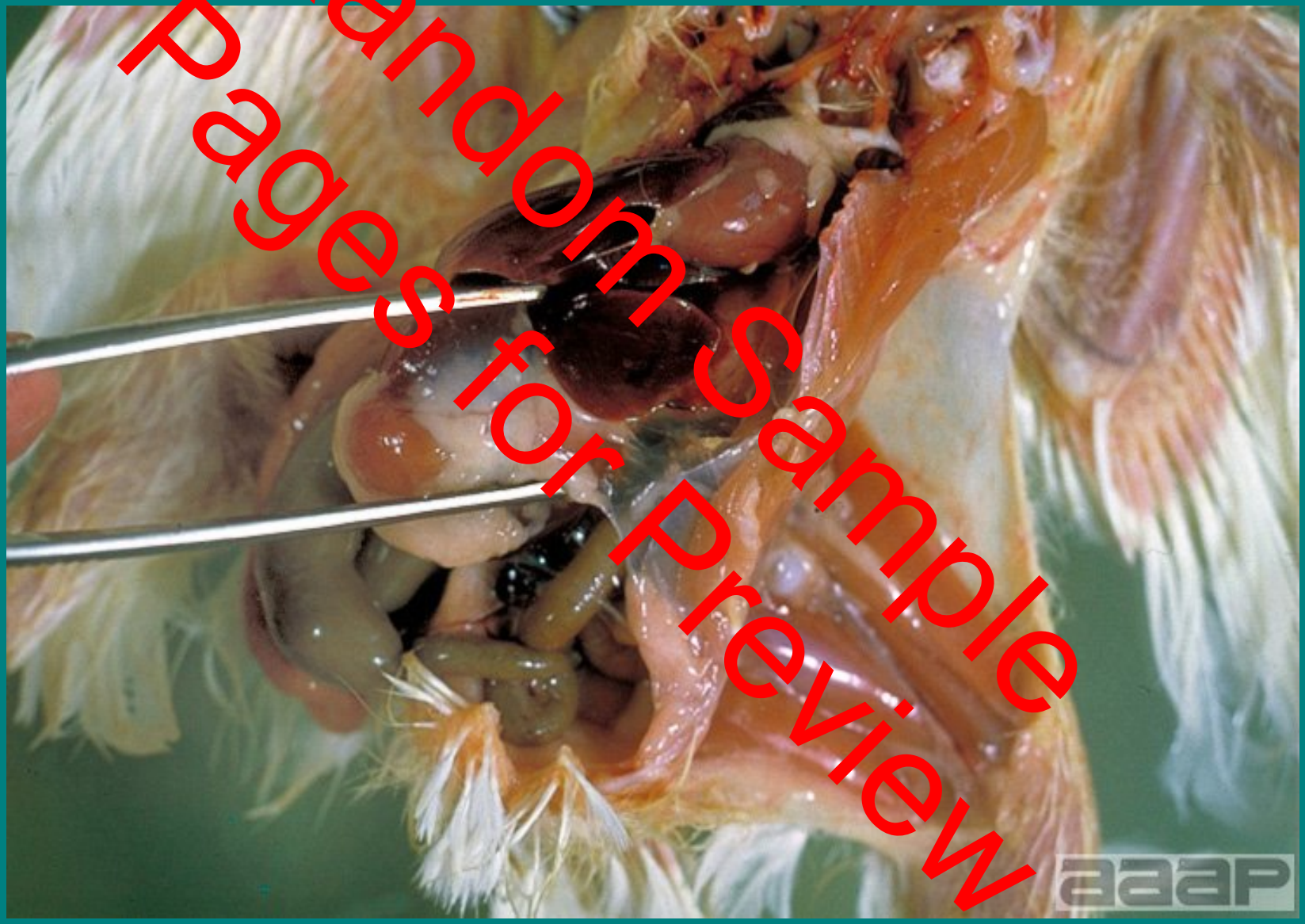
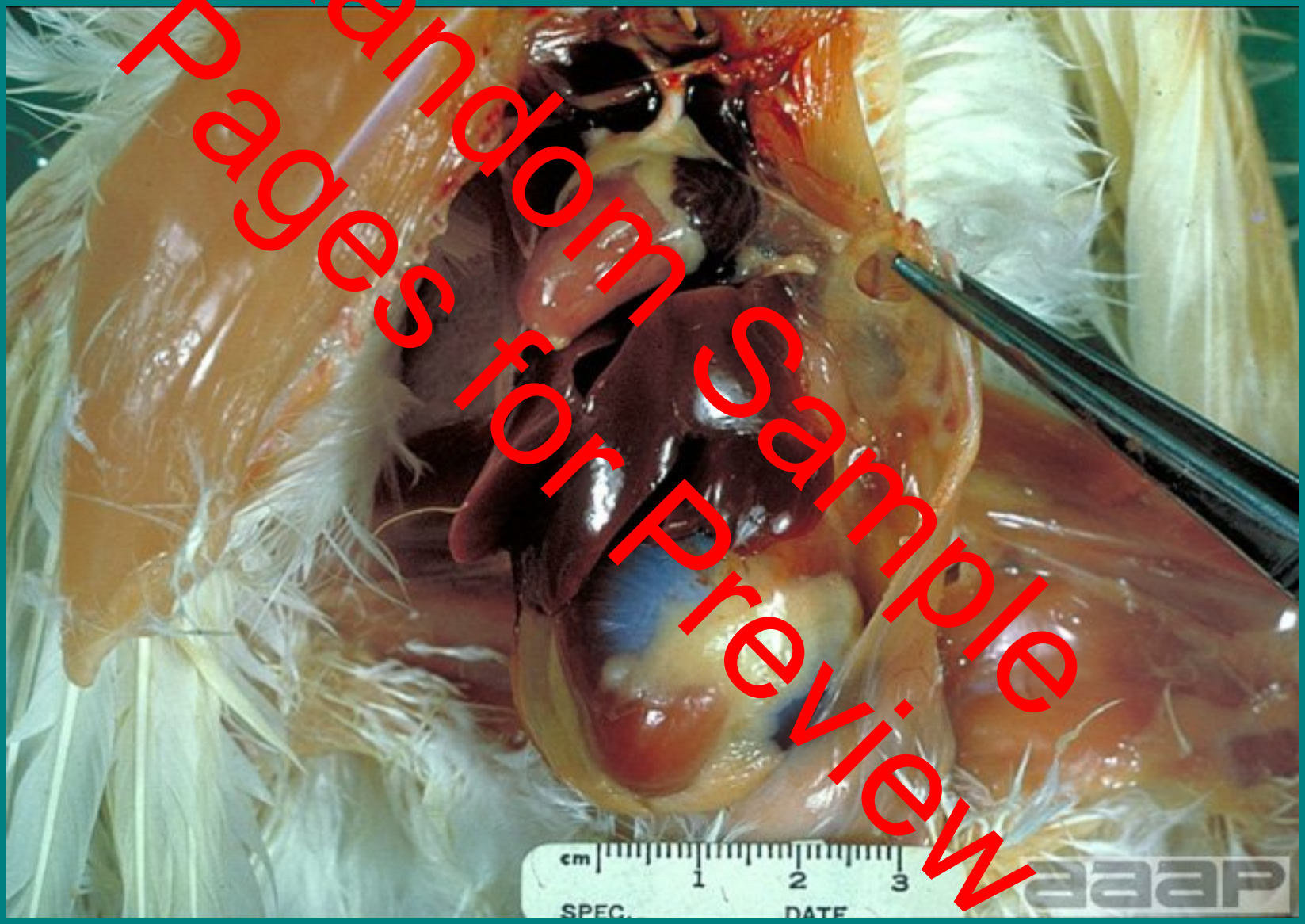
Sinusitis

House Finch Conjunctivitis

Normal air sacs



Acute airsacculitis



adhesins such as PvpA (38), VlhA (previously known as pMGA) (3), GapA, and CrmA (34).

Variability among and within strains of *M. gallisepticum*

M. gallisepticum strains are known to vary in pathogenicity and antigenicity (24). Variability in pathogenicity among strains of MG has been recognized for some time (45). Antigenic variability among MG strains could affect the sensitivity of serological tests, depending on the strain infecting the flock and the strain used to prepare antigen. Infection in house finches (*Carpadacus mexicanus*) with conjunctivitis caused by MG has been shown to be widespread in the Eastern U.S. (28, 31). This strain has been shown to spread poorly to chickens and to be relatively avirulent (33). A house finch-like strain of MG has also been isolated from turkeys with atypically mild clinical disease (11). This is the only known instance of infection of commercial poultry with a finch-like MG strain.

Restriction fragment length polymorphism (RFLP) of whole-cell DNA has been shown to be useful for differentiating MG strains (24). However, the RFLP procedure is time-consuming and laborious, making identification of specific strains a tedious procedure. More recently, randomly amplified polymorphic DNA (RAPD) has been developed for identifying specific strains (8, 9, 14). This procedure is very simple and rapid, and has provided a routine procedure for the rapid identification of MG strains; a disadvantage is that a pure culture of MG is required, which is sometimes difficult when nonpathogenic mycoplasma species are present. RAPD has proven to be very useful for epidemiological studies and for identification of specific MG strains in field outbreaks. More recently, we have utilized PCR for the *mgc*2 (16, 17, 18) gene of MG followed by sequencing of the PCR product to provide a **preliminary** identification of specific MG strains (unrelated strains sometimes have identical *mgc*2 sequences). Using this method we have been able to more closely pinpoint the identity of field and vaccine strains directly from clinical specimens. Another molecular method, amplified fragment length polymorphism (AFLP), may be a more definitive way to type strains by comparing the whole genome instead of small lengths of variable genes (18). There is also a PCR procedure that appears to be specific for the vaccine strain, ts-11, with few exceptions (P. Markham, unpublished). This procedure is suitable for use with pure cultures, but it is not known if it is sensitive enough to be used with clinical specimens.

Studies utilizing Western blots and monoclonal antibodies have shown a high degree of variability in expression of surface antigens among strains of MG; many of these proteins are variably expressed (2, 4, 32). This has led to a large effort to characterize the variable expression of surface antigens and has shown that phase variation also occurs *in vivo*. The significance of such variability in the expression of surface antigens is not well understood; however, it seems logical that it would play a role in pathogenesis, serological responses, and evasion of the immune system of the host. MG has been shown to penetrate cells in vitro (44). This may be an additional mechanism for avoiding the host immune response as well as effective antibiotic treatment.

Distribution

The primary hosts of MG are the chicken and turkey; infection in other avian species occurs much less frequently. The majority of poultry production in the U. S. is mycoplasma-free; however, MG infection is common in commercial egg production flocks. Unfortunately, in spite of increased efforts at control, a small number of outbreaks continue to occur each year. Monitoring of free-flying avian species frequenting infected poultry houses or wild-trapped birds generally do not yield evidence of infection (36, 39). An exception is a relatively recent outbreak of MG infection in house finches (*Carpodacus mexicanus*) in the Eastern United States (31), but this strain of MG has not been a factor in commercial poultry. Even though free flying birds are not usually biological carriers, persistence on feathers for up to 4 days suggests that they may be important mechanical carriers (7). Infection is thought to be quite common in backyard flocks of various types of poultry.

Clinical signs and lesions

Clinical signs include coughing, sneezing, nasal discharge, conjunctivitis, sinusitis in turkeys, poor appetite, and poor growth, with slightly increased mortality. Infection in adult chickens is often subclinical, but in turkeys the infection is almost always clinical. Lesions include sinusitis, rhinitis, conjunctivitis, tracheitis and airsacculitis, and salpingitis (26). Initial histologic lesions are characterized by the presence of surface exudate, edema, fibrin exudation, and heterophilic and lymphocytic cell infiltration. Air sacs may be as much as 8- to 10-fold thicker than normal. Multiple foci of epithelial cell hypertrophy, degeneration, and necrosis probably represent sites of attachment and colonization by mycoplasma organisms. The early epithelial cell changes are followed by hyperplasia. With time post-infection, lymphocytes, macrophages, and plasma cells diffusely infiltrate the connective tissue. Nodular lymphoid cell foci are more common in older lesions. End-stage lesions consist frequently of scattered lymphoid nodules in the increased fibrous connective tissue.

A hallmark of MG infections is the ability to interact synergistically with other respiratory disease agents (including viral vaccines) and *E. coli*, along with poor environmental conditions such as dust, crowding, and/or chilling. Therefore progeny of infected breeding flocks can be expected to have significant respiratory disease, often progressing to colibacillosis with high mortality. Antibiotic treatment may be effective in controlling clinical signs and lesions, but will not eliminate the infection. Antibiotics are most effective when used prophylactically. In areas of the world where aggressive vaccination programs against Newcastle disease is necessary, or in the presence of viral agents such as avian influenza, MG infection is incompatible with profitable poultry production.

Transmission

Transovarian transmission to the offspring occurs in MG infected breeding flocks. There is no economical, practical method of ensuring that this will not occur. This is the primary reason that infected breeders are destroyed. However, it is believed that horizontal transmission occurs primarily by indirect transmission – by the movement of contaminated people, wildlife, supplies, or equipment moving between flocks. Mycoplasma organisms persist in the environment long enough to ensure that this can

occur (7), especially on materials such as cotton clothing or feathers. Aerosol transmission may be possible over short distances. The probability of aerosol transmission depends on distance, environmental conditions and the numbers of organisms present in the respiratory tract available for shedding. Although this has never been scientifically documented for MG, epidemiological studies with *Mycoplasma hyopneumoniae* infection of swine have clearly demonstrated that the greatest risk factor for infection is the proximity of infected swine herds, up to a distance of 2 km (15, 40). Aerosol transmission may also be possible with MG infection in poultry, and may be a significant risk factor when there are large poultry populations in close proximity to each other.

Diagnosis

The basis for control programs has centered around serological methods such as agglutination and hemagglutination-inhibition, with reactors often confirmed by isolation of the organism. More recently, commercial ELISA kits have become available (IDEXX Laboratories, Westbrook, Maine, USA; Synbiotics, San Diego, California, USA) and are becoming widely used. Such kits have excellent sensitivity and specificity, but non-specific reactions may still occur. Improvements in ELISA specificity may result from the utilization of highly purified antigens or the use of a blocking ELISA utilizing a specific monoclonal antibody.

MG strains of low virulence typically produce a poor antibody response, and isolation from clinical specimens may be difficult (4). This may be especially true if the antigenic makeup of the MG strain involved is not a good match with the strains used to produce test antigens.

Polymerase chain reaction (PCR) represents a rapid and sensitive alternative to traditional culture methods, which require specialized media and reagents and are time consuming. At least one company (IDEXX Laboratories, Westbrook, Maine, USA) produces commercial PCR kits. Although several PCR procedures have been developed, the method of Dr. Lauerman at Auburn University (25) is widely used. More recently, PCR procedures based on the *mgc2* gene for MG (10, 18) have gained in popularity. Several different PCR methods for the detection of MG have been recently compared (13).

Improvements in serological methods and rapid detection by PCR have done much to facilitate the rapid and accurate diagnosis of MG infection.

Control

Efforts in the United States to control MG began in the 1960's, primarily as a response to high condemnations from airsacculitis after the initiation of USDA post mortem inspection of poultry. Since then, significant progress has been made in controlling Mycoplasma infections in turkey and chicken breeding stocks. Voluntary MG control programs in the U. S. are administered under the National Poultry Improvement Plan; testing provisions and protocols are provided in their official publication (1). M. *gallisepticum* is currently an OIE notifiable disease.

There have been changes that have resulted in an evolving situation in MG control, both in the United States and world-wide. These include changes in the poultry

industry itself, improved detection methods, better understanding of the agent and its pathogenesis, and improved control methods.

In most modern poultry producing areas of the world, the emphasis on control of Mycoplasma infections has been centered around maintenance of Mycoplasma-free breeding stock and keeping parent and production flocks free of infection by utilizing single-age, all-in all-out farms with good biosecurity. In many parts of the world, this has been very successful, and the majority of broiler, turkey and egg production is free of infection. In contrast, areas with less-developed poultry industries tend to have high levels of infection with MG; this poses special problems for companies attempting to institute modern production methods.

With the rapid growth of poultry production world-wide, there has been concentration of large numbers of birds into small geographic areas, leading to increased risk of exposure to pathogenic Mycoplasmas. In some areas, poultry production is so concentrated that from an epidemiological point of view, it is almost like a very large multi-age farm. Also, general improvements in disease control have sometimes resulted in decreased efforts in biosecurity, thus enhancing the possibilities for the spread of Mycoplasma infections (19).

There has been a tendency to shift away from all-in all-out production and to concentrate production on multi-age sites. This has been especially true for commercial egg production – the majority of egg production in the U.S. is now on multi-age sites, and this trend is developing around the world. Many of such multi-age production sites are MG positive, even though grandparent and parent stocks are generally MG-free.

In many locations, multi-age management of broiler breeders or broilers may occur. In turkey production, multi-stage production farms, on which 2 or even 3 different ages are maintained, are becoming quite common.

Therefore, in spite of sometimes heroic efforts at biosecurity and improved understanding of the survival of Mycoplasmas outside the host, Mycoplasma outbreaks continue to occur.

M. gallisepticum vaccination

With the advent of multi-age commercial layer complexes, control by vaccination became a consideration.

The first commercially available MG vaccines were oil-emulsion bacterins (16). Bacterins protect well against airsacculitis and egg production losses, but provide little protection against colonization by field strains of MG, thus providing little value in eradication programs. Major disadvantages of bacterins are the need for 2 doses for optimal protection and the cost of administration.

Live MG vaccines include F strain (5, 30), which has been available for some time through several manufacturers, strain 6/85 from Intervet America, Millsboro, Delaware (8), and strain ts-11, developed and widely used in Australia and licensed in the U.S. by Merial Select, Gainesville, Georgia (43).

F strain exhibits low to moderate virulence in chickens, colonizes the upper respiratory tract efficiently, spreads relatively slowly from flock to flock, and offers protection against losses in egg production. In addition, progeny of heavy breeders vaccinated with F strain generally remain free of MG infection. It provides excellent protection against colonization by challenge strains (21), and displaces the wild-type field