Current methods to diagnose and manage *Mycoplasma gallisepticum* infection include identification and elimination of infected birds, antibiotic therapy, and vaccination. Antibiotic therapy has been used to mitigate the effects of the disease. However, such therapy does not result in clearance of the mycoplasmas. In addition, antibiotic-resistant strains have been found, and the expense associated with treatment often is prohibitive (1, 13). Currently, an inactivated bacterin and a live attenuated F strain vaccine are used (1, 3, 5, 6, 8, 14). Neither vaccine is completely effective, but both can reduce declines in egg production and lessen disease severity. The method of choice for identification of *Mycoplasma*-positive flocks is serology. The initial screening test commonly used is the plate agglutination test. Because of false-positive reactions in the plate test (4, 12), a positive sample is confirmed by either hemagglutination or enzyme-linked immunosorbent assay (ELISA) serology.

Chloroform-extracted egg yolks have compared favorably with serum for detection of antibody to avian viruses in commercial ELISA kits (11). In a study with a laboratory-prepared ELISA, both chloroform-extracted and saline-extracted egg yolks also compared favorably with serum for detection of antibodies to *M. gallisepticum* and *M. synoviae* (10). Eggs can be obtained and shipped from commercial facilities for testing with relative ease. Therefore, the current study was designed to determine if egg yolks could be used in lieu of serum samples to screen flocks in production for antibodies to *M. gallisepticum* by using a commercially available ELISA and a simplified extraction procedure.

Samples. Serum (n = 1,636) and egg yolk (n = 802) samples were obtained from four commercial egg farms in Florida (Table 1). The number of layers per house was greater than 50,000. The farms were fully automated, with egg collection via a conveyor belt. Birds had not received vaccinations against *M. gallisepticum* but had been given the appropriate vaccine for viral agents. Serum samples were obtained from layers in each house of each farm except for farm 2 (Table 1). Two houses were excluded from farm 2 because the birds were to be moved the next week and would not be available for follow up. The first five rows of each house were sampled. The number of sampling sites per row was dependent on the length of the row and ranged from three to five sites. Within each row, the 10th cage from either end and points equidistant from the ends were chosen as sample sites. Birds were not housed individually but in cages of 5 to 10 birds per cage. Three birds, one each from the top, second, and third tiers, were selected per sampling site. Two eggs were obtained from the egg conveyor belt from the first, second, and third tiers of the first five rows in each house. Thus, the egg and serum samples were paired for vertical location within a row but not for individual bird.

Processing. Blood samples were held at 4°C until returned to the laboratory. Serum was obtained by centrifugation and stored at −20°C in a manual-defrost freezer. Eggs were broken out into individual containers, and the yolk sample, minus membrane, was obtained by aspiration of 0.3 ml of yolk with a tuberculin syringe. The yolk was mixed with 2.7 ml of sterile phosphate-buffered saline and stored at −20°C in a manual-defrost freezer.

ELISA. Antibody levels to *M. gallisepticum* were determined by commercial ELISA (ProFlok MG ELISA; catalog no. 54-85-01; Kirkegaard & Perry Laboratories, Gaithersburg, Md.) according to the manufacturer's instructions. All reagents were provided in the commercial system. Sera were diluted 1:100 in diluent, and 100 μl was placed in the appropriate ELISA well. Yolk samples were diluted 1:10 (to give a final 1:100 dilution), and 100 μl was placed in the appropriate ELISA well. After a 30-min incubation period, wells were washed extensively, and horseradish peroxidase-labeled anti-chicken immunoglobulin G was added for 30 min. After extensive washing, 100 μl of substrate was added and incubated for 15 min. The reaction was stopped, and the A_{405} was determined. All incubations were at room temperature. Three positive and three negative controls were included for each assay plate. The sample value/positive reference value ratio was calculated according to the instructions in the ELISA kit. Briefly, the mean absorbance value for the three negative reference serum samples was subtracted from the mean of the three positive reference serum

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TABLE 1. Summary of farms from which serum and egg yolk samples were obtained for ELISAs

Farm	No. of houses	No. of serum samples	No. of egg yolk samples
1	4	299	120
2	4	171[a]	120
3	3	223	90
4	16	943	472
Total*	27	1,636	802

[a] Serum samples were not obtained from two houses of farm 2.

samples and from the sample value. The sample/positive reference value ratio was calculated by dividing the corrected sample value by the corrected reference mean value. A ratio of ≥0.6 was deemed positive and a ratio of ≤0.199 was deemed negative. Samples which gave ratios of between 0.199 and 0.59 were deemed suspect. The values for determining negative, suspect, and positive values were provided by the manufacturer and were based on experimental infection and field test samples.

Statistical analysis. Serum and egg yolk results for distribution of positive, negative, and suspect samples for a farm were compared by chi-square analysis. Levels of antibody in serum and egg yolk samples were determined by Student's unpaired *t* test.

Distribution and levels of antibody. The distribution of positive, suspect, and negative test samples detected in serum and egg yolks is shown in Table 2. No significant differences were noted between serum and egg yolk samples with respect to distribution of positive, suspect, and negative test results by chi-square analysis.

The distribution of samples with positive, suspect, and negative test results was similar; therefore, it was of interest to determine if the levels of antibody obtained with serum and egg yolk were also similar (Fig. 1). There were no significant differences noted for the mean sample/positive control ratios for any group by Student's unpaired *t* test. No differences were observed between egg yolk and serum samples with respect to background levels. It is interesting to note that, for farms 1 and 2, the only positive samples detected were from egg yolk rather than serum. It is not known if these results represent a false-positive test or flocks which had just begun to seroconvert. Both farms did have suspect sample results which might suggest the latter possibility.

TABLE 2. Serum and egg yolk samples which were negative, suspect, or positive for antibody to *M. gallisepticum*[a]

Farm	Sample type (*n*)	% Samples which were:		
		Negative	Suspect	Positive
1	Serum (299)	90	10	0
	Yolk (120)	88	11	1
2	Serum (171)	88	12	0
	Yolk (120)	93	5	2
3	Serum (223)	3	29	68
	Yolk (90)	1	27	72
4	Serum (943)	51	7	42
	Yolk (472)	52	9	39
Total	Serum (1,636)	55	12	33
	Yolk (802)	58	11	31

[a] No statistically significant differences were noted for any farm between results obtained with serum or egg yolk samples by chi-square analysis.

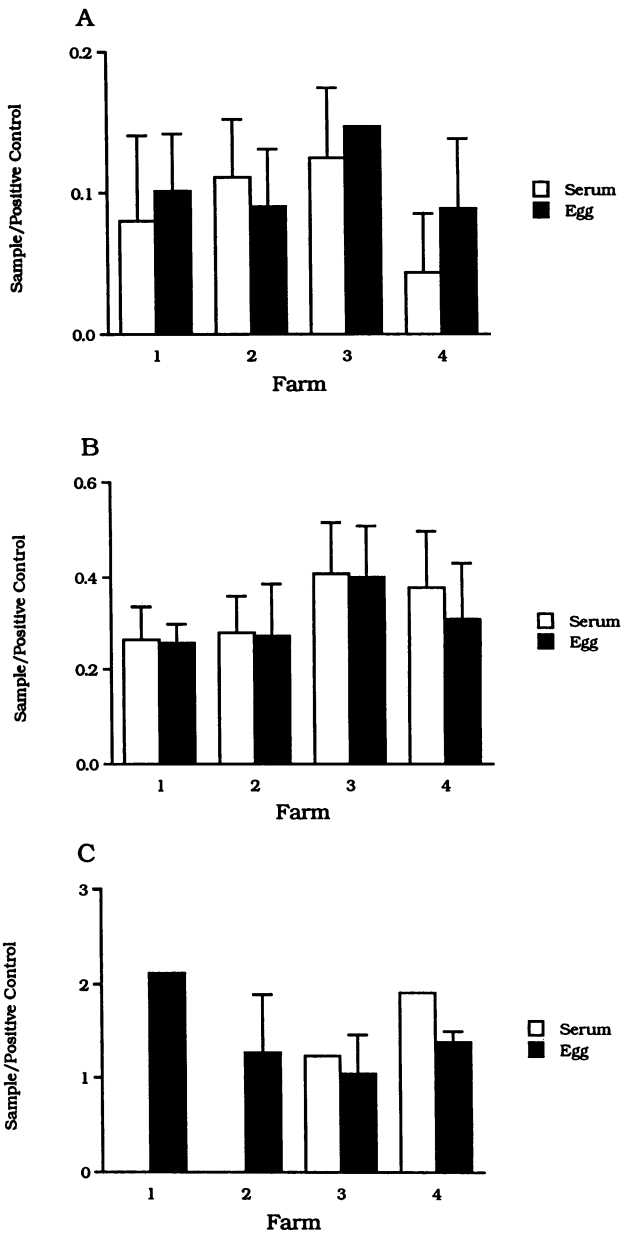

FIG. 1. Mean sample/positive control ratio values of serum and egg yolk samples in birds that tested negative (A), suspect (B), or positive (C) for antibodies to *M. gallisepticum*. Error bars represent the standard deviation of the mean. The absence of error bars indicates that only a single sample was found in the category. No serum samples tested positive for farms 1 and 2. No significant differences were noted between results obtained with serum and egg yolk samples by Student's *t* test.

Age of bird and ELISA results. To determine if there were differences in the pattern of seroconversion with respect to age of bird and sample type, samples were obtained from a facility with birds of differing ages (farm 4). The percentages of birds which tested negative and positive were virtually

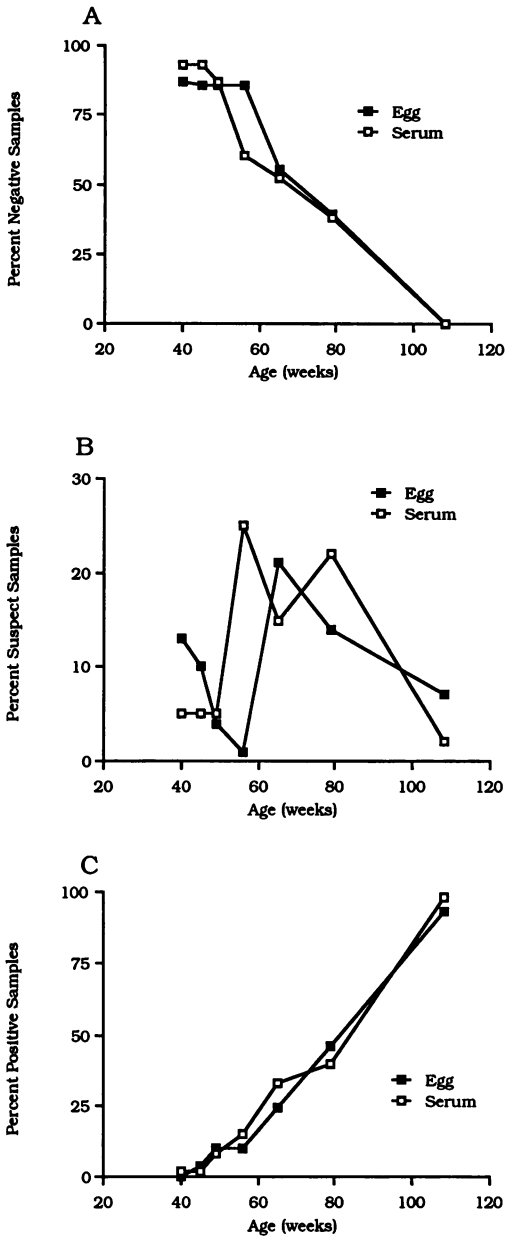

FIG. 2. Relationship between age of bird and the percentage of birds that tested negative (A), suspect (B), or positive (C) for antibodies to *M. gallisepticum* in serum or egg yolk samples.

identical regardless of sample type (Fig. 2). Slightly more variation was observed with birds that had suspect test results (Fig. 2B). The greatest time of variation (45- to 60-week-old birds) in the suspect category also corresponded to the time in which slightly more egg yolk samples than serum samples tested negative (Fig. 2A). The linear response observed for seroconversion is consistent with observations on the lateral spread of *M. gallisepticum* under experimental conditions (9). A single infected bird was introduced into a group of birds; 5 to 10% of exposed birds began to seroconvert within 21 days. The seroconversion pattern in the experimental study (9) was consistent with that observed in our study of naturally infected birds (Fig. 2).

Advantages of egg yolk. Eggs are easily collected and shipped by farm workers without special equipment such as syringes, blood collection tubes, and needles. Collection of eggs by farm workers also prevents potential contamination between farms or houses which could occur during serum sampling. Relevant sample identification can be recorded directly on the egg with a pencil. Collection of eggs is not stressful to birds as is collection of blood. It is more cost effective in man-hours to collect eggs than sera (one person for 4 h versus seven people for 30 h for farm 4 in the present study). No significant differences were observed between sera and egg yolks with respect to distribution of positive samples within a flock or levels of antibody obtained. Thus, we conclude that egg yolks can be used in lieu of serum samples to screen flocks for the presence of antibodies to *M. gallisepticum*.

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REFERENCES

1. **Barile, M. F.** 1985. Immunization against mycoplasmal infections, p. 452–492. *In* S. Razin and M. F. Barile (ed.), The mycoplasmas, vol. 4. Academic Press, Inc., New York.
2. **Cassell, G. H., W. A. Clyde, and J. K. Davis.** 1985. Mycoplasmal respiratory mycoplasmosis, p. 69–107. *In* S. Razin and M. F. Barile, (ed.), The mycoplasmas, vol. 4. Academic Press, Inc., New York.
3. **Cummings, T. S., and S. H. Kleven.** 1986. Evaluation of protection against *Mycoplasma gallisepticum* infection in chickens vaccinated with the F strain of *Mycoplasma gallisepticum*. Avian Dis. **30**:169–171.
4. **Glisson, J. R., J. F. Dawe, and S. H. Kleven.** 1984. The effect of oil-emulsion vaccines on the occurrence of nonspecific plate agglutination reactions for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Avian Dis. **28**:397–405.
5. **Glisson, J. R., and S. H. Kleven.** 1984. *Mycoplasma gallisepticum* vaccination: effects on egg transmission and egg production. Avian Dis. **28**:406–414.
6. **Glisson, J. R., and S. H. Kleven.** 1985. *Mycoplasma gallisepticum* vaccination: further studies on egg transmission and egg production. Avian Dis. **29**:408–415.
7. **Jordan, F. T. W.** 1979. Avian mycoplasmas, p. 1–48. *In* J. G. Tully and R. F. Whitcomb (ed.), The mycoplasmas, vol. 2. Academic Press, Inc., New York.
8. **Kleven, S. H., J. R. Glisson, M. Y. Lin, and F. D. Talkington.** 1984. Bacterins and vaccines for the control of *Mycoplasma gallisepticum*. Isr. J. Med. Sci. **20**:989–991.
9. **McMartin, D. A., M. I. Khan, T. B. Farver, and G. Christie.** 1987. Delineation of the lateral spread of *Mycoplasma gallisepticum* infection in chickens. Avian Dis. **31**:814–819.
10. **Mohammed, H. O., R. Yamamoto, T. E. Carpenter, and H. B. Ortmayer.** 1986. Comparison of egg yolk and serum for the detection of *Mycoplasma gallisepticum* and *M. synoviae* antibodies by enzyme-linked immunosorbent assay. Avian Dis. **30**:398–408.
11. **Silim, A., and D. Venne.** 1989. Comparison of egg-yolk and serum antibody titers to four avian viruses by enzyme-linked immunosorbent assay using paired field sera. Avian Dis. **33**:643–648.
12. **Yoder, H. W.** 1989. Nonspecific reactions to mycoplasma serum plate antigens induced by inactivated poultry disease vaccines. Avian Dis. **33**:60–68.
13. **Yoder, H. W., Jr.** 1984. *Mycoplasma gallisepticum* infection, p. 236–250. *In* M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder, Jr. (ed.), Diseases of poultry. Iowa State University Press, Ames.
14. **Yoder, H. W., and S. R. Hopkins.** 1985. Efficacy of experimentally inactivated *Mycoplasma gallisepticum* oil-emulsion bacterin in egg-layer chickens. Avian Dis. **29**:322–334.