SURVEILLANCE OF SWINE IN MIDDLE TENNESSEE FOR INFLUENZA A VIRUS

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ABSTRACT—Influenza A virus infects a variety of avians and mammals, including swine and humans. Swine have been implicated as a reassortment vessel for the generation of new strains of influenza A virus, an event which can prove disastrous for human populations. Because available information on the incidence of influenza virus among Middle Tennessee swine herds is limited, four Rutherford County swine herds were surveyed weekly from October 1993 through March 1994. Herds were selected based on the degree of interspecies contact. Two hundred fifty nasal swabs were obtained from swine at each of the four sites. Samples were grown in embryonated chicken eggs, tested for hemagglutination activity, and evaluated for the presence of influenza A virus. This report documents no detectable circulation of influenza virus among selected swine herds in Rutherford County in Middle Tennessee.

There are three types of influenza virus, A, B, and C. Types B and C are found only in humans and result in relatively mild respiratory disease (Webster et al., 1992). Influenza A virus infects a wide variety of avian and animal hosts and is responsible for severe respiratory disease in humans. Influenza A virus results in 10,000 excess deaths annually in the United States (Centers for Disease Control, MMWR, 1990). Influenza A subtypes are identified by two envelope glycoproteins, hemagglutinin and neuraminidase. Currently the subtypes H3N2 and H1N1 are circulating in the human population. Swine also are susceptible to infection by these subtypes of influenza.

Influenza is responsible for yearly epidemics and occasional pandemics. Epidemics are due to antigenic drift, or, subtle changes in the antigenic nature of viral glycoproteins. Pandemics, which occur every 20–30 years, arise due to reassortment or exchange of genomic segments between two different influenza viruses that simultaneously infect a cell (Webster and Laver, 1972; Webster and Kawaoka, 1988). Genomic reassortment has been reported for influenza isolates of both avian (Hinshaw et al., 1983) and human origin (Bean et al., 1980; Palese and Young, 1982). The most infamous influenza pandemic occurred in 1918–1919 and claimed over 20 million lives worldwide (Crosby, 1989). The more recent pandemics of 1957 and 1968 involved viruses that were reassortants containing genes characteristic of both human and avian influenza (Kawaoka et al., 1989). However, the host for these reassortment events is unknown. Swine have been suggested as such a mixing vessel (Scholtissek et al., 1985) because swine allow replication of influenza virus of either avian (Schultz et al., 1991) or human origin (Chambers et al., 1991). Further, swine-like influenza has been reported in avians (Wright et al., 1992) and humans (Rota et al., 1989).

Limited information is available on the incidence of influenza virus in swine in the United States. This study determined the incidence of influenza A virus among swine at selected sites in Middle Tennessee. We report the absence of detectable influenza A virus among selected swine herds in Rutherford County in Middle Tennessee.

MATERIALS AND METHODS

Swine sampling sites—Four separate swine herds in Rutherford County were sampled. Each herd numbered approximately 50–90 swine (*Sus scrofa*). Two sites represented indoor facilities allowing limited contact with avians. Two sites represented range facilities where there was free interaction with both wild and domestic avians. Human contact was possible through the swine caretakers at all sites.

Sample collection—A total of 1000 samples were collected from all four sites from September 30, 1993 through March 29, 1994. Ten nasal swabs per week were collected from swine at each of the four sites. Samples were put in transport media (phosphate buffered saline, glycerol, antibiotics) and stored at −70° C until tested. Samples were grown in embryonated chicken eggs and evaluated for hemagglutination activity as described by Hinshaw et al., 1978.

Evaluation for influenza—All samples demonstrating hemagglutination activity were evaluated for influenza in two ways. First, samples positive for hemagglutination were tested with the Directigen Flu A kit which detects the presence of the influenza nucleoprotein antigen but does not differentiate host of origin (kindly supplied by Becton Dickinson Microbiology Systems). Additionally, positive hemagglutination samples were evaluated by genetic amplification and probing as previously described, (Wright et al., 1992). Briefly, viral nucleic acid was extracted from allantoic fluid. cDNA was prepared and amplified by the polymerase chain reaction (PCR) using influenza-specific primers for the Non-Structural (NS) gene (synthesized by Keystone Laboratories, Inc.). Final evaluation of samples as influenza was done by dot-blot hybridization using an influenza-specific probe. Sequences for primers and probes have been reported (Wright et al., 1992).

RESULTS

Forty of the 1000 samples collected demonstrated hemagglutination activity (Table 1). Slightly more than half (57.5%) of

TABLE 1. Number of samples demonstrating hemaggluti- nation activity from each of the four sites evaluated and the month of sample collection.

	Month collected	Collection sites[1]			
		1	2	3	4
1993	October	5	1	5	0
	November	0	0	0	0
	December	0	1	1	1
1994	January	0	4	4	1
	February	0	0	2	1
	March	4	2	5	3
	Total each site	9	8	17	6

[1] Sites 1 and 2 were closed facilities, allowing little contact between swine and avians. Sites 3 and 4 were range facilities, allowing frequent contact between swine and avians.

the samples that were positive for hemagglutination were obtained from swine housed under range conditions. There was no seasonal preference for hemagglutination activity.

The hemagglutination agents were evaluated for the influenza virus by both the Directigen Flu A kit and polymerase chain reaction/dot blot hybridization using an influenza-specific probe. Although reactions were appropriate for positive controls by both Directigen and PCR/dot blot, all samples remained negative (Fig. 1).

DISCUSSION

Pandemic strains of influenza A virus most often result from gene reassortment of avian and human influenza viruses. Generally, avian viruses do not replicate well within humans, although the isolation of avian H5N1 from humans has been reported (Centers for Disease Control, MMWR, 1998). Swine are the leading candidate as mixing vessels for the reassortment event between human and avian influenza. In order for interspecies transmission to occur, the virus must be maintained in the swine population. However, no influenza virus was detected among our sampled swine herds in Rutherford County. Thus, the likelihood of swine participating in interspecies transmission of influenza was low.

The lack of detectable influenza A virus in this study correlates with other reports. Chambers et al., 1991, reported only 10% of swine that were sampled in Tennessee demonstrated antibody to influenza (no virus isolation was attempted). In a previous study of 9400 tracheal swabs collected from swine at
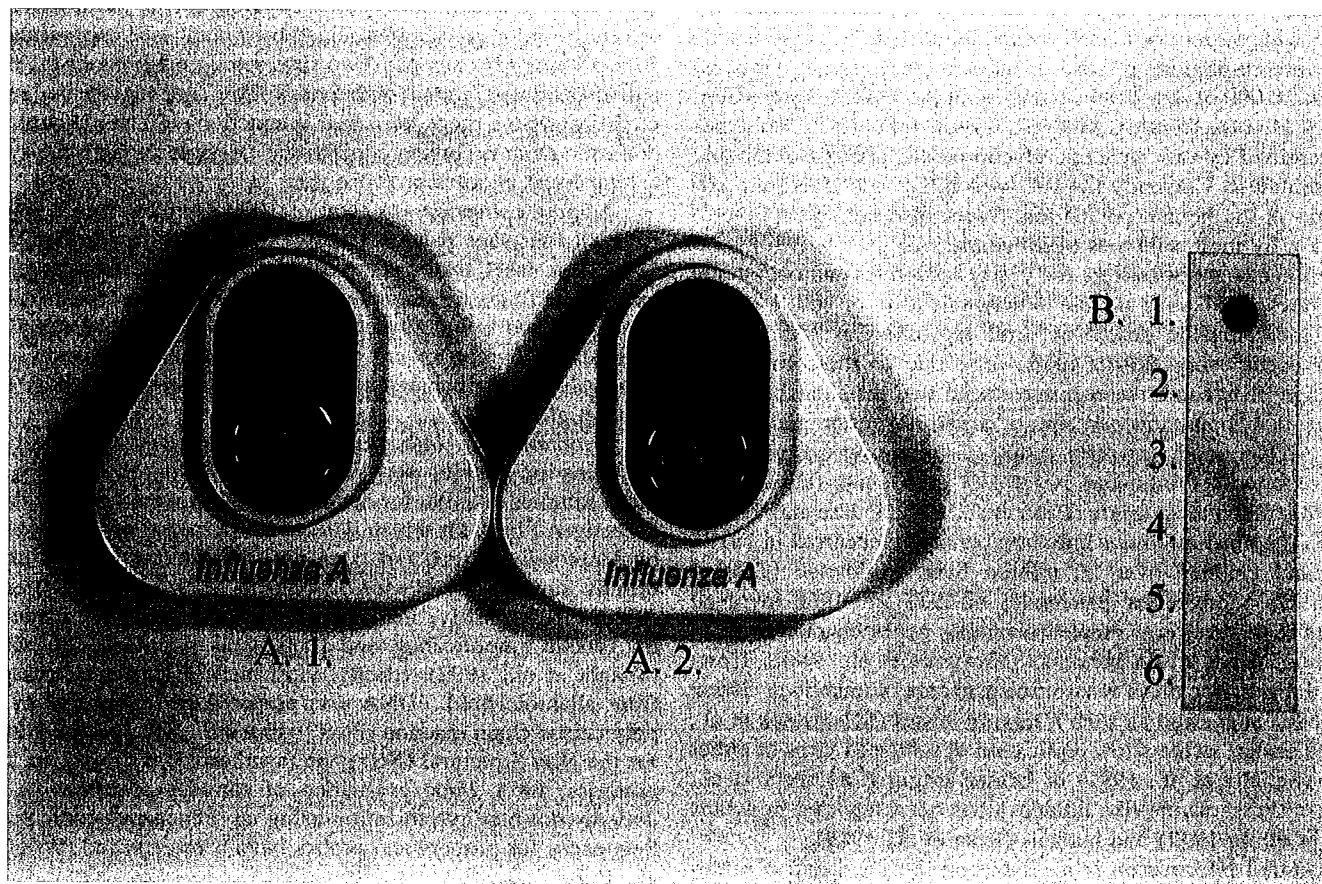

FIG. 1. A. Directigen Flu A kit. 1. MAL/NY/6750/78, an avian influenza A virus, was used and exhibits a positive test (triangle). 2. A positive hemagglutination agent from this study was used and a negative result (dot) was obtained. B. Dot blot hybridization. Row 1: amplified nucleic acid from MAL/NY/6750/78 NS gene; Row 2: amplified nucleic acid from herpes simplex virus-1 (heterologous DNA); Rows 3, 4, 5, 6: samples, after amplification, that exhibited positive hemagglutination from this study.

slaughterhouses in both southern and northern states, approximately 5% were positive for influenza (Hinshaw et al., 1978). However, isolates from southern states accounted for less than 1% of the total. Further, Hinshaw et al., (1978) reported no seasonal preference in isolation of influenza among swine in the southern states. We found no seasonal preference for the isolation of hemagglutination agents. However, in the study by Hinshaw et al., (1978) and the present study, the prevailing month for isolation of these agents was March.

Although the influenza virus was not detected, the question remains as to which agents were responsible for the hemagglutination reactions observed. A number of virus families are capable of agglutinating red blood cells including the Poxviridae, Togaviridae, Flaviviridae, Paramyxoviridae, Coronaviridae and Reoviridae (White and Fenner, 1986). However, no effort was made to characterize the agents. The slightly higher incidence of hemagglutination agents detected among swine maintained under free range conditions could contribute to decreased herd productivity due to infectious disease. Animal husbandry practices that discourage the transmission of infectious agents, particularly when humans are at risk, are most prudent.

ACKNOWLEDGMENTS

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