Chapter 1
Principles of Disease Prevention, Diagnosis, and Control

Introduction
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Poultry product prices have not kept pace with rapidly escalating input costs, causing profit margins to diminish throughout the poultry industries. Producers are faced with the challenge of consistently achieving expected performance while satisfying stringent food safety and animal welfare requirements; they can no longer tolerate even the slightest deviation from expected performance.

In an effort to cost-effectively meet demand, producers have had to increase the size and throughput of their production systems. These large close confinement rearing systems, designed to improve economies of scale and maximize productivity by optimizing bird comfort, unfortunately also increase the risk of disease challenge. The physiological stress of keeping pace with genetic potential for production makes birds more vulnerable to disease challenge, and the close proximity of susceptible hosts increases the chance and rate of infectious disease spread. Diseases previously recognized as unimportant, because they have been adequately controlled, have now reemerged as significant concerns. Most of today's disease challenges are not new problems; rather, they have merely expanded their geographic distribution or reemerged primarily because of management techniques and production system design constraints.

Methods of disease control have evolved with intensification of the industry. While initially focused on diseases of catastrophic nature, attention has rapidly shifted from defined, clinical disease at the individual house or farm level to less well-defined subclinical disease and bird welfare. Similarly, cost justification in decisions on whether or not to implement prevention or control measures has become more complex, requiring the aid of formal economic appraisal.

To maximize flock efficiency, disease challenge management requires a carefully designed, multi-tiered approach, which includes consideration of elements ranging from flock health and productivity to chick or poult viability. In addition, the term “efficiency” implies the all-important economic component. Because the production system is profit driven, decisions regarding disease prevention can rarely be made based solely on biological grounds. Disease management intervention also requires sound economic justification, which begins with clearly defining the financial risk associated with disease.

Unless a disease poses a specific risk to human health or animal welfare, its mere presence in a flock may not be significant from a business perspective. It is often difficult for the veterinarian, trained in disease prevention, diagnosis, and control, to appreciate that the presence of a disease in a flock could be considered superfluous. Unless it is economically advantageous to take action against a disease challenge, its presence in a flock is tolerated. Intervention strategies are consequently chosen based on both their economic and biological efficiency. This process requires a dynamic, integrated combination of an epidemiologic and economic analysis to determine and quantify the production effect of the disease challenge as well as a proposed intervention strategy. Such integrated analysis has become far more significant in today's intensive production systems because the outcome of disease challenge is so markedly influenced by the environmental conditions.

The economic impact of disease is difficult to assess. This is particularly so in a production system in which the economic return is governed not only by flock productivity but also by product quality and viability. In addition, the consequential loss from disease challenge will be, at best, only partially recoverable. Using the cost of disease to justify intervention over-emphasizes the consequence of inaction and it is only useful in justifying intervention strategies directed at disease prevention. As the process of economic analysis has evolved, the focus has shifted from the cost of disease to the benefit derived from disease control strategies.
Bird performance continues to increase linearly because of efficient breeding and selection programs, but each increment in improvement is 1 step closer to the point of physiological limit and diminishing returns. Production systems need to be modified to better satisfy bird requirements, and disease prevention, diagnosis, and control strategies changed to preclude physiological, nutritional, and agent-induced pathologies from affecting performance.

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Flock Health

Disease is the antithesis of health but neither state is easy to define in production animals. Health is defined in the human individual as a state of physical, mental, and spiritual well-being. It is impossible to apply this definition to an animal, and production animals have in the past been classified as healthy if they were free from clinical disease and performing to standard. Although individual animals are frequently described as healthy or diseased, these terms are not mutually exclusive. The impact of disease challenge on productivity is apparent long before clinical signs of disease appear. Production animals are expected to perform at their genetic potential, and to achieve this they need to be physically and mentally well or stress free.

Stress has been defined as a nonspecific response of the body to any demand made upon it. From a physiological point of view this can be restated as the metabolic response of the body to external factors that impact well-being (34). Stress is cumulative and only measurably impacts performance once the aggregate of each individual stress exceeds the host's coping mechanisms. An interesting study by Klasing et al. (52) has shown that the degree to which an adverse stimulus or stress will negatively impact bird performance is directly proportional to the existing stress load. Any stress will impact productivity once the stress threshold is surpassed. In a production system in which animals are expected to produce at genetic potential, the definition of health needs to be expanded to freedom from disease or stress. In this context, health is proportional to the difference between stress level and stress threshold.

Disease prevention and control strategies tend to be too focused on addressing the precipitating cause, and too little attention is given to the predisposing causes of disease. The traditional paradigm of disease has been shaped by the study of specific diseases in individual animals and tends to overemphasize the importance of the infectious agent. In intensive animal agriculture it is the influence of environmental disease determinants that decides the economic outcome of infectious agent challenge. The focus of flock health management has consequently shifted. Initially aimed at avoiding mortality because of an inadequate immune response, health management is now directed at avoiding an exaggerated or inappropriate immune response because it may depress productivity. The task of the veterinarian has shifted from the prevention, diagnosis, and control of specific disease conditions in the individual bird to preventing and limiting the consequence of more complex multifactorial disease outcomes to maximize the productivity of the flock.

Resistance and Resilience

An animal’s resistance to disease can be defined as its capacity to prevent an overwhelming infection by a disease-causing organism. Disease resistance is determined by immune competence and health status at the time of challenge. Because stress negatively impacts health it also negatively impacts resistance. Ironically, the process of mounting an effective immune response is itself a source of stress because of the demands made on the immune system and the consequence of the resulting fever response. An immune response that is adequate to contain disease can be considered as the cost of health. There is a delicate balance between too little and too much because an inappropriate immune response, whether inadequate or excessive, will depress performance.

The resilience of an animal is a measure of its capacity to continue to perform while preventing a disease challenge from causing an overwhelming infection. As with resistance, resilience is negatively impacted by poor health but in this case the negative impact of the resulting stress is more significant. The chemical messengers (cytokines) released in response to a disease challenge depress production directly by influencing metabolism and indirectly by suppressing appetite and feed intake (52). While immune response is crucial to maintaining health, its consequence is depressed productivity.

The skin and respiratory, urogenital, and gastrointestinal tracts form the interface between foreign (antigenic) material and animal cells (self). To protect the bird from disease the immune system has to develop exquisite sensitivity as to whether foreign antigens are friend (nutrients or normal flora)
or foe (pathogenic). An inappropriate immune response to gastrointestinal antigens will, for example, have a negative impact on feed efficiency. The fever response induced by foreign antigens will depress feed intake, while the inflammatory response damages the gut lining, thus reducing the nutrients available for production. The capacity of an animal to fight off a disease challenge while avoiding the negative impact of the induced immune response on productivity (resilience) depends on how close the prevailing level of stress is to the bird's stress threshold. The success of any health program thus hinges on balancing immunity and health to maximize resilience. There is a dynamic interface between nutrition, immunity, and productivity. The aim of any production veterinarian should be to optimize feed utilization by modulating the immune response: enhancing the protective response to prevent clinical disease while simultaneously suppressing the acute phase or fever response.

Population Dynamics
Like human medicine, traditional veterinary medicine is focused on the study of the disease process in individuals. In modern flock medicine, where the emphasis is on prevention, diagnosis, and control of disease in finite and confined populations, the focus shifts to the epidemiology of the disease. Flock health is a rather nebulous term. Because health and disease are not mutually exclusive, individual birds within the flock will at any point in time be in various stages of health/disease (Poisson distribution). At what point is a flock diseased or healthy? Productivity gives a good estimate of an individual's state of well-being and welfare. Similarly, a flock that is performing to standard is assumed to be healthy, based on the fact that the birds act and produce as an equivalent nonstressed sibling would in a laboratory situation. This approach unfortunately takes little cognizance of the flock variance, because flock performance indicators are based on flock averages. Population variance or range is a much better indicator of flock health.

Modern population-based health management requires the complex integration of animal husbandry (housing environment), medical management (host and agent factors), and epidemiological practice (analysis of causal relationships). Modern risk management approaches facilitate the transformation of epidemiologically derived statistical data into a population health management tool. The probability of exposure to health risk factors and their expected outcomes are used first, to guide the response to a disease challenge, and second to improve the health program design/management to better respond to future potential population health challenges.

Each component of the production process can be evaluated on the basis of cost and contribution. In the past, intensive agriculture has been production driven, and contribution measured in terms of performance. In today's market-driven enterprise, value is regarded as a function of quality, yield, and cost of production with the emphasis shifting from performance to profit through the chain of realization. In this scenario the simplest strategy for improving productivity is to reduce within- and between-flock variance. By reducing variability and thus eliminating the extremes, it is possible to improve the quality, speed, and cost of production. Improved uniformity translates to improved productivity and hence profitability. Health (the difference between stress level and stress threshold) is probably the single most important determinant of flock uniformity. Within a group of animals the threshold and level of stress experienced by each individual will vary. The relative efficiency of a production manager to minimize in-house environmental variation, and therefore host-, agent-, and environment-dependent stress, is reflected in flock uniformity.

Challenges of Disease Prevention, Diagnosis, and Control in Modern Poultry Production

Because the goal of a poultry operation is to convert feed into food as economically as possible, it is critical to manage both the risk and consequence of disease challenge. While the biological efficiency of feed conversion is governed primarily by intrinsic or genetic determinants, in an intensive production system it is the extrinsic disease determinants that ultimately decide the efficiency of the operation in both biological and financial terms. Capital investment in the housing's environmental control capability, and the effective operation of these controls, is fundamental to economic success. Even subtle disease challenge such as vaccination with live respiratory disease vaccines can compromise efficiency if exacerbated by environmental disease determinants.

Viral diseases are challenging to control because there are no effective treatment options, while bacterial, protozoal, and parasitic diseases present a challenge because the treatment options are either no longer available or no longer effective. The approach to controlling diseases within these 2 categories is very different.

The molecular structure of a virus particle is relatively simple, making immunological recognition very acute and the control of known viral diseases possible through immunization. Provided the immune system has been primed by vaccination, immunological protection against viral disease challenge is usually highly successful. Emerging and reemerging viral diseases arise when novel or immunologically distinct viruses are introduced into naïve populations (48). In the absence of prior exposure, immune recognition and activation is delayed and the extent of the primary immune response is frequently inadequate to prevent clinical disease (45). Under such conditions virus replication and spread occurs rapidly with potentially devastating consequences (22). While the majority of emerging viral diseases in humans are the result of exposure to novel viruses, it is the emergence of variant strains that pose the biggest threat to the poultry industry (88). Although controlled environment housing and good biosecurity practices have been highly effective in preventing
In contrast to viruses, bacteria and protozoa are structurally and immunologically complex, making protection through vaccination much less successful. Although a great deal of research effort is and has been focused on developing effective immunization strategies for these diseases, antibiotics have remained the primary means of control (24)—a point well illustrated by the escalating difficulties experienced in the European Union with the current systematic withdrawal of in-feed antibiotics (33). It is no coincidence that the downward trend in prophylactic (in-feed) antibiotic usage has been matched by an increase in therapeutic use (58). Many expert committees blame the use of in-feed antibiotics in food animal agriculture for the proliferation of antibiotic-resistant strains of bacteria and for the increase in prevalence of antibiotic-resistant infections in humans (47). This is undoubtedly providing the impetus to ban in-feed antibiotic use, even though a link to increased antibiotic-resistant bacterial disease in humans has not been conclusively established (29).

Consumer pressure to remove antibiotics from the food animal nutritionist’s arsenal is, however, winning the battle and the trend toward reemergence of previously controlled bacterial and protozoal diseases will likely continue. The industry must adapt to remain competitive.

The Principles

Disease prevention and control involves the 3 interrelated processes of bioexclusion, surveillance, and biocontainment. Disease prevention is difficult, expensive, and requires total commitment because it invariably involves eradication. Eradication programs are appropriate when the economic consequence of the disease is so devastating that it is economically advantageous to implement such drastic control measures. It is only feasible if there is an effective means of detecting infection, containing the infection through cleanout and disinfection, and preventing dissemination of the disease causing agent (75). There are 3 categories of disease for which eradication is an appropriate means of control: those that significantly threaten public health, those that have a devastating effect on bird performance, and those that severely compromise product quality. With diseases of this nature, the control effort is focused on the complete elimination of the agent from the environment (75). This places the emphasis on preventing contact between the agent and the host (bioexclusion). Early diagnosis and containment is in this case the contingency plan for failure in bioexclusion.

In contrast to eradication, control programs are aimed at limiting disease challenge to a tolerable level. There is a subtle shift in emphasis from prevention, through bioexclusion, early detection, and elimination, to reducing the consequence or economic impact of the disease, i.e. damage control. Although monitoring and surveillance are still used to gather prevalence data, the primary focus is to measure the level of protection and challenge, not the mere presence of the disease. The principles of prevention through biosecurity still apply, but in a disease control program the focus shifts to limiting the extent and consequence of exposure. In reality, many of the biosecurity measures taken to eradicate the more devastating diseases provide a solid foundation for the control of the erosive diseases, and immunization is usually used to bolster host resistance.

Methods of disease management have evolved with intensification of the industry. While initially focused on diseases of catastrophic nature, focus has rapidly shifted from defined, clinical disease at the individual house or farm level to less well-defined sub-clinical disease and bird well-being or welfare. Similarly, cost justification in decisions on whether or not to implement control measures has become more complex, requiring the aid of formal economic appraisal.

The presence of disease in a flock only becomes significant when the functional derangement of normal metabolic and homeostatic processes causes a decline in productivity that is sufficiently large to affect economic efficiency. This occurs either as a result of disease-induced anorexia or through specific effects on the physiological processes of nutrient metabolism, respiration, and excretion. Only in severe cases does disease cause mortality, and yet mortality rate is used universally as a measure of flock health status. Health and disease are not mutually exclusive, yet a flock is assumed to be healthy when it is performing to standard and is free of clinical disease. Productivity provides a much more sensitive measure of flock health, but because it is the composite output of a population it gives no indication of the variance in health status between individuals within the flock. Within-flock variance provides the most sensitive measure of flock health status but flock uniformity is still a fairly coarse measure of health status.

Disease challenge management must be considered to be an integral part of any poultry business risk management program. It involves the development and implementation of a stringent biosecurity plan which comprises a hierarchy of components directed at preventing or limiting the risk and consequence of disease. Economic analysis is a critical step in biosecurity plan design because resource allocation must match risk (10). Although it is difficult to accurately determine the precise risk and consequence of a disease challenge, it is possible to rank disease challenge according to relative risk (39). From a risk analysis point of view, emerging diseases can be considered in 2 broad categories, those that are unlikely to occur but are catastrophic in nature and those that are highly likely to occur but have less of a financial impact.

Disease prevention, tantamount to eradication, is an appropriate means of risk management for diseases that have a devastating effect on profitability through their impact on productivity or sales (75). In contrast, disease control is aimed at keeping the prevalence and economic impact of disease at a tolerable level. There is a subtle shift in emphasis from prevention through early detection and elimination to limiting the
consequence or economic impact of the disease, i.e. damage control.

No disease control or prevention/eradication program would be successful without diligent diagnostic surveillance. The objective of the data collection system is to provide incisive and epidemiologically informative indicators which will permit objective judgment and decision making. To support an eradication program, surveillance must be sufficiently intense to detect the source case of an outbreak so that biocontainment through quarantine and slaughter can be carried out before disease spread occurs. The difficulty lies in confident early detection because this requires frequently testing a large sample of the population. The heavy economic burden of such intense surveillance is difficult to carry and frequently biases risk management decisions, especially when the probability of a disease outbreak is low. Potentially devastating diseases such as high pathogenicity avian influenza (HPAI) can be effectively eradicated provided adequately robust bioexclusion, surveillance, and biocontainment programs are in place. The fact that this disease has public health connotations has helped to justify sufficient commitment to surveillance, the linchpin between bioexclusion and biocontainment.

For disease control purposes, a surveillance program is aimed at identifying when disease prevalence changes are sufficient to initiate corrective action. The difficulty is in distinguishing common cause (background variation) from special cause (a disease effect). Surveillance for the purpose of disease control, or more appropriately flock health management, remains an art. There are no specific tests that can be carried out to determine the health status of a flock, thus placing the emphasis/burden on skilful clinical assessment. Flock health monitoring systems involve a combination of clinical observation and necropsy findings. The sample size and frequency constraints of these procedures severely limit sensitivity, thus emphasizing the need for careful sample selection and attention to detail. The focus should be on identifying and eliminating subtle disease challenge because even a mildly exaggerated or inappropriate immune response will compromise performance.

In contrast to respiratory disease, where early signs of disease are outwardly apparent and relatively easy to detect, low-grade gastrointestinal disease is much more insidious. Breeding and selection for performance has down-regulated the clinical signs of intestinal disease: birds continue to eat and drink at normal levels even when gastrointestinal disease is quite advanced. Early changes in intestinal absorptive capacity, normally indicated by litter moisture changes because of compromised water balance, are often masked by litter buffering capacity and good ventilation. Similarly, accelerated cellular sloughing usually indicated by the presence of orange mucus in the feces is to a degree masked by high feed through-flow rates.

**Biosecurity**

In poultry production, biosecurity includes all procedures implemented to reduce the risks and consequence of introducing an infectious disease into a flock. These preventive measures, which are based on applied microbiology and epidemiology, must be practical, enforceable, and cost effective and thereby form an integral part of the production system. Because the implementation of biosecurity carries a cost, it is necessary to relate this cost to the risk and consequence of infectious disease. Unfortunately there is no way of accurately defining the relative risk and financial consequence of disease exposure or, for that matter, the effectiveness of preventive measures. Clearly, the development of a cost-effective biosecurity system must entail a calculated estimate of these parameters.

A comprehensive biosecurity program comprises a hierarchy of conceptual, structural, and operational components directed at preventing infectious disease transmission from bird to bird, house to house, site to site, complex to complex, operation to operation, region to region, company to company, or country to country.

Every event in the production process that involves movement across the house/site/farm/complex boundary creates risk of contact between an infectious organism and the host. Avoidance is the best form of prevention. Where the event is unavoidable, biosecurity measures need to be implemented to alleviate risk. This can be achieved by reducing the frequency of the transgression or the probability of the event resulting in colonization or infection.

**Conceptual Biosecurity**

Conceptual biosecurity is the primary level of biosecurity and involves the location of a poultry operation and its various components. Physical isolation is the most effective means of limiting disease risk and should therefore be the primary consideration in establishing a new complex or farm. This physical separation will limit the use of common vehicles and facilities, preclude visitation of personnel not directly involved with the operation, and reduce the possibility of indirect spread of disease by vermin, wild birds, or wind. Farms should not be located adjacent to a public road, especially in an area that has a high density of poultry.

**Structural Biosecurity**

The second level of biosecurity includes farm layout, perimeter fencing, drainage, change rooms, and housing design. Long-range planning and programming of the operation, whether large or small, is very important and should consider movement patterns of various vehicles and equipment, work traffic of regular and holiday caretakers and special work crews, feed delivery and storage, and the system for moving eggs and flocks from the farm. An avian pathologist can be helpful in avoiding some common pitfalls, but to avoid high-risk disease situations, consultation should be done when the farm is being designed and the production programmed, rather than after it is developed and serious trouble is evident.

**Procedural Biosecurity**

The third level of biosecurity comprises implementation and control of routine procedures intended to prevent the introduction (bioexclusion) and spread (biocontainment) of infection.
within a complex or enterprise. These activities can be adjusted on short notice to respond to disease emergencies, and constant review of these procedures is necessary.

**Risk**

The success of a disease control program hinges on the ability to identify and then address the risk of infection. Disease risk in a flock situation is characterized by the probability of point infection and subsequent spread occurring. Aggregate risk is the sum of the individual risk of adverse health effects in an exposed population. The spread and consequence of point infection is influenced by several factors referred to as disease determinants.

**Disease Determinants**

An infectious disease is the result of a complex interaction between several factors. Any factor that influences the risk and consequence of disease challenge is thus a disease determinant. Disease determinants have traditionally been classified as primary or secondary; intrinsic or extrinsic; and host, agent, or environment associated. The latter best describes infectious disease in intensive poultry production units. In an intensive poultry production system the house environment, agent, and host determinants are largely under the control of the manager. The management thus becomes the most important disease determinant influencer.

**Risk Assessment**

Risk assessment involves determining the probability of exposure to an infectious agent, the probability of that exposure resulting in infection and spread of the disease, and the consequence of the disease outbreak.

While the chi-squared test can be used to test statistically whether a specific factor or process is a disease risk (correlated with disease), it gives no indication of the extent of the risk. The strength of association or degree of risk is determined by the ratio of association (51).

\[
\text{Risk Ratio} = \frac{P_e(D | E)}{P_e(D | \neg E)} = \frac{\text{the probability of disease given exposure}}{\text{the probability of disease given no exposure}}
\]

For disease control purposes it is appropriate to evaluate each part of the production process in terms of the probability or chance of the process or event causing infection, and the frequency with which that event occurs.

**Risk of infection = probability of the event causing infection × frequency of the event.**

Limiting the frequency of an event that carries any form of health risk is the obvious first step in any flock health program. Establishing the degree of risk requires further analysis. The probability of infection occurring after exposure is influenced by the resistance of the host and the challenge dose and virulence of the organism.

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\text{Risk of infection} = \frac{\text{challenge dose} \times \text{agent virulence} \times \text{challenge frequency}}{\text{host resistance}}
\]

The probability of infection occurring can thus be reduced by improving host resistance through immunization, reducing the challenge dose through cleaning and disinfection, or reducing organism virulence by medication or competitive exclusion.

**Host Resistance**

Bird resistance to disease challenge is primarily governed by the efficiency of its immune response. An appropriate immune response, adequate to contain infectious disease and minimize its impact on productivity, is the cost of health. An inappropriate (excessive or inadequate) immune response will depress performance unnecessarily. Inherent resistance to disease challenge varies among individuals, and baseline variance is due primarily to genetic differences and thus invariably demonstrates normal (Poisson) distribution within a flock.

Immune suppression as a result of stress, nonspecific disease challenge, or disease of the immune system will reduce both individual immunity and flock immunity. Because the impact of individual stressors is cumulative, the “poor doers” in the flock will be more adversely affected by stress or disease challenge when compared with the best birds in the flock. The distribution of resistance within stressed flocks thus becomes skewed to the right and flock immunity drops dramatically because of the presence of highly susceptible individuals within the population.

**Disease Challenge (Dose × Virulence × Frequency)**

The outcome of an event that causes a disease challenge is influenced by host resistance, challenge dose, and agent virulence. In this case challenge dose is the number of organisms that an individual bird is exposed to and agent virulence is the inherent capability of the agent to infect the host (infectivity) and cause disease (pathogenicity). Because the challenge dose required to cause disease in an individual varies, the infective dose 50 (ID\(_{50}\)) is traditionally used as an estimate of agent virulence. ID\(_{50}\) is the challenge dose required to infect 50% of the birds in a specific population. Although the ID\(_{50}\) helps in estimating the risk of exposure for the average bird in a flock, it is in fact the challenge dose required to infect the least resistant bird in the flock that is important when designing a flock health program. A chain is only as strong as its weakest link. Once 1 bird in a flock becomes infected or diseased, the process of agent replication increases the challenge of exposure (dose and possibly agent virulence) for other birds in the flock. The level of challenge escalates with each infection until even the most resistant birds in the flock are at risk.

**Epidemiology**

Epidemiology is the unbiased study of the interrelationships between the various factors (disease determinants) that affect the frequency and distribution of disease in a population. Because the prevalence and consequence of any infectious disease involves a complex interaction between several disease determinants it is critical to have a thorough understanding of
epidemiology (causal relationships between exposures and outcomes) to design an effective flock health or biosecurity program.

For flock health management purposes, each disease must be analyzed in terms of its relative risk first to determine whether it is necessary to implement control procedures and second in terms of its epidemiological characteristics to ensure optimum resource allocation. The important epidemiological characteristics for disease control purposes include:

- Source of infection. Although an infected bird is the obvious source of infection, the shedding pattern, host range, mode of transmission and farming practices will vary and ultimately determine the relative importance of a particular source.
- Transmission. While within-flock spread might be the result of direct bird-to-bird contact, indirect contact through contaminated objects (fomites) can accelerate the rate of transmission within a flock and increase the extent of transmission to other noncontact birds/flocks. This type of transmission is commonly referred to as horizontal or lateral transmission. This is in contrast to vertical transmission in which the disease agent is transmitted from parent to offspring. While vertical transmission may occur as a result of eggshell contamination, some disease-causing agents are able to reside inside the egg or embryo and spread by transovarial transmission.
- Spread. The incubation period, replication rate, resilience, and virulence of the disease agent will determine the course of the disease within an individual (acute, subacute, or chronic) and the spread of the disease within a flock (defined population). An acute disease caused by a resilient organism with a short incubation period and high replication/shed rate will, for example, spread very rapidly in a susceptible flock.
- Susceptible host. The host range of a disease agent (species, breed, type) is important in control program design. The proximity of species that are not susceptible is irrelevant to control.
- Predisposition. Several host, agent, and environmental disease determinants can enhance the detrimental outcome of exposure to a disease-causing agent. It is necessary to know the causal relationships between exposures and outcomes to design an effective flock health program. For example, any environmental stress could compromise the immune system and predispose to infection. Similarly, host factors such as breed, sex, size, and age, and agent factors such as concomitant infection with different organisms or immune suppressive disease can predispose birds to infection.
- Prevalence. The prevalence of a disease is directly proportional to the risk of challenge. Endemic diseases (those that are always present in the area under consideration) are difficult to prevent, whereas those that are exotic (do not occur in the area under consideration) or occur sporadically as an epidemic are easier to contain and eradicate through surveillance and biocontainment.
- Morbidity. Morbidity describes the number of birds in a flock that show clinical signs of disease at a point in time (specific) or at the peak of the epidemiological curve (general) and is usually expressed as a percentage. The morbidity rate will be high in rapidly spreading diseases and low in diseases that spread slowly.
- Mortality. The percentage of birds, in a finite population, that are expected to die during a particular disease outbreak.
- Recovery. The course of a disease is influenced by a multitude of factors (disease determinants). Epidemiological statistics on the expected outcome of a disease outbreak aid in determining the best course of action for limiting the current and future financial risk of that particular disease.

Disease Prevention: Bioexclusion

Preventing or reducing disease challenge requires a systematic approach to eliminating or decreasing the number of disease-causing organisms within the bird’s environment. This is achieved through the implementation of cost-effective procedures to prevent pathogen movement across physical or imaginary barriers demarcating protection zones around the bird. The establishment of zone boundaries should be based on sound epidemiological principles while making use of existing physical and geographical barriers.

Global Perspective: Top Down

The poultry industry has become a global industry. Poultry and poultry products are shipped internationally on a daily basis. Whereas the World Trade Organization (WTO) is focused on ensuring free trade, the World Organization for Animal Health is the guardian of flock health. It is important to understand the workings of this organization because it pertains to disease prevention, diagnosis, and control. In order to trade internationally in poultry and poultry products, control measures implemented at the farm level must ultimately comply with the organization’s stipulated requirements.

The World Organization for Animal Health is an intergovernmental organization responsible for improving animal health worldwide. This organization was initially called the Office International des Epizooties (OIE). Despite the name change in 2003 the OIE acronym remains in use. It is recognized as a reference organization by the WTO and currently represents 178 member countries, including the United States. It is led by the World Assembly of Delegates, consisting of representatives from each member country.

The reader is referred to the official OIE Website for details of the rules and regulations as laid out in the OIE Terrestrial Animal Health Code (Terrestrial Code). In summary the objectives of the OIE are to: (1) ensure transparency in the global animal disease situation by reporting detected disease, (2) collect, analyze, and disseminate veterinary scientific information on animal disease control, (3) encourage international solidarity in the control of animal diseases by providing technical support to member countries requesting assistance with animal disease control and eradication operations, including diseases transmissible to humans, (4) safeguard
world trade by publishing health standards for international trade in animals and animal products that member countries can use to protect themselves from the introduction of diseases and pathogens, without setting up unjustified sanitary barriers, (5) improve the legal framework and resources of national veterinary services, and (6) provide a better guarantee of food of animal origin and promote animal welfare through a science-based approach. The OIE works in conjunction with the Codex Alimentarius Commission to improve the safety of food of animal origin and is viewed as the leading international organization for animal welfare.

Country Perspective: Responsible Trade Through Risk Reduction and Disease Containment

The movement of animals or animal products across country borders carries a risk of disease spread. The OIE plays an important role in establishing the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) worldwide. This agreement of the WTO provides definitions and describes the OIE in-house procedure for settlement of disputes. It also provides guidelines and principles for conducting transparent, objective, and defensible risk analyses for international trade. The principal aim of import risk analysis is to provide importing countries with an objective and defensible method of assessing the disease risks associated with the importation of animals, animal products, animal genetic material, feedstuffs, biological products, and pathological material.

Risk analysis involves identification and estimation of the risk and may be qualitative or quantitative. Qualitative assessment is usually adequate in the case of OIE-listed diseases because the risk is well recognized and there are well developed internationally agreed-upon standards.

OIE-Listed Diseases

Diseases are included on the OIE list based on international prevalence and capacity for spread, resultant morbidity and mortality, zoonotic potential, and emergent properties. The details of the criteria and decision process are outlined in Chapter 2.1.1 in the Terrestrial Animal Health Code (5). The following avian diseases are included in the OIE list (7): avian chlamydiosis, avian infectious bronchitis, avian mycoplasmosis (Mycoplasma gallisepticum), avian mycoplasmosis (M. synoviae), duck virus hepatitis, Eastern equine encephalomyelitis, Fowl cholera, Fowl typhoid, highly pathogenic avian influenza and low pathogenic avian influenza in poultry as per Chapter 10.4. of the Terrestrial Animal Health Code, infectious bursal disease (Gumboro disease), Marek’s disease, Newcastle disease (ND), pullorum disease, Q fever, tularemia, turkey rhinotracheitis, and West Nile fever.

Region or State Perspective: Zoning and Compartmentalization

The Terrestrial Code makes allowances for zoning and compartmentalization to address the difficulties in controlling the disease status and management practices of poultry flocks across the vast expanse of large countries like the United States (8). By defining subpopulations based on flock health status, member countries are able to limit the damaging effect of a listed disease outbreak on international trade without exposing the importing country to the risk of disease spread. Compartmentalization applies to a subpopulation separated by biosecurity procedures, while zoning applies to a subpopulation separated on a geographical basis. The details of the requirements to establish these subpopulations vary according to the disease in question and the requirements of the trading partners. These details are ideally decided on prior to the disease outbreak. Of particular interest are the epidemiology of the disease, environmental factors, applicable biosecurity measures (including movement controls, use of natural and artificial boundaries, commercial management, and husbandry practices), and surveillance and monitoring. To establish a zone or compartment within its territory for international trade purposes, the veterinary services of an exporting country should clearly define the subpopulation as stipulated in the Terrestrial Code. These claims must be communicated to the veterinary services of an importing country and supported by detailed documentation published through official channels.

Because the borders of a zone are based on natural, artificial, or legal boundaries, they can be established relatively easily and made public by the veterinary services through official channels. Compartments are a little more difficult to define in that they must be established based on biosecurity procedures. This involves developing a partnership between the company and the veterinary authority to develop clearly stipulated responsibilities. To meet the requirements for a compartment, the biosecurity plan, operating procedures, and management practices must be adequate and documented and evidence of compliance documented.

The plan must adequately demonstrate robust disease surveillance, animal identification, and traceability. This requires that detailed records are kept on bird movement, flock production, feed source, disease surveillance results, chick source, visitor’s log, flock morbidity and mortality, vaccination and medication, and personnel training. Risk mitigation also requires that the biosecurity plan is regularly audited, reviewed, and adjusted when necessary.

Company Perspective: Bottom Up

From a biosecurity perspective a company operates as a compartment within the country, state, or region. In order to trade internationally, companies must at the very least comply with international standards and in so doing meet the OIE requirements for biosecurity. Compartment, as defined by the Terrestrial Code, means an animal subpopulation contained in 1 or more establishment under a common biosecurity management system with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control, and biosecurity measures have been applied for the purpose of international trade (8). The implication of this is that in order to trade internationally each company, region, state, or country should classify OIE-listed diseases according
Mycoplasma gallisepticum

Viral diseases: high pathogenicity avian influenza (HPAI), low pathogenicity avian influenza (LPAI) of H5 and H7 types, velogenic viscerotopic Newcastle disease (vvND), west Nile fever, duck virus hepatitis, duck virus enteritis, eastern equine encephalomyelitis, tularemia, and Q fever.

Disease challenge reduction requires a systematic approach to decreasing the number of disease-causing organisms within the bird’s environment through the implementation of cost-effective procedures to prevent pathogen movement across physical or imaginary barriers demarcating protection zones around the bird. The establishment of zone boundaries should be based on sound epidemiological principles while making use of existing physical and geographical barriers. Biosecurity program design thus begins with the identification of critical control points or epidemiological unit boundaries at which bioexclusion practices can be implemented. For the purposes of disease control an epidemiological unit is a group of birds with a defined epidemiological relationship that shares approximately the same likelihood of exposure to a pathogen.

Primary Control Zone: Poultry House and Hatchery

Bioexclusion begins at the boundary of the smallest epidemiological unit within the company, in this instance the poultry house. The birds in the house form an epidemiological unit because they share a common environment and common management practices and have approximately the same likelihood of exposure to a pathogen. In addition the roof and walls of the house provide a well-defined barrier to entry and an ideal site for the implementation of critical control procedures. From a biosecurity standpoint every crossing of the house perimeter (event) should be considered as a potential means of pathogen transfer or disease risk.

Risk of infection = probability of the event causing infection \times frequency of the event

The process of risk reduction begins with an all-in-all-out placement strategy, so that decontamination of the house environment by thorough physical cleaning followed by chemical disinfection and/or “down time” is possible between successive placements. After placement the emphasis shifts first to limiting the frequency of any house perimeter crossing (event) and second to reducing the probability of pathogen transmission and infection if the event is unavoidable or essential.

The Poultry House

Management of the House Environment in Disease Prevention. While it is important to keep disease out, it is equally important to prevent the house environmental conditions from...
causing discomfort or stress. Traditional thinking, stimulated by widespread acceptance of Koch’s Postulates in the 1900s, over emphasizes the importance of infectious agents in the disease process. As production systems have evolved, environmental and host disease determinants have played a more obvious role in the disease process, emphasizing the multifactorial nature of disease. The prevalence of specific infectious disease entities has declined as knowledge and control measures have improved. In contrast, the predisposition to and prevalence of noninfectious disease has increased with intensification and genetic change. The distinction between infectious and noninfectious disease has become somewhat blurred in intensive agriculture, and a more fully encompassing epidemiological approach to disease diagnosis and control has become necessary.

The poultry house environment, with all its intricacies, is a crucial disease determinant because stress of any kind stimulates a cascade of physiological and biochemical changes which erodes host resistance and productivity (74). The negative impact that a particular stressor has on performance is directly proportional to the existing stress load, because stress is cumulative and measurably impacts animal performance once the aggregate of individual stresses exceeds coping mechanism capacity (52). Stress level, and hence competence to cope with additional stress, varies with each individual within a flock, thus emphasizing the need to consider the epidemiology of disease within a confined, finite population. Stress lowers the minimum dose of infective agent required for development of infection and increases the risk of infectious or noninfectious challenge developing into clinically detectable disease. The risk and consequence of infectious disease spread within the population is increased by the presence of stress, because susceptible individuals act as amplifiers for the infectious organisms, and thus increase the challenge dose to which the pen mates are exposed. While the introduction of a noninfectious disease to a flock may also lower individual resistance, there is no risk of spread (75). The influence of the house environment on viral disease of poultry has been reviewed by Anderson and Hanson (2).

**Turnaround Time and Down Time.** Turn-around time is the time lapse from the start of depletion/transfer to the start of subsequent placement. Down time, which is of greater significance, is the time between the removal of all poultry, poultry by-products, and litter to the start of the next placement. The process of bioexclusion is pointless if the production system does not start off disease or specific pathogen free (SPF). The risk of pathogen carry-over from one production cycle to the next is directly linked to the time interval between the removal of 1 flock and the subsequent placement of the next. Pathogen attrition occurs with time and the chance of pathogen carry-over from one grow-out cycle to the next is reduced by extending down time. The longer the bird-free period, the greater the reduction in disease challenge. In a low-challenge situation or if prevailing conditions preclude the removal of litter from the house, an extended turn-around time can be used to substitute for cleanout and disinfection.

In the United States, true cleanout and decontamination is seldom practiced at broiler level and extended turn-around times (minimum of 14 days) are commonplace. Decontamination of the house through cleanout and disinfection hastens the attrition rate of pathogens within the house environment and therefore serves to reduce the need for long down time. While the process of cleanout and disinfection carries a cost, it reduces turn-around time and hence improves return on investment. The decision as to whether to implement a cleanout and disinfection program or to reuse litter is complex and should involve a detailed analysis of the fixed vs. variable cost benefit, the level of disease challenge, the nature of the prevailing diseases, the type of housing, stocking densities, etc.

**Decontamination: Cleanout and Disinfection.** Cleanout is designed to reduce the risk of disease through the physical removal of all poultry, poultry by-product and litter, and the sequential washing, disinfection and possibly fumigation of all the houses. The relative importance of this process increases as the length of turn-around time diminishes. Decontamination is a sequential process which requires careful planning, execution, and control. As outlined in Chapter 5 of the American Association of Avian Pathologists (AAAP) publication *A Practical Guide for Managing Risk in Poultry Production*, decontamination involves 5 steps: removal of debris, detergent application, washing with water, drying, and disinfecting (60).

After depopulation, the litter or droppings should be removed. Once the bulk of the litter has been removed as much of the remaining solid material as possible should be brushed out of the house before the washing process begins. With development of huge specialized poultry farms, proper and economical disposal of litter and poultry manure has become a serious problem. There is no clear-cut answer. A general recommendation is to remove it far enough from the buildings so that insects will not crawl or fly back into the houses, and to dry it, compost it, or spread it onto fields and work it into the soil. If cleaning is done while chickens are still present (cages), remember that contracted personnel, trucks, and equipment may recently have been on another farm where a disease outbreak occurred.

In some cases, the nature of a pathogen may dictate that some extra precautions (wetting down or soaking with disinfectant, delaying removal, burying, burning) be taken with litter, even though they may be expensive. Any treatment of manure or litter must consider residual effects of the applied compounds on plant life when treated manure is spread on the land. For most disease agents, composting of litter or droppings is sufficient. Whatever method is used, it is important to remember that wherever litter is spilled or piled, it remains as a pathogen reservoir for varying lengths of time.

In the case of outside runs such as turkey and game bird ranges, the topsoil should be scraped off and hauled some distance from the site. Sunlight and soil activity combine over a long period to destroy most pathogens. Anything that can be
done to aid the destruction process is helpful. Removal of organic residues, such as leaf beds and manure accumulations, helps to reduce the danger for future broods. It is best to rotate the ranges or dirt yards so that they stand idle for 1 complete flock cycle.

Washing begins with blow-down, a process by which water (preferably hot) and detergent sprayed through high-pressure nozzles is used to wet the surfaces and remove most of the dirt and dust from the house. This is followed by cleaning with water (preferably hot) sprayed at high pressure to remove residual dust and dirt. The detergent helps to dissolve the organic biofilm and aids the cleaning process. If washing is not possible, dry cleaning must be thorough and include scraping and sweeping or vacuuming surfaces, corners, ledges, nests, and feeders.

Once the house is physically clean and free of organic matter the process of disinfection can begin. Disinfection involves the application of correctly diluted disinfectant to all internal surfaces of the house by low-pressure spray (preferably as foam to increase contact time). The concentration and volume of chemical applications must be correct to ensure adequate success. Dry cleaning will significantly compromise the disinfection process. The amount of disinfectant used on dry-cleaned surfaces must be increased over that required for washed surfaces.

Disinfectants. Many effective disinfectants are sold under variety of trade names; follow the manufacturers’ recommendations. A disinfectant is a physical agent or chemical agent that destroys vegetative forms of harmful microorganisms, usually on inanimate objects but sometimes on the animals (4).

In the United States, disinfectants are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Title 40 of the Code of Federal Regulations (CFR). Individual states also have regulations which may be stricter than the federal regulation. The Worker Protection Standards (WPS) are a specific portion of FIFRA (Title 40 CFR Part 170) which requires the protection of employees from agricultural pesticides (including disinfectants). Supervisors of individuals who will apply disinfectants must read the label on the disinfectant closely and look specifically for references to the Worker Protection Standards. If the labeling refers to WPS, compliance is mandatory. Copies of WPS How to Comply may be obtained from local cooperative Extension offices.

Complete discussions of various disinfectants and sterilization methods should be consulted (4, 16, 49, 60). Additional references on disinfectants and their use (28, 50) and textbooks on pharmacology and therapeutics should be consulted. The virucidal activities of several commercial disinfectants against vvND have been determined. A list of commercial disinfectants approved for use against avian influenza virus is available from the Environmental Protection Agency (USA) (49). The list provides product names, basic formulations, and dilutions along with the names, addresses, and telephone numbers of appropriate distributors or formulators. For a detailed review of disinfection or to order the Scientific Technical Review, volumes 14(1) and 14(2), 1995, see the OIE website (http://www.oie.int/en/publications-and-documentation/scientific-and-technical-review-free-access/list-of-issues/).

After disinfecting the house, all subsequent processes and movement should be controlled to prevent recontamination. This requires that clean and dirty areas are clearly demarcated. Stringent sanitary practices are frequently ineffective because the pathogen in tracked in after the buildings and equipment are cleaned and disinfected, or because some step in the total program was omitted.

Feed bins should be emptied and cleaned between grow-out/production cycles and special care must be taken to ensure that the inside is totally dry before new feed is delivered.

Water lines should be drained, cleaned, and disinfected. It is important to strip the lines of biofilm before disinfecting the system. The water line biofilm is composed of both a mineral and organic component so the cleaning process entails dissolution of the mineral component (acid or alkali) and destruction of the organic fraction (oxidation and disinfection) (57, 84).

Built-Up Litter and Uncleaned Buildings. Commercial producers require that chicks and poults are delivered disease free. To maintain this status, it is preferable to place these healthy, new flocks in cleaned and disinfected buildings with fresh, clean litter. This is an expensive and time-consuming task. Litter material is becoming scarce and litter disposal requires detailed nutrient management program compliance. Rearing of several successive flocks on the same (built-up) litter has become an economically acceptable practice with broilers, where the life span is very short and single ages of birds/farm permit complete depopulation at the end of each brood. Cleaning and disinfesting of houses is in such instances reserved for disease outbreak control.

Whereas young poults are usually placed in cleaned and disinfected buildings with fresh, clean litter, the litter in turkey grow-out buildings is frequently used for several successive flocks. Rearing of meat birds on reused litter has become commonplace with the development and use of litter-processing machinery. This equipment is used after flock depletion to break up or remove caked litter to ensure that a deep friable and absorbent layer of bedding material remains for the next brood. This practice of reusing litter will unfortunately result in the accumulation of microbial pathogens and parasites within the litter and is strongly discouraged in egg-producing operations.

Culling. Culls are birds that are removed from the flock for humane reasons because they are injured, diseased, or poor performers. The practice of using hospital pens to separate sick birds from the main flock should be discouraged because they act as a source of infection for the rest of the flock. Instead, birds that are injured, diseased, or dying should be
humanely destroyed and removed from the chicken house as soon as possible to avoid unnecessary suffering and disease spread. Such culling must be done judiciously, performed in a humane manner, and started from placement.

**Mortality:** Any dead bird which is left in the poultry house poses a serious threat to the flock health. The carcass undergoes decomposition with the production of millions of decomposition bacteria and potentially pathogenic organism within the carcass. These are released when the carcass breaks. Occasionally toxins may be formed in the carcass and cause problems for the flock. Dead birds should be removed daily and categorized/house according to the likely cause of death. If daily mortality is abnormally high (greater than 0.3/1,000 in broilers and greater than 0.3/1,000 in breeders), further investigation is indicated.

**Nest and Egg Hygiene.** The most important consideration in hatching egg sanitation is to manage the flock so that eggs are clean when gathered. It is crucial to keep the litter dry to prevent soiling of nests, nest material, and eggs. Table-egg breeding stock is traditionally raised on slatted or sloping wire-floor houses which greatly reduces the number of dirty eggs. Broiler and turkeys breeders do not perform as well on these floors, so combinations of part-slat and part litter are used to aid in litter management.

Automatic nest boxes are, generally speaking, more biosecure than manual collection nest boxes. The plastic mats lining the automatic nest boxes are less likely to cause egg contamination and the eggs spend much less time in the nest box. It is essential to keep the nest box environment as clean as practically possible. Dirty or contaminated nest boxes can result in egg contamination, vertical transmission of disease agents, and infection of the hen’s oviduct. The shell of an egg laid by a healthy breeder hen is warm, moist, and clean when it first makes contact with the nest box shavings. Debris will adhere to the moist surface and as the egg cools, particles that are small enough, such as microorganisms, may be drawn in through the pores before the cuticle has had time to dry. During oviposition there is a tendency for the distal section of the reproductive tract to prolapse. In many instances this delicate moist tissue actually makes contact with the nesting material and is therefore very easily contaminated. Microorganism contamination of the nest box can thus be the cause of infectious reproductive disorders and peritonitis.

It is essential that the nest boxes are thoroughly cleaned of all organic material, and disinfected during terminal disinfection of the laying houses. If wood shavings are used as nesting material, there should be a very low proportion of sawdust and the shavings must be dry and free of contaminants including fungus and preferably even fungal spores. Ideally the shavings should be fumigated with formalin prior to use to ensure there are no live microorganisms present. The nesting material should be “topped up” with clean material every 2 weeks to keep it filled to a depth of 5–10 cm and ideally replaced (and the nest box disinfected) monthly. Any extraneous material (i.e., broken eggs, fecal material, etc.) should be removed from the nest box as soon as possible.

From a biosecurity point of view, the house environment is classified as a dirty area and the eggs need to be removed from this environment as soon as possible. If manual collection is practiced, eggs need to be collected as frequently as possible—at least 4 times a day. Each egg collection must be a complete process so that each nest box is emptied. The operator’s hands need to be washed before commencing with egg collection and every effort must be made to keep hands clean during collection to prevent contamination of the eggs. Every effort must be made to ensure that the hatching eggs do not get wet. Eggs and egg trays must be dry cleaned (compressed air) prior to fumigation to remove all the dust and debris that has accumulated during collection.

Very dirty eggs and floor eggs must not be used as hatching eggs. They should be collected separately and must not be placed on the egg trays that are used for collection of clean nest eggs. It is important that the floor eggs are stored away from the nest eggs to avoid cross contamination.

The eggs need to be sanitized or fumigated within 2 hours of being laid (i.e., immediately after collection) to derive maximum benefit from any disinfection procedure. Effective formalin fumigation of hatching eggs is a proven method of reducing eggshell contamination with the vegetative and spore forms of bacteria and fungi. While formalin fumigation has been the backbone of most egg hygiene programs in the past, the classification of formalin in 2004 as a known human carcinogen by the International Agency for Research on Cancer has made its use much more arduous. Stringent health and safety regulations apply even in the United States, where the EPA has classified formalin as a probable human carcinogen (6, 28).

After fumigation the eggs should be transferred to the egg store room. Control of temperature and relative humidity during storage is critical to the survival of the embryo. Temperature and relative humidity fluctuation during storage reduce embryo viability and cause condensation and wetting of the shell surface (sweating). This increases the chance of egg contamination and vertical transmission of bacteria and fungi. The egg storage room should be maintained at a constant 13°C–16°C with a relative humidity 75%. Movement of eggs in and out of the store room should be done as quickly as possible to avoid excessive fluctuation in temperature and humidity. Dirty/floor eggs should be stored separate from clean nest eggs. Care during delivery and collection of eggs is essential to avoid cross contamination.

Egg washing is routine practice in the commercial egg industry. These table eggs are washed with warm (43°C–51.8°C) detergent solution and then sanitized with a chlorine compound, quaternary ammonia product, or other sanitizing agent. It is critical that the washing water is at least 16.6°C higher than the egg itself but not higher than 54°C. While this procedure is often employed successfully with turkey hatching eggs, it is seldom used in the broiler
industry. If hatching egg washing is attempted, a brush conveyer machine that uses continuous-flow water is preferable, and very careful supervision and meticulous management is essential to avoid contaminating rather than sanitizing the eggs. It is also important to consider water quality. If, for example, the iron content of the wash water exceeds 5 ppm, a serious egg spoilage problem is likely. A complete review of egg sanitizing agents is presented by McKenzie (60) and Scott (72).

**Feed and Drinking Water.** The potential for feed- or water-borne challenge occurs every time the birds eat or drink, so the frequency of challenge is very high. This means that even a low level of contamination poses a high risk for introduction or spread of disease. Contamination of feed with fecal pellets from rats, mice, and other rodents is particularly worrying. First, they are likely carriers of dangerous pathogens such as *Salmonella* species. Second, the fecal pellet provides a concentrated source of pathogens in a package that birds are highly likely to selectively pick out and consume.

Litter scratched into feed and water troughs and feed spilled into litter increases intake of litter and litter-borne disease agents (e.g., more coccidial oocysts and less coccidiostat are ingested, and a clinical infection may result). If poultry are permitted to consume litter, considerable mortality and depression can occur from impaction of the ventriculus (gizzard), and litter fragments may cause enteritis by mechanical irritation.

Feed troughs should have some type of guard to keep poultry out and should not be overfilled so that feed is spilled into the litter. Feeders without guards permit defecation into feed, which encourages spread of diseases shed in feces. Wet feed in litter provides a good medium for growth of molds, which can cause liver, kidney, immune system, and other damage to the well-being of poultry. Growing and laying cages for egg production flocks in light- and temperature-controlled houses eliminate most of the problems associated with litter. Many good automated feeding and watering systems are available commercially, but sometimes these are not installed or oriented as the manufacturer intended, and consequently health problems develop.

Roost areas over screened dropping pits are common in floor-laying and breeder hen houses to keep chickens away from their feces. Screened roost areas are also desirable in rearing houses for layers and breeders to prevent piling by the birds and excessive fouling of litter with feces, which in turn leads to packing and caking. Feeders and drinkers over the pits keep the birds on the roost area much of the daytime as well as at night, so most droppings collect out of reach. Spilled water also falls under the roosts, so the litter area stays drier.

Drinkers are frequently set or hung over the litter area. In this case, drinkers should be managed so that spillage onto the litter is minimized.

Drinkers can be put into 2 basic categories: those that provide a constant reservoir of water, which is maintained automatically (troughs, cups, and hanging plastic bells), and nipple drinkers (Figure 1.1), which supply water on demand when activated by a bird. Drinkers that provide an open reservoir of water must be cleaned and disinfected regularly to prevent the buildup of potentially pathogenic organisms in the water supply. These drinkers are also more prone to spillage and the associated problems of wet litter. Starting day-old birds is somewhat easier with drinkers that have an open and visible water reservoir. The advantages of nipple drinkers are found in the significant improvement they offer in providing water free of organisms commonly found in the poultry house environment and in decreased water spillage.

**Feed and Water Medication.** Facilities for quick treatment by medication in water or feed should be provided in case birds become sick. When thousands of birds are grouped in 1 pen, segregation and treatment of individuals is impractical so mass medication is essential.

Feed medication is not the best method of treatment because sick birds have little or no appetite and are unable to compete for feed. Water medication is better because the sick will still frequently drink. Mass medication, while not completely successful in curing the sick, may hold the disease in check until the host can respond with a successful immune response. Provision should also be made for mass vaccination through drinking water, because this is an accepted and successful labor-saving practice. If drinking water is chlorinated or otherwise treated, the sanitizing agent may destroy the vaccine, so provision must be made to permit the use of untreated or distilled water for mixing and administering water vaccines.

Several methods can be used to reduce, remove, or neutralize chlorine in chlorinated water supplies. The only practical method for dealing with this problem on poultry farms is to...
add protein to the water when mixing water vaccines. A common practice is to add 1 cup of nonfat dried milk to 50 gallons of water in tanks or canned liquid nonfat milk mixed with vaccine in a proportioner.

If a building is constructed with a bulk water tank for gravity-flow watering devices, the tank should be plastic or lined with a nonreactive protective substance and be readily accessible for cleaning and for mixing medicaments. If the watering devices are operated on high pressure, the pipe leading into the pen should have a bypass system with proper valve arrangement so that a medicament proportioner can be installed quickly when needed. A metering device to measure feed and water consumption is useful to keep track of the health of the flock.

Bulk feed delivery, metal bulk storage tanks, and automatic feeders are common in modern poultry operations. They eliminate the possibility of rodent contamination because feed is always in closed tanks rather than in bags or open bins, but the system leads to difficulties when short-term emergency medication in feed is desirable and the bulk tank is full. Two alternative systems are useful: an additional smaller bulk tank may be installed just for emergency medicated feed, or a small dispensing tank may be interposed between the bulk tank and feed troughs so that emergency medicated feed can be put in the smaller tank by hand.

**House Access: People.** People, especially visitors, pose the greatest biosecurity risk to any poultry operation. Their mobility, duties, curiosity, ignorance, indifference, carelessness, or total concentration on current profit margin make them one of the most likely causes of disease spread. Rarely is this because they become infected and shed the pathogen, but rather because they track in infectious agents, use contaminated equipment, or manage their flocks in such a way that spread of disease is inevitable. At least 1 avian pathogen (i.e., Newcastle disease virus; NDV) has been found to survive for several days on the mucous membrane of the human respiratory tract and has been isolated from sputum (83). It is a sound principle of disease prevention that no employee of a commercial unit should have any contact with non-company poultry, pet, or hobby birds, at home or elsewhere. The backyard flock maintained without regard for disease control can perpetuate a disease that constitutes a threat to a large, productive industry. The greatest hazard to commercial producers that is created by fancy breeds and backyard flocks is the possible perpetuation of diseases that have been eradicated from the industry.

Disease outbreaks in a community have been known to follow the path of a careless visitor. If visitors do not enter premises or buildings, they cannot track in pathogens. The easiest and most effective means of reducing this risk is to reduce the frequency of visits to those that are essential. When it is necessary to enter the house steps must be taken to reduce the probability of inadvertently transporting infectious agents into the house. As an absolute minimum any person entering the house should don coveralls, a hair net, gloves, and boot covers to reduce the risk of pathogen transfer. While it is not practical to change protective clothing between houses on the same farm, special attention must be given to hands and feet because they are the most likely means of infectious agent transfer.

When moving from one house to another it is best to change boot covers or use house-dedicated footwear. Foot baths might work well when the boots or boot covers are clean and the disinfectant is clean and at the correct concentration. However, foot baths are notoriously difficult to manage and frequently end up enhancing disease transmission rather than preventing it. When using house-dedicated footwear it is best to set up a step-over partition barrier just inside the entrance to the house. It is thus possible to maintain a clear barrier between clean and dirty by stepping over the partition barrier into a new pair of shoe covers or into a house-dedicated pair of boots on entering the house and stepping out of them on exiting the house.

Bird contact is invariably made with the hands so it is essential to pay close attention to hand hygiene. Using disposable latex or nitrile gloves is the best option, but hand washing and disinfection between houses is acceptable. It is preferable to have hand wash basins with running water and soap/liquid soap next to each entrance/exit. Where this is not possible there should at the very least be a hand sanitizer dispenser appropriately placed for use on entrance and exit.

Personnel that frequently visit many different types of poultry enterprises, farms, and farm units such as veterinarians, managers, supervisors, and company owners are high risk for disease transfer. Apart from needing to set an example they must to be meticulous in following procedural biosecurity practices to prevent spreading disease-causing agents. Procedural biosecurity is as much a culture as it is a discipline.

The source of a new or dreaded disease is often puzzling. World trade and travel are becoming more commonplace. It is not uncommon for a person to leave one farm in the morning and be visiting another farm or place of business in another part of the country or another continent on the same day. Some disease agents can survive that time frame easily. All who travel should be cognizant of this and guard against introduction of pathogens into their own flocks or onto the premises of clients, competitors, friends, or fellow producers when returning from a trip. Protective footwear and clothing are not readily available in all countries and poultry areas. A good preventive measure when returning from a trip is to sanitize shoes and launder all clothing worn on farms.

Many poultry farm procedures require sporadic use of specialized crews (e.g., blood testing, beak trimming, vaccinating, inseminating, sexing, weighing, and moving birds from one location to another). These crews travel about the poultry community handling many flocks and must be regarded as a potential source of infection. Thus, they should take stringent precautions to safeguard the health of every flock with which they work.

**House Access: Animals.** No animals should be allowed into the poultry house. All openings in the outer structure of the house must be sealed to exclude animal entry into the house.
Dogs and cats, like rodents, are capable of harboring enteric organisms that are infectious to poultry. When these pets are not confined to the household area, but roam continually among the poultry in the pens and yards, they constitute a serious health hazard. Such pets are just as capable of tracking contaminated material on their feet and in their hair as people.

Wild birds are capable of carrying a variety of microbial pathogens and parasites. Some cause infection or illness in the wild birds themselves, while with others, the birds act as mechanical carriers. Every effort should be made to prevent their nesting in the poultry area. The first rule in poultry house construction is to exclude free-flying wild birds. Poultry raised on range or with access to the outdoors are especially vulnerable to pathogens carried by wild birds. For this reason and for improved sanitary practices, the trend has been to house poultry in closed or partially closed bird-proof houses. However, the advent and growth of the free range and organic industries threaten to compromise carefully designed national programs to eradicate devastating diseases such as high-pathogenicity avian influenza.

Imported zoological specimens destined for zoos are not a direct contact threat because the zoos are located in cities, but they should be considered as a potential source of introduction of an exotic microbial pathogen or parasite. Exotic ornamental pet birds constitute a real hazard because they become widely dispersed and may be purchased by poultry workers. On numerous occasions, exotic birds in or those destined for pet stores have been found infected with a vvND virus, which in at least 1 instance was the source of a serious and costly outbreak in poultry. Stringent entry quarantine requirements to apprehend and destroy infected birds provide a good barrier against the introduction and dissemination by carrier birds, but failures can occur (illegal smuggling), and producers should be wary of such personal pets. Domestic pigeons also can be a source of dangerous strains of NDV.

Rodents contaminate feed and litter with their excrement. They are particularly hazardous to Salmonella control, because they are frequently infected with these organisms and can perpetuate the disease on a farm by serving as a continual source of Salmonella. The house should be monitored for signs of rodent presence. Regular baiting of rodent stations and breeding areas must be enforced. Housekeeping must be of such a standard as to deter the vermin from settling. This can be achieved by the removal of rubble and waste materials from the house, the avoidance of feed spillage, and the regular rotation of chemical control products and traps used. For more detail on rodent control the reader is referred to Chapter 9 of the AAAP publication A Practical Guide for Managing Risk in Poultry Production (86) and Chapter 26 of this book.

House Access: Insect, Mite, and Tick Control. Many insects act as transmitters of pathogens. Some are intermediate hosts for blood or intestinal parasites, others are mechanical carriers of pathogens through their biting parts. They also act as reservoirs of infectious agents in that they can transfer an infectious agent from one flock to the next. The litter beetle is not only a major pest in the poultry house environment but can play a vital role in the spread or carryover of some disease-causing agents. The control of litter beetle is based primarily on spraying insecticide during the clean-out process. Flies and mosquitoes are less of a problem but can still play a role in disease transmission. Mites and tick can also pose a threat to flock health. Control measures need to be directed primarily at the breeding areas of these insects.

The EPA defines a pesticide as any substance intended for preventing, destroying, repelling, or mitigating any pest. A pest can be any insect, animal, plant, or microorganism. Insecticides destroy animal parasites such as lice, mites, ticks, and fleas. They also destroy other undesirable insects (flies, beetles, ants, and sow bugs) in the environment. The limited number of available commercial parasiticides and their active chemical properties, limitations, tolerances, and various applications are discussed in detail in Chapter 26. Also see Chapter 32 for toxic effects of some insecticides. For more detail on insect control the reader is referred to Chapter 8 of the AAAP publication (69).

Building Construction. An apron of concrete at the entrance to a poultry house helps prevent tracking of pathogens into the unit. Rain and sunshine help keep the apron clean and sterilized. A water faucet, boot brush, and covered pan of disinfectant available on the apron for disinfecting footwear are further aids in keeping litter and soil-borne pathogens out of the house. Boots must be thoroughly cleaned before the wearer steps into the pan of disinfectant. The disinfectant is useless, however, unless renewed frequently enough to ensure a potent solution at all times.

All surfaces inside the building should preferably be of impervious material (such as concrete) to permit thorough washing and disinfection. It is impossible to sterilize a dirt floor.

Raised slatted floors have been used successfully for years for laying chickens, both for adults and for rearing birds. Such floors have alternating wooden pieces and spaces, each about 3/4-inch wide (Figure 1.2), to permit droppings to fall through, out of reach of birds and to prevent recycling infection of intestinal parasites and diseases. Commercial meat birds are inclined to develop leg problems and breast blisters if raised on slatted or wire floors. A modification of this system, with part of the floor or yard raised slightly and covered with slats, has been used for broiler breeders.

Keeping laying hens in some type of cage has become an accepted practice in closed houses (Figure 1.3) and open-type houses found in hot climates. Cages and wire floors also are widely used to rear pullets destined for cages as adults.

The Hatchery

The building and equipment in which the fertile egg is converted to a day-old chick, poult, or other fowl and the equipment used to process and deliver it to the farm must be clean and sanitary. An individual hatched from a pathogen-free egg
Design and Location. A hatchery should be located away from sources of poultry pathogens such as poultry farms, processing plants, necropsy laboratories, rendering plants, and feed mills. It is not good practice to retail poultry equipment and supplies from a hatchery building because this draws producers and service workers who may introduce contaminating material.

A good hatchery design has a one-way traffic flow from the egg-entry room through egg-traying, incubation, hatching, and holding rooms to the chick-loading area. The clean-up area and hatch-waste discharge should be off the hatching room, with a separate load-out area. Each hatchery room should be designed for thorough washing and disinfecting. The ventilation system is equally important and must be designed to prevent recirculation of contaminated and dust-laden air. Gentry et al. (38) found that hatcheries with poor floor designs and faulty traffic patterns were highly contaminated compared with those with one-way flow.

Importance of Good Sanitation. Factors that aid in obtaining pathogen-free chicks and poults are hatchery cleanliness and sanitation, well-arranged traffic flow, and well-controlled ventilation. Techniques have been devised for evaluating the sanitary status of commercial hatcheries by culturing fluff samples (91), detecting microbial populations in hatchery air samples (27, 38, 54), and culturing various surfaces in the hatchery (56). By relating results of these techniques to hatchery management, it has been observed by Magwood (55) that bacterial counts of egg shells dropped quickly in clean air, and low counts persisted on all surfaces to completion of hatching. Chute and Barden (26) found fungal flora of hatcheries to be related to management and sanitation programs.

To minimize bacterial contamination of eggs and hatching chicks, hatchery premises must be kept free of reservoirs of contamination, which readily become airborne (55). Trays used for hatching should be thoroughly cleaned with water and then disinfected before eggs are placed in them. This can be done by dipping in a tank of suitable disinfectant (see “Disinfectants”), washing with hot water or steam followed with disinfectant spray, or fumigating with formaldehyde in the hatcher. Trays and eggs are frequently fumigated together immediately after eggs are transferred to the hatcher. Fumigation is sometimes done during the hatch (at about 10% hatch), but concentrations low enough to avoid harming the hatching chick probably serve only to give the down a pleasing yellow color. Formaldehyde fumigation in one case increased the severity of mold infection rather than overcoming it (89). Wright (90) emphasized the practical meaning of hatchery sanitation and how to attain it. He concluded that no fumigation program should be used to replace cleanliness, but rather to supplement it.

As chicks hatch, the exposed embryo fluids collect bacteria from contaminated shells, trays, and ventilating air. The combination of the nutritious fluids and warm temperature forms an excellent environment for bacteria and they multiply very
The cleaner the air and environment the less likely the navel is to become infected (omphalitis).

**Breeder Codes.** The breeder code is a designation used to denote the source of hatching eggs. It usually denotes breeders of the same age on the same or different farms, all breeders on a particular farm, or any other grouping. There is a tendency to keep breeders in larger flocks and to avoid as much as practicable the mixing of hatching eggs from flocks of many different microbial, nutritional, and genetic backgrounds. If breeders are kept free of disease and fed a good ration, hatching eggs are produced clean and properly disinfected, and chicks are hatched and handled in clean surroundings. Keeping chicks of different breeder codes separate has little practical meaning other than providing that all have more nearly the same level of maternal antibodies against the same diseases. This may permit a more uniform response to vaccines applied to chicks the first 2–3 weeks of life when maternal antibodies have a protective effect.

Occasionally, a disease is believed to be egg transmitted from a breeder flock to the offspring. When this occurs, the disease nearly always appears in several offspring flocks derived from the same breeder flock(s) and delivered to different farms. A hatch of chicks is frequently divided into deliveries to several farms, and if a disease occurs in only those delivered to 1 farm it indicates that the disease is farm associated and not hatchery or breeder-flock associated.

**Chick Sexers.** Unless the output of one hatchery is so great as to demand them full time, chick sexers may go from one hatchery to another, which introduces the possibility of carrying disease-causing pathogens. Most sexers are aware of this hazard and are eager to follow proper biosecurity procedures. If sexers must also service other hatcheries, facilities should be provided so that their equipment can remain at the hatchery. They should have a clean area in which to change clothes and wash themselves and their equipment and should have clean protective garments to wear. Their habits should be as clean as those of the hatchery crew.

**Surgical Procedures.** Beak trimming is commonly practiced in breeder flocks, meat turkeys, and cage layers. Proper beak trimming promotes maximum performance. Done improperly, it provides a portal of entry for normally nonpathogenic organisms such as *Staphylococcus aureus* or primary pathogens such as *Erysipelothrix rhuseopathiae*. Similarly, other surgical procedures, such as removing wattles, combs, or toenails of certain toes must be done as aseptically as possible.

**Storage Facilities.** After fumigation or other shell sterilization, hatching eggs are frequently stored in a cool room (about 10°C) at the hatchery until set. Cool rooms should be clean and free of mold and bacteria and periodically disinfected to prevent recontamination of shells. Holding hatching eggs too long or under improper storage temperature, humidity, and environment can result in poor quality chicks. Clinical histories indicate that infection in young chicks may sometimes be traceable to fungus-contaminated hatching eggs; infections have been produced experimentally by contaminating shells with fungus spores (89). Whenever cold eggs are moved into a warm, humid atmosphere, moisture condenses on the cold shells (called “sweating”). This moisture provides a medium for the growth of bacteria and fungi already present on the shell or from contaminated warm air around the eggs. Therefore, cold eggs should be warmed (preheating) to room temperature in clean, low-humidity air before placing them in an incubator.

**Secondary Control Zone: Farm or Site**

The company farms or sites constitute the next logical zone or compartment for disease control. For this purpose, the farm and not the house is defined as the epidemiological unit. First, the farm has a defined boundary, and second, because the houses are in close proximity, the birds on the farm share a defined epidemiological relationship (common environment, with common management practices) and thus have approximately the same likelihood of exposure to a pathogen.

The boundary of the farm/site serves as a physical (fenced) or imaginary (nonfenced) line of access control to the secondary control zone—the most crucial zone for disease control. The farmer should enforce full biosecurity with no controlled access from the start of the disinfection process—the site is “closed.” The farmer should enforce general biosecurity from the start of transfer/depletion with access only granted to necessary vehicular traffic—the site is “open.” The farmer should enforce routine control from the point of last bird removal—the site is “fully open.” In the event of a disease outbreak, the site should remain closed until the responsible veterinarian declares the site clean.

**Isolation**

Not all producers follow the same disease control practices. A close neighbor may disregard sound principles and be burdened with diseases until forced out of business by economic pressures. Disease agents present on his or her premises may be blown or carried by various vectors and fomites to adjacent premises. Until a disease has been eradicated from a flock like this, it serves as a reservoir and potential source of infection for future flocks on the same premises and those on adjacent premises. The closer houses or premises are to one another, the more likely it is for disease to spread.

Highly concentrated poultry production areas frequently deteriorate into problem zones of disease of one type or another. Farms are so close together that the area forms an epidemiological unit from a disease perspective. Within these areas there are several different age groups of birds and many managers, each vaccinating, treating, or exposing birds without regard to the programs of others. In such situations a system of a single age of fowl, permitting complete depopulation at the end of each rearing or laying cycle, goes a long way...
toward solving the problem. This is even more successful if coordinated area depopulation and restocking is practiced.

**One Age of Fowl/Farm**

Removing carriers from a flock and premises is an effective way of preventing a recurrence of some diseases, but it is impossible or impractical for others. The best way to prevent infection from carrier birds is to remove the entire flock from the farm before any new replacements are added and to rear young stock in complete isolation from older recovered birds on a separated farm segment or preferably on another farm and in an isolated area. This practice is often called all-in-all-out production.

Where birds of different ages exist on a large farm, depopulation seems drastic, but considering mortality, poor performance, and endless drug expense, it could be the most economical solution. Where only 1 age of bird is maintained, depopulation occurs each time pullets or poults are moved to the layer or breeder premises, each time the broilers or turkeys are moved to slaughter, and each time the old layers or breeders are sent to market. Should a disease occur, the flock can be quarantined, treated, and handled in the best way possible until it is disposed. Depopulated premises are then cleaned out, washed, and disinfected, and left idle for at least 2 weeks before new healthy stock is introduced.

**Functional Units**

For certain economic reasons (breeding farm or small specialized market trade) it is not always possible to limit the entire farm to a single age of poultry. In such instances, it should be divided into separate quarantinable units or areas for different groups of birds (rearing area, pedigree unit, production groups, and experimental birds) (Figure 1.4). Each area can periodically be depopulated, cleaned, and sanitized. Much stricter security procedures for personnel, bird, and equipment movements are necessary for this type of operation. A very rigid monitoring system is also essential to detect any disease early enough to bring it under control while it is still confined to 1 quarantinable segment.

**Farm Environment**

The farm or site must be maintained to minimize the breeding areas and any overt protection given to vermin, predators, or other organisms. The grass must be kept short and the aprons free of grass and weeds. Vermin are vulnerable to predation when crossing these exposed areas.

Water must not be allowed to accumulate on site in open pools. Drainage must be sufficient to remove excess water, especially during storms and cleanout. Stagnant water is an ideal breeding ground for insects and other organisms. No rubble or waste debris should be stored on site and equipment must be stored to avoid offering shelter or protection to unwanted creatures.

**Farm Access Control**

The site should ideally be completely fenced with sufficient deterrents to access from predators, vermin, and unauthorized people. There should preferably be only 1 access point into the site. This entrance should be protected by gates which should be locked at all times and access control must be exercised by site personnel to limit vehicle, equipment, and people movement.

Only poultry considered to be part of the site flock must be allowed on site and they must be confined to the house or free-range enclosure. No domestic or wild animals must be allowed within the perimeter fence and wild birds must be actively discouraged from the site through the control of any activity that may attract (feed spillage) or harbor (nesting) these birds.

**People.** Farm/site personnel should ideally be the only people permitted on site and even they must not have had contact with other avian species for at least 2 days prior to entering the farm. Only essential visits by authorized personnel such as mechanics, managers, working crews, etc. should be allowed on site. They must not have had contact with other noncompany poultry or domestic birds for at least 2 days and must observe the prevailing biosecurity procedures for house access if entering a poultry house.

All nonessential visits by company employees and all noncompany personnel visits must be authorized by the relevant authority (live production manager or veterinarian). No such visitor must have had contact with other poultry or domestic birds for at least 2 days.

The visitation sequence to sites should always be from youngest flock age to oldest flock age. In the event of a disease outbreak, the disease control should always supersede the age sequence (i.e., affected flocks must always be visited last, even after visiting an older healthy flock).

A visitor and vehicle register must be maintained to record all visitors (defined as a person not working on site on a daily basis).
and all vehicle movements onto and off the site. Such records should include the reason for and the duration of the visit.

In situations where a “shower-in-shower-out” policy is in place, the shower unit is the crucial point separating the site from the outside environment. The shower complex must therefore be unidirectional with the shower unit in-line. All transit or personal clothing and personal items must be stored on the external side of the shower. Any item not suited to washing must not be taken onto the site unless it can be fumigated or suitably decontaminated. Anyone or anything entering the shower unit must be thoroughly cleansed prior to exiting onto the site side of the unit.

After showering, or if there is no shower in place, any person entering the farm/site must don site-dedicated protective wear: coveralls, hairnets, and protective footwear or plastic shoe covers. Hands should be cleaned with running water and soap/liquid soap prior to entering and on leaving the site.

The purpose of protective clothing is to provide site personnel with a standard uniform that has not had outside contact or contamination and therefore poses no disease risk to the poultry. The protective clothing colors also can be used to distinguish between departments and the various biosecurity zones.

The office should be a separate room and must only be accessible from the site side. Nothing should enter this room until it has passed through the designated cleaning and disinfection procedures.

Specialist crews and people performing specialist tasks are frequently called upon to visit more than 1 site/day and sometimes not in the prescribed visitation sequence. Such crews and their equipment pose a serious disease risk to the site so they need to be particularly vigilant about following biosecurity protocols.

Vehicle Access. To reduce the risk of disease agent transmission it is best to prohibit vehicle access to the site. It is seldom necessary for vehicles to drive onto the site. However, all vehicles entering the site should be suitably disinfected prior to entry. This means that the vehicle must pass through a full spray bay, which has the capacity to deliver a coarse to fine spray of disinfectant over the entire vehicle to ensure total wetting of the exterior. The disinfectant used must be applied at the recommended dosage rate and should not be unduly corrosive or damage the painted surfaces of vehicles. A vehicle wheel dip should be built into the spray bay to ensure that all vehicles entering and leaving the site at any stage of production cycle pass through this dip. Any vehicle that carries live birds or nonwettable exposed cargo (shavings) or with no roof or side protection for the driver (tractor) must have a full undercarriage spray. The spray bay must be designed to spray the entire vehicle including the undercarriage. Vehicle drivers must not leave their vehicles while on site unless the cab has been suitably disinfected on entering the site and the driver has gone through the correct access control procedures applicable to personnel. Site-dedicated vehicles must not leave their area of dedication except for repairs, servicing, and fueling.

On return, site-dedicated vehicles must be completely disinfected at the point of re-entry.

Equipment. All equipment entering a site should be suitably decontaminated by a soap wash disinfectant spray and/or fumigation. Some equipment does not lend itself to fumigation or wetting and such items (cell phones, beepers, vaccine syringes, pens, etc.) must be suitably cleaned at the point of dispatch to remove gross contamination or stored in a sealed plastic bag, or the exposed surfaces may be wiped with a moist disinfectant cloth.

All site equipment must be sanitized during the clean-out process. Site equipment must be dedicated to a site or have at least a 14-day outside storage period to reduce the risk of disease spread. House equipment such as chick fonts, feeder or scratch pans, crates, plastic sheeting, partitions, nest boxes, etc. should not leave a site to be used on another site.

Placement Transfers and Depletion
All placements, transfers, and depletions must be synchronized to ensure that sites are placed in a suitable sequence within complexes and operations. All placements and transfers require that the live birds be kept for some time within the company’s transport equipment and therefore, all vehicles and equipment must be cleaned and disinfected between loads. This should be done at the point of origin for placements and transfers and at the point of delivery and again at the complex/site entrance for depletions.

Egg Room
The egg room is a holding room for eggs prior to dispatch to the hatchery. The eggs originate from the houses on site (dirty area) and eggs should preferably be fumigated prior to entering the egg room (clean area). Although the egg room is part of the site, no eggs or buggies placed into an egg room should be taken back on site. The external door is the physical demarcation of the site side of the egg room and must only be opened for the purpose of removing filled egg bogeys. It is preferable to wheel the egg bogeys leaving the egg room through a wheel-dip containing a suitable disinfectant to reduce the chance of spreading a disease agent off site.

The egg truck, egg bogeys, and egg trays form an important epidemiological link between all the company farms (broilers and breeders) via the hatchery. It is thus essential to implement and enforce strict controls at this interface. All bogeys and egg trays coming from the hatchery must enter the site through the fumigation room to ensure decontamination. The egg room must be cleaned and disinfected at least once a day, preferably immediately after eggs are dispatched to the hatchery (i.e., when the room is empty).

Fumigation Room
Fumigation is the process of decontamination of an object through the use of a gas compound. Because gases can penetrate tiny holes, this form of disinfection is ideal for most
objects that are otherwise difficult to clean. The fumigation room must have 2 access points: one on the site side and the other to the outside. The external access must be used for loading all objects that need to be taken onto the site. The site access must be used for receiving fumigated goods onto the site and for dispatching potentially contaminated goods from the site. Only 1 access point must be open at any given time. Nothing should be allowed to be taken onto the site unless it has been showered (soap wash), disinfected, or fumigated. Certain exceptions exist and include live birds and nonwettable cargo such as shavings and feed.

**Dead Bird Disposal**

All dead birds should be taken to a designated collection point on the farm/site and: (1) stored in suitable containers in a cool environment (shade or refrigerator) to delay the rate of decomposition, avoid ground contamination through leakage and spillage, and prevent predation; (2) mortalities must be disposed of on a daily basis, either on site through incineration, pickling, pit or tank (Figure 1.5A), composting (Figure 1.5B) or burial, or off site through burial, composting, central depots, or rendering plants (63); and (3) mortality collection vehicles must not enter any site and must always follow strict visitation sequences (young to old, and healthy to diseased) and disinfection procedures.

**Tertiary Control Zone: Complex**

An epidemiological unit also may refer to groups of birds that share a communal animal handling facility. For example, the sites/farms within a complex will invariably share a hatchery, feed mill, and processing plant, and thus form an epidemiological unit. Similarly, production processes within the complex such as pullet rearing farms, breeder or laying farms, and broiler farms also form separate epidemiological units. Depending on the level of biosecurity these areas can be demarcated and classified as tertiary control zones.

Tertiary control zones are frequently set up around high-value sectors of the operation because resource allocation to biosecurity is easier to justify. For example, grandparent stock are substantially more valuable than breeder breeders, which are in turn more valuable than broilers, so the implementation of tertiary control zones becomes easier to justify as one moves up the production pyramid. Tertiary critical control points (transit facilities) may be established beyond the outer confines of the site perimeter to reduce the risk of disease agent transmission. Control procedures such as showering or merely changing into protective clothing at this point and using site-/zone-dedicated transport to move to the site significantly reduce the chance of pathogen transmission. The tertiary control zone is seldom fenced so the access control boundary is usually imaginary.

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**Figure 1.5.** (A) Poultry disposal pit. Such a pit can be made any size that is convenient. (B) A simple above-ground poultry carcass composter of 200 ft³ (5.7 m³) capacity. Five such bins will process ca. 1,000 lbs. (455 kg) of carcasses/day. (Courtesy of the University of Maryland Poultry Science Department)
**Complex Environment**

In rare situations a group of sites within a geographical location can be protected as a single unit by the erection of an enclosure (perimeter fence) with access control (transit facility). In other situations the boundary may be operational in nature. Such a complex does not merely exist because 2 or more sites are on the same location, but rather as a means of implementing bioexclusion procedures. The entrance to such a complex is referred to as a transit facility, and the complex a disease-free area or control zone. A complex must always be considered to be closed in terms of the enforcement of biosecurity control measures and dedicated vehicle transport used within this control zone.

Although a complex can be a large expanse of land, the same principles to housekeeping on the site are applicable. The land around each site must be maintained to minimize breeding sites and overt protection given to vermin, predators and other organisms. The grass outside the site perimeter fence must be kept short and free of any rubble and debris.

**Complex Access: Transit Facility**

The transit facility is the entrance to the complex and serves as a biosecurity critical control point to reduce the risk of disease. Site and complex personnel should ideally be the only people within the complex. Only essential visits by authorized personnel such as mechanics, direct managers, working crews, etc. should be allowed onto a complex. All nonessential visits by company employees must be authorized by the veterinarian. All noncompany visits must be authorized by the relevant authority. In the event of a disease outbreak, the veterinarian is responsible for the imposition of additional control measures appropriate to the disease.

**Access to the Complex**

Procedures for people, vehicle, and equipment access to a complex are the same as those for a farm. Anybody intending to visit any part of the tertiary control zone (complex) must comply with transit facility controls. This should involve a clear separation between clean and dirty areas/items. Anybody or anything entering the complex should ideally be “decontaminated” by washing with soap and water. People entering the complex should at the very least leave all personal clothing and personal items in the transit facility and change into complex clothing. Complex-dedicated vehicles should be used to move between the transit facility and the farms/sites and a visitor and vehicle register similar to those at farm level must be maintained.

**Monitoring and Surveillance**

Monitoring and surveillance are both terms used to describe the ongoing collection of data to describe the prevalence and severity of disease in a population. A monitoring program is usually designed to accumulate statistically reliable disease prevalence data over time to indicate a change in the incidence or severity of a disease. In contrast, a surveillance program is usually designed to collect prevalence data from a readily available sector of the population (potential sample bias) with the primary purpose of implementing timely corrective action when there is a perceived increase in incidence of a disease. As flock size and production intensity increases, management control becomes more remote, so monitoring and surveillance programs become more important.

With eradication programs implemented to control diseases of catastrophic nature, the objective of the surveillance program should be to detect the source case of an outbreak so that biocontainment through quarantine and slaughter can be initiated before the diseases spreads. If the goal is less than eradication, the degree of deviation from normal prevalence necessary to stimulate corrective action needs to be set at such a level to differentiate common cause (background variation) from special cause (a disease effect).

Several parameters such as the sample size, necessary to detect specific levels of prevalence, can be calculated by equation and it is important to realize that the significance of this in program design. The objective of the data collection system is to provide incisive and epidemiologically informative indicators which will permit objective judgment and decision making.

For disease eradication and trade purposes it is often necessary to demonstrate freedom from infection (absence of the pathogenic agent) in the country, zone, or compartment (company). It is not possible to prove with 100% confidence that a population is free from infection (unless every member of the population is examined simultaneously with a perfect test with 100% sensitivity and specificity). Therefore, a surveillance system to demonstrate freedom from infection should be designed to predict with an acceptable level of confidence that infection is below a specified level of prevalence in the target population. Any evidence of infection at any level in the target population automatically invalidates any freedom from infection claim.

For disease control purposes surveillance is used to determine the distribution and occurrence of infection or immunity within a zone or compartment to aid in the decision-making process. In this instance surveillance is designed to collect data on several variables relevant to flock health, including prevalence or incidence of infection, morbidity and mortality rates, flock immunity as indicated by frequency distribution of antibody titres, farm production records, etc.
Poultry flock health tracking requires that the poultry are monitored for disease at regular intervals. A change in prevalence over time indicates a change in incidence which signals the need for corrective action to prevent disease spread. Unless monitoring includes true random sampling, results cannot be taken to be absolute measures of disease incidence and prevalence, but may serve as adequate indicators for intervention.

The following formula provides a simplified method of estimating the number of animals that need to be tested for the probability of selecting at least 1 diseased animal in a finite population of birds to be greater than a preset confidence level (commonly 95%).

\[
n = \left[1 - (1 - p)^{1/d}\right] \times \left[\frac{N - d}{2}\right] + 1
\]

where \(n\) = sample size, \(N\) = flock size, \(p\) = probability of selecting at least 1 diseased animal, and \(d\) = the number of animals affected for the desired level of prevalence.

Although this method of sample size determination is widely used, its accuracy is based on several assumptions. Violation of the assumptions (that the disease is present at a certain minimum prevalence, the diagnostic tests used are 100% sensitive and 100% specific, sampling is performed with replacement and the data is collected by simple random sampling) renders the estimate inaccurate (19, 21). A more accurate determination of sample size is given with a computer program such as FreeCalc. This program uses trial and error to calculate the exact sample size required for a specified probability, can be used on finite populations, and takes account of test imperfections (19).

Sample frequency must be calculated based on the epidemiology of the disease under consideration. With *Mycoplasma gallisepticum* (MG), for example, the index case could produce infected eggs within 17 days but peak shedding occurs when colonization peaks at 3–6 weeks after flock exposure (41, 42, 71). After flock exposure to MG, there is a latent phase of 12–21 days in which less than 5% of the flock has a detectable antibody response (61). To prevent vertical transmission the monitoring system must be capable of detecting infection at the 5% level with 99% confidence. The sample size \((n)\) that must be tested to have 99% confidence in determining whether MG is present at a prevalence of 5% in a flock of 7,000 birds can be estimated by calculation as 90 birds (21). To prevent infected eggs from entering the hatchery it would be necessary to sample flocks every 2 weeks (assuming 100% sensitivity for the test system). The testing interval can be extended by 2 weeks where hatchery tracking systems allow infected egg removal from the setters.

**Performance Parameters**

Metrics generally used to judge overall health, which encompasses vaccine program efficacy, are culls at the hatchery, 7-day mortality, 14-day mortality, final flock livability, feed-conversion efficiency, rate of gain, condemnation, egg production, and egg quality. Many of these metrics have standards or comparative histories established through each company’s own historical data or, in the United States at least, national reporting services such as AgriStats (AgriStats, Fort Wayne, IN), or Agrimetrics (Agrimetrics Associates, Inc., Midlothian, VA), and government reporting services such as the poultry slaughter reports published monthly by the National Agricultural Statistics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. An additional metric that can be used over time is antimicrobial and antiparasitic drug usage. Although this is influenced by many things, including management changes and climatic shifts, monitoring usage is an essential tool for evaluating overall health and vaccination program efficacy.

**Examination of Field Birds**

Health surveys (9, 50) that include extensive gross and microscopic evaluation of necropsy specimens, and controlled challenge studies (64) to measure a relative protection level, are both useful in assessing vaccine program effectiveness. Perhaps the most frequent controlled challenge work done is to measure passive protection of broiler chicks from hens hyperimmunized to infectious bursal disease (64). Trends in program efficiency may be identified over time if sufficient groups of chicks are sampled.

**Serologic Monitoring**

Serologic monitoring (76) is only useful in production medicine if adequate samples have been analyzed over time to establish a normal baseline for a specific program, in a specific location, in a specific bird, using specific and consistent application techniques, with samples run consistently by a specific laboratory. After a baseline is established, flocks can be identified that have serologic profiles above or below the established baseline.

In broiler and turkey production flocks, an effective monitoring program can be the regular sampling and testing of blood as the birds are slaughtered at the processing plant. This serologic monitoring will establish a baseline of antibody titers that are the result of both vaccination and field challenge. Changes in the usually observed antibody titers may indicate a decrease in the efficacy of vaccine administration or an increased field challenge by a particular pathogen. A regular serologic monitoring program is also helpful to determine whether a flock has been exposed to a new pathogen not previously present in the region.

Serologic monitoring of layer flocks should be performed before the flock is placed in the layer building, with periodic serologic monitoring throughout the production cycle. This type of program will assess both the efficacy of vaccine administration and the disease challenge the flock experiences in the field. Breeder flocks should be monitored in the same way as layer flocks and, in certain instances, breeders can be revaccinated during production to boost the maternal antibody titers of their progeny if they are found to be low.

**Interpretation of Serologic Data**

It is usually impossible to differentiate between antibodies that are produced by vaccination vs. those induced by field exposure to a given infectious agent. The only difference that
may be observed is that the antibody titer following a field challenge may be higher than that observed following vaccination. A valid interpretation of serologic results requires a complete knowledge of the flock’s vaccination history.

It usually takes poultry 1–3 weeks to produce detectable levels of antibodies in their serum. It is possible, therefore, to collect blood during the middle of a disease outbreak and not be able to detect any antibodies to the causative disease agent. If this same flock is tested 2 weeks later, however, serum-antibody levels will be high. A useful practice in establishing a disease diagnosis is to take acute and convalescent serum samples from the flock as it is undergoing an unknown disease challenge. Typically, the acute serum sample collected during the initial phase of the disease outbreak will be negative for antibodies to the suspected disease agent. The convalescent serum sample, taken shortly after the flock has recovered, if positive, will provide a definitive diagnosis when interpreted in conjunction with the clinical signs and lesions of the case. An important concept in the interpretation of serologic results is that a single positive serologic test only indicates that the flock was exposed to that disease agent during its life.

Different laboratories often conduct serologic tests using different reagents or techniques. Because of this, comparing antibody titers (a titer is a measure of the level of concentration of antibody in the serum) reported from different laboratories may be confusing. It is best to use 1 laboratory for a given test so that a familiar range for negative, low, or high titers is established. With experience and training, production managers can become skilled at the interpretation of serologic results.

**Flock Profiling**

Today’s disease problems often represent the sum of various subclinical disorders occurring at different times throughout the life of a flock. Acquisition of the fullest understanding of this sequential collection of serologic and other data concerning multiple pathogens requires disciplined and careful organization. The systematic, graphic presentation of this data is commonly called a flock profile. The establishment of such profiles is facilitated by enzyme-linked immunosorbent assay (ELISA) technology because a single basic test system is used to monitor for a broad array of diseases (61).

Snyder et al. (77) demonstrated the value of correlating ELISA profiling data with flock performance. The further evolution and diagnostic advantages of the graphic presentation of ELISA-based flock profiling data in combination with gross and microscopic pathology data was described by Mallinson et al. (57). The method has broad applicability to epizootiologic investigations, field research, and quality control. Baseline profiles can be established both as targets for vaccination goals and as a base from which deviations from the norm may be demonstrated when a field problem is subsequently encountered. Several flock-profiling kits and systems are now commercially available. Their value is enhanced when good data retrieval and graphic presentation of data (Figure 1.6) are combined with the diagnosticians’ veterinary skills and experience in assimilating medical information and establishing a plausible diagnosis.

**Diagnostic Procedures**

Many satisfactory diagnostic and necropsy methods exist. The techniques and instruments used by one pathologist may vary considerably from those used by another. Some suggestions are offered here to guide the student and beginner. The goal of the necropsy is to determine the cause of impaired performance, signs, or mortality by examining tissues and organs and to obtain the best specimens possible to carry out microbiologic, serologic, histopathologic, or animal inoculation tests. It is important that in the process, infectious materials do not endanger the health of humans, livestock, or other poultry. By proceeding in an orderly fashion, possible clues are less apt to be overlooked, and tissues will not be grossly contaminated prior to examination. Remember that a blood sample or tissue specimen determined later to be superfluous can always be discarded. It’s better to save tissues and then discard them if they are later determined to be unnecessary or unimportant to the diagnosis.

A key to good poultry diagnosis is the art of “seeing the forest as well as the trees.” Try to identify the most significant flock problem(s) rather than becoming engrossed in individual bird disorders. Watch for telltale patterns of pathology as presented by the total diagnostic consignment.

The techniques and procedures necessary to make an accurate diagnosis and identify specific disease agents are found in the technical information contained in succeeding chapters of this book and in the following excellent reference manuals: *A Laboratory Manual for Isolation, Identification and Characterization of Avian Pathogens* (32), *Avian Disease Manual* (25), *Avian Histopathology* (35), and *Color Atlas of Diseases and Disorders of the Domestic Fowl and Turkey* (67). *Avian Hematology and Cytology* (20) should be consulted for detailed information on avian blood elements and methods for preparation and study. New information is continually being presented in the journals *Avian Diseases, Avian Pathology, and Poultry Science*; in the proceedings of several regional poultry disease conferences; and in other avian pathology and science journals.

**Case History**

The pathologist who has not seen the farm or the flock before attempting to diagnose the problem and recommend corrective measures is at a disadvantage. This can be partially overcome by getting a complete history of the disease and all pertinent events leading to the outbreak. The more information pathologists have about the history and environment, the more directly they can proceed to determining solutions for the problems. Unfortunately, the history includes only the situations, events, and signs that the caretaker, owner, service worker, or neighbor
has observed and remembered. Knowledge of management factors such as ventilation; feeding and watering systems; accurate records of egg production, feed consumption, feed formulation, and body weight; lighting program; beak trimming practices; brooding and rearing procedures; routine medication and vaccination used; age; previous history of disease; farm location; and unusual weather or farm events may make the difference between diagnosis of the flock problem and the finding of a few miscellaneous conditions in a sample that may or may not be representative. Duration of the signs, the number of sick and dead, and when and where they were found dead can be important clues.

Poultry producers have developed a high degree of knowledge about poultry diseases and usually recognize those resulting in dramatic or clear-cut signs and lesions. The veterinarian, therefore, is often confronted with obscure, undramatic, and complicated disease cases requiring extensive investigation. Even if all indications are that reduced performance is most likely due to a management factor, the veterinarian must check all reasonable disease possibilities. This requires a systematic approach to be sure that nothing is overlooked.

**External Examination**

Look for external parasites. Lice and northern fowl mites (*Ornithonyssus silivarum*) can be found on the affected chicken. If red mites (*Demanyssus gallinae*) or blue bugs/fowl ticks (*Argas persicus*) are suspected, examination of roosting areas and cracks and crevices in the houses and around the yards must be made, because these species do not stay on birds. See Chapter 26 for diagnosis and identification of external parasites.
The general attitude of live birds and all abnormal conditions should be noted carefully. It is very important to observe evidence of incoordination, tremors, paralytic conditions, abnormal gait and leg weakness, depression, blindness, and respiratory signs before the specimens are killed. It is very helpful to place birds in a cage where they can be observed after they have become accustomed to the surroundings and perform at their best. It is sometimes advisable to save some of the affected birds to observe possible recovery from a transitory condition (transient paralysis), respiratory infection, chemical toxicity, feed or water deprivation on the farm, or overheating during transport to the laboratory.

Examination should be made for tumors, abscesses, skin changes, beak condition, evidence of cannibalism, injuries, diarrhea, nasal and respiratory discharges, conjunctival exudates, feather and comb conditions, dehydration, and body condition. These are all useful clues.

**Blood Samples**

Blood specimens may be taken at this time (or immediately after the bird is euthanized). Frequently, it is desirable to have 2 (paired) blood samples several days apart to determine a rising or falling titer of antibodies to some disease (Newcastle disease) in the serum. In this case, a blood specimen may be taken from the main (brachial) wing vein or jugular vein or by heart puncture, and the bird then is saved for a second sample.

Venipuncture of the brachial vein is usually the simplest and best method for obtaining blood from turkeys, chickens, and most poultry under field conditions, especially when the bird is to be returned to the flock. Ducks are bled from the saphenous vein near the hock. Expose the vein to view by plucking a few feathers from the ventral surface of the humeral region of the wing. The vein will be seen lying in the depression between the biceps brachialis and triceps humeralis muscles. It is more easily seen if the skin is first dampened with 70% alcohol or other colorless disinfectant. To facilitate venipuncture, extend both wings dorsally by gripping them firmly together in the area of the wing web with the left hand. Insert the needle into the vein of the right wing holding the syringe in the right hand (Figure 1.7). The needle should be inserted opposite to the direction of blood flow.

Heart puncture can be made anteromedially between the sternum and metasternum (44), laterally through the rib cage, or anteroposteriorly through the thoracic inlet. Only through experience can one learn exactly where and at what angle to insert the needle. It is best to practice these techniques on freshly killed specimens before attempting to bleed live birds. A general rule for the lateral puncture is to form an imaginary vertical line at the anterior end of—and at a right angle with—the keel, and then palpate along that line. The heartbeat can be felt, and the needle inserted to the proper depth.

For heart puncture through the thoracic inlet, the bird should be held on its back with the keel up. The crop and contents are then pressed out of the way with a finger while the needle is guided along the ventral angle of the inlet. After penetrating the inlet, the needle is directed horizontally and posteriorly along the midline until reaching the heart.

The site for heart puncture between the sternum and metasternum is (in a mature chicken) about an inch above and posterior to the anterior point of the keel. The needle is directed at approximately a 45-degree angle in the anteromedial direction toward the opposite shoulder joint. The needle should pass through the angle formed by the sternum and metasternum and directly into the heart. For further details and illustrations, see Hofstad (44).

The size and length of the needle required for heart and venipuncture depends on the size of the bird: for young chicks and poults, use a 1/2-inch, 20-gauge needle; for mature chickens, a 2-inch, 20-gauge needle is needed. Mature turkeys may require larger needles. For quick and accurate bleeding, it is essential that the needle be sharp. A very slight vacuum should be developed intermittently to determine when vein or heart puncture has occurred. After vein puncture, a steady, slight vacuum should be continuous to withdraw blood. If the vacuum is too great, the vessel wall may be drawn into the needle and plug the beveled opening. It is sometimes necessary to rotate the needle and syringe to be sure the beveled opening is free in the lumen of the vessel.
For most serologic studies, the serum from 2 ml blood is adequate. The blood should be removed aseptically and placed in a clean vial, which then is laid horizontally, or nearly so, until the blood clots. An occasional sample may require a long time to clot. This is especially true of turkey blood. Clotting can be hastened by adding a drop of tissue extract, made by killing and pooling a number of 10- to 12-day-old chicken embryos, grinding in a blender, and freezing for future use. After the clot is firm, the vial may be returned to the vertical position to permit serum to collect in a pool at the bottom. Plastic vials are available for blood collection. The clot does not adhere to the vial, and special positioning during clotting is unnecessary. Frequently, the serum from fat hens will appear milky due to lipids. Placing vials in an incubator will hasten the separation of the blood clot and serum. A fresh blood sample should never be refrigerated immediately after collection because this will hinder the clotting process. Sera should not be frozen if agglutination tests are to be performed because this frequently causes false-positive reactions.

If an unclotted blood sample is required, it should be drawn into sodium citrate solution at the rate of 1.5 ml 2% solution/10 ml fresh blood, or deposited in a vial containing sodium citrate powder at the rate of 3 mg/1 ml whole blood, and the mixture should be shaken gently. One way to prepare tubes for collecting sterile citrated blood is to add the proper amount of 2% sodium citrate solution to the collecting tubes ahead of time and then sterilize the solution and evaporate the moisture in an oven.

Blood-collecting vials containing the anticoagulants heparin or ethylenediaminetetraacetic acid (EDTA) also can be obtained commercially from laboratory supply companies. For certain types of serologic tests, fresh blood can be absorbed on the tips of filter paper strips, dried, and sent to the diagnostic laboratory, where antibodies can be recovered for testing by placing pieces of the treated paper into saline solution.

If a blood parasite or blood dyscrasia is suspected, smears of whole blood should be made on clean glass slides previously warmed to promote rapid drying. For staining techniques, see Campbell (20).

A drop of blood for a wet mount or smear may be obtained from very small chicks by pricking the vein on the posteromedial side of the leg or by pricking or cutting the immature comb.

**Killing Birds for Necropsy**

**Cervical Dislocation**

Several methods can be used to kill poultry, and each has certain advantages. The objective is to kill the bird instantaneously so it will not suffer in the process. Cervical dislocation, as described, is considered a humane method of poultry euthanasia by the American Veterinary Medical Association (AVMA) (3).

Bovine Burdizzo castration forceps can be used for killing large chickens and turkeys. It is difficult for one person to perform this operation and hold the bird at the same time, but it is quite easily done with the aid of an assistant. This technique also prevents agonal regurgitation and aspiration of crop contents into the respiratory passages if the forceps are left clamped until reflex muscle spasms cease. The neck of a young chick also can be broken easily by pressing it firmly against a sharp table edge, or by pinching between thumb and index finger, or by using the inside, noncutting angles of a surgical scissor such as a small Burdizzo.

**Others**

Specimens selected for diagnosis also may be killed by intravenous injection of euthanasia solutions. Another satisfactory method is euthanasia by placing the bird in a chamber filled with carbon dioxide (CO₂). Local availability of a CO₂ source may limit use of this technique. Other methods of euthanasia can be found in a report of the AVMA (3). The method selected depends upon the existing situation: species, size, and number of birds to be necropsied or sacrificed; tissues, fluids, and cultures to be taken; etc.

**Necropsy Precautions**

If there is reason to suspect that birds to be necropsied are infected with disease that may be contagious for humans (chlamydiosis, erysipelas, or equine encephalitis), stringent health precautions are essential. The carcass and the necropsy table surface should be wet thoroughly with a disinfectant. The carcass and the necropsy table surface should be wet thoroughly with a disinfectant. Good rubber gloves should be worn and care should be taken that neither the pathologist nor assistants puncture the skin of their hands or inhale dust or aerosols from tissues or feces. It is advisable to wear a fine-particle respiratory mask to prevent inhalation of contaminated dust. All laboratory personnel who may come in contact with carcasses, tissues, or cultures should be informed of their possible infectious nature and precautions to be taken.

With some notable exceptions (see sections on the specific diseases), most commonly encountered poultry disease agents are not considered pathogenic for humans. Nevertheless, it is wise to wear rubber gloves at all times while performing necropsies. For a review of poultry diseases in public health, see Galton and Arnstein (37) or the Public Health Significance of Poultry Diseases chapter below.

Adequate instruments for routine work are necropsy shears to cut bones, enterotome scissors to incise the gut, a necropsy knife to cut skin and muscle, and a scalpel for fine examination of tissues. These should be supplemented with forceps, sterile syringes, needles, vials, and petri dishes for collecting blood samples and tissue specimens as the situation dictates.

**Necropsy Technique**

**Internal Organs**

The specimen is laid on its back and each leg in turn drawn outward away from the body while the skin is incised between the leg and abdomen. Each leg is then grasped firmly in the area of the femur and bent forward, downward, and outward until the head of the femur is broken free of the acetabular attachment so that the leg will lie flat on the table (Figure 1.8A).
Figure 1.8. Each pathologist will develop his or her own systematic technique for conducting a necropsy. The illustrated technique will aid the beginner. (A) The skin and fascia between the leg and abdomen are cut, and the legs are pulled and twisted to disarticulate the head of the femur (arrow) from the hip. (B) The skin from the vent to the beak is incised and reflected. (C) The body cavity is entered at the ventral tip of the sternum. The incision is made at the margin of the pectoral muscle and continues through 2–3 ribs. A similar incision is made on the opposite side of the breast. (D) The shears are reoriented (arrows), and the incision is continued through bone and muscle to the thoracic inlet. The breast is broken over to the opposite side (or removed), exposing the viscera. At this point of the necropsy, microbiological samples are collected. (E) The intestinal viscera are freed by cutting through the esophagus and vessels of the liver just anterior to the proventriculus and liver. Heart (H), liver (L), and proventriculus (P) are indicated. (F) The intestines can be removed by gentle traction, which tears mesenteric and air sac attachments. The lungs, heart, and kidneys remain in the body cavity for later examination.
The skin is cut between the 2 previous incisions at a point midway between keel and vent. The cut edge is then forcibly reflected forward, cutting as necessary, until the entire ventral aspect of the body, including the neck, is exposed (Figure 1.8B). Hemorrhages of the musculature, if present, can be detected at this stage.

Either of 2 procedures is now used to expose the viscera. The poultry shears are used to cut through the abdominal wall transversely midway between keel and vent and then through breast muscles on each side (Figure 1.8C). Bone shears are used to cut the rib cage and then the coracid and clavicle on both sides (Figure 1.8D). With some care, this can be done without severing the large blood vessels. The process also may be completed equally well in reverse order, cutting through the clavicle and coracid and then through the rib cage and abdominal wall on each side. The sternum and attached structures can now be removed from the body and laid aside. The organs are now in full view and may be removed as they are examined (Figure 1.8E and F).

If a blood sample has not previously been taken and the bird was killed just prior to necropsy, a sample can be promptly taken by heart puncture before clotting occurs. Large veins leading into the leg may be incised, allowing blood to pool in the inguinal region for subsequent collection.

**Laboratory Procedures**

**Bacterial Cultures**

If gross lesions indicate bacterial cultures are needed, they can be made from unexposed surfaces of the viscera without searing the surface. If contamination has occurred, the surface of the organs should be seared with a hot spatula or other iron designed for that purpose before inserting a sterile culture loop. Care must be taken not to sear and heat the tissue excessively. It is often desirable to transfer large tissue samples aseptically to a sterile petri dish and take them to the microbiology laboratory for initial culture in cleaner surroundings.

**Respiratory Virus Isolation**

If a respiratory disease is suspected and virus culture or bird passage is desirable, an intact section of lower trachea, the bronchi, and upper portions of the lungs is removed aseptically with sterile scissors and forceps and transferred to a sterile container. Other tissues (air sac tissue) can be added aseptically to the sample or transferred to other sterile containers for separate study. The trachea can now be incised. If exudate is present, it can be added to the preceding collection or saved in separate vials. Similar procedures can be followed for initial virus isolation from various parenchymatous organs.

**Salmonella Cultures**

All other visceral organs should be examined for abnormalities (microabscesses, discoloration, swelling, and friability). If abnormalities are observed, inoculum from the affected tissues should be transferred to suitable solid or liquid media for culture before the intestinal tract is opened. Once opened, gross contamination of other organs with gut contents is almost certain to occur. If Salmonella infection is suspected, selected sections of the gut are removed with sterile forceps and scissors and placed directly into a sterile petri dish for later culture. For routine examination, a single section comprising the lower ileum, proximal portions of the ceca and cecal “tonsils,” and proximal portion of the large intestine may be used. All are minced or ground aseptically to produce an inoculum. Additional areas of the intestinal tract or tissues of other visceral organs may be added to the gut collection or cultured separately. Alternatively, sterile swabs may be used to obtain samples from the exposed gut lining for Salmonella cultures. See Chapter 2 of *A Laboratory Manual for Isolation and Identification of Avian Pathogens* (32) for detailed culture technique.

**Gross Necropsy**

After necessary cultures have been collected, a thorough gross examination of all tissues should be performed. Enlargement of the liver, spleen, and kidney should be evaluated. A clear indication of hepatomegaly is rounded liver margins. The intestine may be examined for inflammation, exudates, parasites, foreign bodies, malfunctions, tumors, and abscesses. The various nerves, bone structure, marrow condition, and joints can now be examined. The sciatic nerve can be examined by dissecting away the musculature on the medial side of the thigh. Inside the body cavity, the sciatic plexus is obscured by kidney tissue. These nerves can best be exposed by scraping away the tissue with the blunt end of a scalpel. Nerves of the brachial plexuses are easily found on either side near the thoracic inlet and should be examined for enlargement. Examination of vagus nerves in their entirety should be made, or otherwise short enlargements may be missed.

The ease or difficulty with which bones can be cut with the bone shears is indicative of their condition. The costocondral junctions should be palpated and examined for enlargement (“beading”) and the long bones cut longitudinally through the epiphysis to examine for abnormal calcification. Rigidity of the tibiotarsus or metatarsus should be tested by bending and breaking to check for nutritional deficiency. A healthy bone will make an audible snap when it breaks. Bones from a chicken deficient in vitamin D or minerals may be so lacking in mineral elements that they can be bent at any angle without breaking.

Joint exudate, if present, can be removed after first plucking the feathers and searing the overlying skin with a hot iron. After searing, the skin may be incised with a sterile scalpel and exudate removed with a sterile inoculating loop or swab. Paranasal sinus exudates can be removed and examined in a similar manner.

**Exposure and Removal of Brain**

Removing the intact brain is not easy, because meningeal layers are attached firmly to bony structures in some places. The following technique can be performed quickly and is satisfactory for examination and removal of the brain in most instances.
Remove the head at the atlanto-occipital junction and remove the lower mandible. Sear the cut surface and trim away excess loose tissue. Reflect the skin forward over the skull and upper mandible and hold it firmly in that position with 1 hand. Sterile instruments should be used for the succeeding steps if a portion of the brain is desired for animal inoculation, virus isolation, or fungal or bacterial culture.

With the sterilized tips of heavy-jawed bone shears or strong surgical scissors, nip just through the bone to the cranial cavity on both sides of the head, beginning at the occipital foramen and proceeding forward laterally to the midpoint at the anterior edge of the cranial cavity (Figure 1.9A). Lift off the cut portion of bone and expose the entire brain (Figure 1.9B).

If a portion is needed for culture or animal inoculation (e.g., avian encephalomyelitis virus suspect) and also for histopathologic examination (e.g., vitamin E deficiency), cut the brain medially from anterior to posterior along the midline with a sharp, sterile scalpel blade. With sterile, sharp curved scissors, cut the nerves and attachments carefully from 1 of the brain halves while the head is tipped upside down, so that the loosened portion falls into a jar of formalin as it is freed (Figure 1.9C). The second half can now be removed aseptically (but without concern for preservation of tissue structure) to a sterile petri dish or sterile mortar and pestle. Be careful not to contaminate brain tissue intended for virus isolation with instruments that have been in contact with formalin. The separate halves also may be removed in reverse order (Figure 1.9D). If all of the brain is required for either purpose, proceed with proper precautions for the purpose intended. If the brain is destined only for sectioning, it may be fixed in situ and then removed. Large brain portions should be incised longitudinally to permit good penetration of fixative.

Figure 1.9. With a little practice, the brain can be removed with a minimum of trauma. (A) Incise bone all the way around the periphery of the cranial cavity with heavy bone shears. (B) Remove loosened portion of the bony skull. (C) Incise brain longitudinally with sterile, sharp scalpel and remove one-half for sterile culture technique. (D) Remove second half by dropping it into 10% formalin for histologic techniques.
Tissues for Histopathologic Examination
Frequently, stained tissue sections are needed. The quality of the slide is no better than the quality of the specimen and the care taken to preserve it. For good preservation, the tissue pieces from killed birds should be saved immediately after death, especially gastrointestinal, brain, and kidney tissues, which deteriorate rapidly. Specimens should be small to allow quick penetration of fixative, gently incised with a sharp scalpel or razor blade to preserve tissue structure, and preserved in 10 times their own volume of 10% formalin or other fixative. Bone pieces should be saved with a sharp bone saw unless thin or soft enough to cut with scissors or scalpel. After proper labeling and dating, they should be sent immediately to the processing laboratory.

Lung tissue usually floats on the surface of the fixing solution because of trapped air. Satisfactory fixation can be accomplished by placing absorbent cotton over the tissue, which serves to keep it immersed. Methods to exhaust air from air spaces in lung tissue by creating a vacuum over the fixative can be used but are less satisfactory and may result in artifacts.

After fixing, bone tissue must be decalcified by immersion in a decalcification solution made by mixing equal parts of aqueous 8% hydrochloric acid and aqueous 8% formic acid (66). Decalcification typically takes 1–3 days, depending on the size and density of the bone sample.

If eye tissue is to be saved for sectioning, the whole eye should be removed and all ocular muscles trimmed off the globe to allow for rapid penetration by the fixative.

Any tissue held too long in formalin fixative becomes excessively hard. If processing is to be delayed, tissues should be transferred to 70% alcohol after 48 hours in fixative. Textbooks on histologic techniques (65, 66, 80) should be consulted for detailed procedures.

Progressive Examination Hints
The following procedures during the course of necropsy may be helpful to the beginner in checking for some commonly encountered diseases. They are not intended as definitive diagnostic methods. To arrive at a diagnosis, the student and beginning diagnostician must refer to the characteristic signs and lesions, diagnostic procedures, and characteristics of the infectious agent discussed under the specific diseases in succeeding chapters, and also to the manual Isolation and Identification and Characterization of Avian Pathogens (7).

Coccidia. Observe and note the subserosa before incising the intestine. Make wet mount smears of mucosal scrapings from various segments of the intestine and cecal contents and examine directly under the microscope for suspended oocysts and merozoites and stages undergoing development in epithelial cells (tissue stages).

Other Protozoa. Make wet mounts of affected areas, adding a little warm physiologic saline solution if necessary to provide fluid, and examine under a microscope for hexamita, histomonads, and trichomonads.

Capillarids and Ascarid Larvae. Collect mucous exudate and deep mucosal scrapings and press into a thin layer between 2 thick pieces of plate glass. Examine before a strong light or under low-power magnification for the presence of parasites. Under magnification, look for bipolar, lemon-shaped eggs in the female capillarids.

Fungi. Make wet mount smears of scrapings of affected areas and add 20% sodium or potassium hydroxide. Digest with frequent warming for 15 minutes or more and examine under high-power magnification for mold hyphae.

Campylobacter. Examine fresh bile wet mounts under dark-field or phase illumination. Only positive findings may have significance.

Bacteremia and Blood Parasites. Make fresh mounts, preferably with citrated blood, and examine under light- and dark-field illumination for viable organisms. Make fresh blood smears and air dry for staining by Giemsa’s, Gram’s, Wright’s, or other method.

Exudates. If infectious coryza is suspected, make thin smears of clear nasal or sinus exudate for staining by Giemsa’s, Gram’s, methylene blue, or other method. Inoculate appropriate media or susceptible chickens for isolation of the organism.

Abscesses. Select appropriate culture media suitable for the growth of a variety of infectious organisms that may be suspected of causing the abscess. Sear and incise the surface of the abscess and inoculate culture media with the extracted material, using a sterile inoculating loop or swab. Make smears from the abscess on clean glass slides, diluting with a drop of water if the material is too thick. Air dry and flame slides and make Gram’s, acid-fast, or any other stain as desired.

Embryo Inoculation for Virus Isolation. For routine virus isolation, centrifuged and/or filtered fine-ground suspensions of suspect tissues (trachea, bronchi, lung, liver, spleen, kidney, brain, bone marrow) or body fluids and exudates may be inoculated into the chorioallantoic cavity and yolk sac and onto the chorioallantoic membrane (CAM) of embryos at various stages of incubation. See discussions of the specific diseases for virus culture techniques. Also see A Laboratory Manual for the Isolation and Identification of Avian Pathogens (32) for selection of the proper age of embryo and route of inoculation for various disease agents as well as detailed inoculation procedures. Embryos from specific pathogen free hens should be used for culture to be sure that any agent recovered originated in the inoculum and not in the hens that produced the eggs. Equally important is assurance that negative cultures are due to absence of infectious agents in inoculum, rather than to interference of passive antibodies in eggs. Because the purpose of virus isolation is to determine which may be present, it is advisable to inoculate
various ages of embryo by the various routes. Several blind passages may be necessary before the culture attempt can reasonably be considered negative. A simple technique that does not require dropping the CAM has been described (43).

The CAM may be drawn away from the shell (dropped) to facilitate inoculation. First, drill or punch a small hole in the shell over the air cell and then slowly drill or punch a second hole through the shell at a point on the side over the embryo. Applying mild suction through a rubber tube over the hole into the air cell causes the CAM to drop away from the inner shell membrane under the second drilled hole. A bright candle light should be used while suction is applied to determine when the CAM has dropped.

For yolk sac inoculation, the needle can be directed through the air cell and directly to the center of the egg. Some yolk may be withdrawn into the syringe to verify the location of the needle.

For chorioallantoic cavity inoculation, a hole is drilled over the edge of the air cell at a spot previously marked with the aid of a candle light. The cavity lies adjacent to the shell and can be easily penetrated from that point. All holes should be sealed with suitable sterile material before reincubating.

Cell culture procedures are becoming more common in diagnostic laboratories. Technicians with this capability may inoculate the cell cultures directly with tissue extracts or body fluids, or they may use embryos for primary screening and transfer embryo fluids or extracts to cell culture for further study and identification.

Disposing of the Specimen
If a disease infectious for humans is suspected, the carcass should be autoclaved, incinerated, or otherwise rendered incapable of transmitting the pathogen to laboratory or other personnel. Similar precautions should be followed during disposal of carcasses infected with a virulent poultry pathogen that presents a health hazard to the industry. The necropsy area, instruments, and gloves should then be cleaned, washed, and disinfected.

Communication
Flock owners are not always interested in technical data. They want to know what the problem is and what should be done to correct it and/or how to prevent reoccurrences. Sometimes technical data are necessary to clarify the diagnosis, but the report should be in language and terms that owners will understand. A minimum of complicated scientific and medical technology words should be used. When medical terms are apt to be confusing, they should always be explained in lay terms.

The report should include the necropsy findings, results of laboratory studies, (histopathologic, serologic, and cultural), diagnosis (temporary or final), and conclusions and recommendations. The owner is seeking professional advice. The veterinarian should give his or her best conclusions and recommendations based on the facts available. A verbal report or telephone call to the flock owner, manager, or service worker soon after completion of the necropsy and initial tests is highly advisable. A tentative diagnosis can be offered pending further confirmation.

Disease Control: Biocontainment
Disease control strategies are designed to reduce the consequence of disease challenge by limiting challenge (bioexclusion), enhancing bird resistance (immunization), and preventing spread (quarantine). In the case of eradicable diseases, quarantine is usually followed by emergency slaughter. Control measures are implemented routinely for diseases that are endemic to the epidemiological unit and sporadically when there is an unexpected epidemic disease outbreak.

The word quarantine has several different meanings: (1) enforced isolation of animals that may have been exposed to a contagious or infectious disease (e.g., when entering a country), (2) a place in which animals spend a period of isolation to prevent the spread of disease, and (3) the period of time during which animals are kept in isolation to prevent the spread of disease. For live bird and product importation, quarantine is routine. To prevent the introduction of a pathogen into a country, region, zone, or compartment it is essential that potentially infectious material is kept in isolation until it have been shown to be clear of the pathogen(s) in question.

In a production setting quarantine is the first step of biocontainment and it involves the immediate enforced isolation of birds that have been exposed to a contagious disease. The movement of anything into, onto, from, or through the area of control must be restricted and monitored. The extent of the control zone depends on the risk associated with the disease but usually involves the house, farm, site, or complex within a particular company. If the disease is of national or regional importance, the control zone is usually a circle with a 2-mile radius around the affected farm. It is important to establish the extent of the disease outbreak through disease monitoring, first within the quarantine zone and then in a demarcated surrounding contact zone. In the case of foreign/notifiable diseases the relevant veterinary authority assumes control. In the United States, each state has a predetermined emergency response plan carefully designed to handle all of the relevant details of containment and eradication.

Chemoprophylaxis
Prophylactic medication in the form of in-feed medication, and in specific cases, water medication, may be used to reduce the risk of disease by reducing agent virulence.

\[
\text{Risk of infection} = \frac{\text{challenge dose} \times \text{agent virulence} \times \text{challenge frequency}}{\text{resistance}}
\]

Chemoprophylaxis is used routinely in the control of coccidiosis worldwide. Chemicals and ionophores are added to broiler diets to reduce virulence of the *Eimeria* species challenge.
Immunization

Immunization through vaccination is a commonly used method of reducing the risk (increased ID$_{50}$) and consequence (reduced pathogenicity) of bird or flock exposure to a disease-causing agent. Vaccination is the practice of administering live and/or killed vaccines which have been modified to minimize disease manifestation yet maximize immunity. The primary purpose of immunization is to raise the ID$_{50}$ of the flock to prevent clinical disease following subsequent challenge. While some vaccines are given to protect that individual bird against disease, others are given to pass the protection on to the next generation, and others are given to prevent disease in the hen and subsequent transmission of the disease to the chick.

Vaccines and vaccine programs vary widely in their effectiveness, and this is frequently by design. Some vaccines are designed to incite high levels of immunity to protect birds in the face of aggressive endemic disease challenges, such as vvND. These vaccines may cause a mild form of the disease themselves but are deemed appropriate and useful because of the risk associated with eventual infection of the deadly field pathogen. Vaccine selection and how they are programmed frequently becomes an exercise in risk management and cost efficiency. Local conditions must always be considered when evaluating and critiquing a vaccination program.

A second reason for the vaccination of poultry flocks is to hyperimmunize hens to maximize maternally derived antibody passed through the egg to the hatching progeny. Chicks frequently receive up to 3 weeks of protection from maternal antibodies, allowing their immune system to mature to a level capable of eliciting an efficient active immune response if exposed to a potentially harmful virus or bacteria. Antibodies are not always completely protective but for viruses such as infectious bursal disease (IBD), many areas of the world have found maternal antibodies a very useful tool in IBD prevention and control.

The success of vaccination does not rest solely with the manufacturing or research of vaccines. More important is the maintenance of the cold chain, protection of the vaccine from the elements, and the correct application of the vaccine to the bird. Vaccination programs should be documented for each operation by the responsible veterinarian and operations manager. All vaccines must be stored at the correct temperature. Most vaccines require refrigeration at 2°C to 8°C. Some vaccines, mostly killed oil vaccines, can be safely stored at room temperature. Some vaccines need to be stored at temperatures below 0°C. Vaccines are adversely affected by exposure to sunlight and heat. Vaccines must be administered using suitably cleaned equipment and be given to every bird in the defined epidemiological unit.

Types of Vaccines

Poultry vaccines are typically characterized as live or inactivated. General characteristics of vaccines are summarized in Table 1.1 (18). Live vaccines are available for numerous viral, bacterial, and coccidial organisms.

Techniques used in the development of live vaccines have varied widely. Table 1.2 shows some of the most common methods used to generate an acceptable live vaccine candidate and examples of each method.

Live vaccines are widely used throughout the world because they are commonly effective when mass applied, and they are relatively economical. Immunity from live vaccines is generally short-lived, particularly following initial exposure. Some exceptions to this exist for vaccines such as for infectious laryngotracheitis, fowlpox, and Marek’s disease, which give long-lived immunity.

For live vaccines to work as they were designed, they must be stored, mixed, dosed, and applied appropriately. Storage of live vaccines is generally in a dark, refrigerated area. Liquid nitrogen freezing of live vaccines preserves and prolongs cell culture viability that is essential for cell-associated vaccines such as Marek’s disease vaccines. Licensed live vaccines have an expiration date printed on the vial that, if stored according to label directions, ensures that the appropriate minimum dose is maintained through the dating period. Shelf life varies widely with live vaccines, but most generally are licensed with 18 months to 2 years before expiration. Mixing directions also vary widely, but many recommend the use of a water stabilizer such as powdered skim milk. Water stabilizers minimize some of the negative effects of residual chlorine, metals, and high temperature on the reconstituted virus. Cell-associated Marek’s vaccines generally have very specific diluents aimed at maintaining cell culture viability through the time period between reconstitution and inoculation. The dose needed to get an appropriate immune response from a live vaccine is frequently dependent on the virus, genetic background of the bird, age of the bird, existing circulating antibody within the bird, and the method to be used when applying a vaccine. Vaccines generally are licensed based on protection studies performed in an SPF-type leghorn bird, without any circulating antibody to that particular agent, at the youngest age on the label, and at the minimum titer expected at the end of the dating period allowed for each given vaccine. With all of these variables, it is not difficult to imagine why clinical veterinarians and other health professionals may adjust dosages of live vaccines according to local field conditions.

Severe vaccine reactions or insufficient protection can result from misjudging any of these variables. As a final note, poultry house conditions and local disease risks need to be taken into account when optimizing the use of live vaccines.

A second type of live vaccine is emerging with the development of genetically engineered, live virus- and bacteria-vectored vaccines and gene deletion mutants of a pathogenic parent organism. The recombinant vaccines are made using live virus or bacteria as a vector to transport the gene coding for the protective antigen of a second infectious agent, for which immunity is desired. Examples of live virus-vectored vaccines include recombinant fowlpox virus vaccine expressing genes to protect against H5 avian influenza (13), fowlpox virus expressing Newcastle disease virus antigen (17), fowlpox virus expressing infectious bursal disease virus antigens (11), and baculovirus expressing infectious bursal disease virus antigens (84). Bacteria-vectored vaccines described in poultry include bacteria such as E. coli (46) and Salmonella spp. (68) expressing antigens from
coccidia and *E. coli*, respectively. A vaccine to reduce *Salmonella* infection, made from a gene deletion mutant of *Salmonella typhimurium* (30), is commercially available. These recombinant and gene deletion mutant vaccines have been shown to be relatively protective, when compared to controls, against pathogenic challenge under experimental conditions. The efficacy and cost effectiveness of the recombinant vaccines under field conditions are yet to be determined. This type of vaccine may offer advantages where the spread of traditional vaccines to susceptible populations cannot be properly managed. Additionally, these technologies allow for diagnostic differentiation of vaccine from virulent field challenge. This property may be useful when used in eradication programs such as infectious laryngotracheitis. Regulatory considerations when acquiring a federal license for vectored vaccines include demonstrating the genetic and phenotypic stability of recombinant viruses or bacteria and documenting any alterations in the host range or tissue tropism of the recombinant organism, as compared to the parent organism (62).

Inactivated vaccines or killed vaccines used in poultry are generally whole bacteria or virus preparations combined with an adjuvant that are designed for subcutaneous or intramuscular injection. They are frequently, but not always, used in commercial egg layer and breeding birds to stimulate long-lasting immunity and/or antibody levels to specific antigens. Inactivated vaccines generally consist of 2 distinct components, often referred to as aqueous and adjuvant phases, emulsified into a homologous liquid. The aqueous phase contains the antigen and the adjuvant generally enhances the bird’s response to this antigen. The ratio of antigen to adjuvant differs greatly depending on the vaccine. This ratio generally is determined by factoring in the properties of the adjuvant(s), the antigen(s), viscosity, immune response, and tissue reactivity. Mineral oil is the most commonly used adjuvant, although aluminum hydroxide is a common alternative in notoriously reactive inactivated vaccines such as fowl cholera and infectious coryza. Adjuvant technology continues to grow, and vegetable, fish, and animal oils used as adjuvants offer some opportunities for lower viscosity, immunogenic vaccines (65).

### Table 1.1. General characteristics of live and inactivated vaccines for poultry.

<table>
<thead>
<tr>
<th>Live vaccines</th>
<th>Inactivated vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller quantity of antigen. Vaccination response relies on multiplication within the bird.</td>
<td>Large amount of antigen. No multiplication after administration.</td>
</tr>
<tr>
<td>Can be mass administered—drinking water, spray.</td>
<td>Almost always injected.</td>
</tr>
<tr>
<td>Adjuvantage live vaccines is not common.</td>
<td>Adjuvanted killed vaccines is frequently necessary.</td>
</tr>
<tr>
<td>Susceptible to existing antibody present in bird.</td>
<td>More capable of eliciting an immune response in the face of existing antibody.</td>
</tr>
<tr>
<td>In immune bird, booster vaccination is ineffective.</td>
<td>In immune bird, additional immune response frequently seen.</td>
</tr>
<tr>
<td>Local immunity stimulated (i.e., trachea or gut).</td>
<td>Local immunity may be re-stimulated if used as a booster but poor if not a secondary response.</td>
</tr>
<tr>
<td>Danger of vaccine contamination (e.g., egg drop syndrome, reticuloendotheliosis virus).</td>
<td>Little danger of vaccine contamination.</td>
</tr>
<tr>
<td>Tissue reaction commonly referred to as a “vaccine reaction” is possible and frequently visible in a variety of tissues.</td>
<td>No microbe replication; therefore, no tissue reaction outside that which is adjuvant dependent.</td>
</tr>
<tr>
<td>Relatively limited combinations—due to interference of multiple microbes given at the same time (e.g., infectious bronchitis, Newcastle disease virus, and laryngotracheitis).</td>
<td>Combinations are less likely to interfere.</td>
</tr>
<tr>
<td>Rapid onset of immunity.</td>
<td>Generally slower onset of immunity.</td>
</tr>
</tbody>
</table>

### Table 1.2. Methods of generating live vaccine candidate.

<table>
<thead>
<tr>
<th>Method</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virulent organism inoculated to a less susceptible target tissue or at a controlled dose</td>
<td>Laryngotracheitis-cloacal route</td>
</tr>
<tr>
<td>Naturally occurring mild pathotype</td>
<td><em>Mycoplasma gallisepticum</em> F strain</td>
</tr>
<tr>
<td>Egg passage of virulent parent</td>
<td>Infectious bronchitis—Arkansas strain</td>
</tr>
<tr>
<td>Temperature-sensitive mutant of virulent parent</td>
<td>Turkey coryza vaccine—<em>Bordetella avium</em></td>
</tr>
<tr>
<td>Chemically derived mutants of virulent parent</td>
<td>M-9 Fowl cholera vaccine</td>
</tr>
<tr>
<td>Tissue culture / passage of virulent parent</td>
<td>Laryngotracheitis</td>
</tr>
<tr>
<td>Combination of egg passage and tissue culture passage of virulent parent</td>
<td>Infectious bursal disease—Lukert virus</td>
</tr>
<tr>
<td>Plaque selected “clones” of parent virus</td>
<td>Newcastle disease virus—cloned Lasota vaccines</td>
</tr>
<tr>
<td>Selection of subpopulations or organisms based on replication characteristics <em>in vivo</em></td>
<td>Precocious strains of <em>Eimeria</em> spp.</td>
</tr>
<tr>
<td>Relatively virulent organisms given at an age that minimizes disease</td>
<td>Avian Encephalomyelitis</td>
</tr>
</tbody>
</table>
Accidental injection of humans that are administering these inactivated vaccines should be prevented. Serious injuries have been reported from accidentally injecting vaccine into a finger or hand. The site of injection can become swollen, red, and painful, and the function of the area may be affected. Victims should seek medical treatment at once and inform attending physicians of the organism(s) and adjuvant contained in the inactivated vaccine.

DNA vaccines are an entirely new type of vaccine that evolved in the late 1990s. These vaccines can achieve both humoral and cell-mediated immunity, are similar to live vaccines, and have the relative safety associated with inactivated or vectored vaccines. Experimental DNA vaccines have been tested with success in poultry for avian influenza and Newcastle disease in chickens (36, 70) and duck hepatitis B in ducks (82). Although promising, DNA vaccines have both technological and economical challenges to overcome before they are commercially viable.

**Vaccine Delivery Systems**

Improper vaccine application is the most common reason vaccines and vaccine programs fail. With the success and growth of the poultry industry throughout the world came tremendous challenges in efficient and economic application of poultry vaccines. The most commonly used application techniques in commercial poultry include *in ovo* at 17–19 days of embryonation, subcutaneous or intramuscular injection at day of hatch, spray in the hatchery, intraocular or nasal drop in the hatchery or on the farm, spray on the farm, through the drinking water on the farm, wing web stab, and subcutaneous or intramuscular injection on the farm.

**In Ovo Vaccination.** *In ovo* vaccination is performed during the process of transferring incubating eggs in the hatchery from the setter to the hatch. After poking a hole in the shell, vaccine, most frequently Marek’s disease vaccine, is injected just under the membranes at the floor of the air cell. Depending on the embryo age at transfer, generally between 17 and 19 days of incubation for chickens, approximately 25%–75% of the vaccine (0.05 ml in most cases) is injected into the area of the neck and shoulder. In the remaining 25%–75%, vaccine is administered into the extra embryonic compartment (40). The original experiments on *in ovo* vaccination with Marek’s disease vaccine showed that chicks were protected earlier than those vaccinated after hatch (73). However, in the United States, where more than 80% of broiler chickens are vaccinated against Marek’s disease *in ovo*, the primary reason for its acceptance has been the labor savings when compared to day-old injection (81). Using an egg injection system (Embrex Inovoject Egg Injection System, Research Triangle Park, NC), 1 machine with 3 people generally inoculates 20,000–30,000 eggs/hour (Figure 1.10). This method of vaccination leaves a hole in the egg for the final few days prior to hatch and in poorly sanitized hatcheries has resulted in poor early livability due to bacterial or fungal contamination while in the hatch. Hatcheries must be acutely aware of their aspergillosis levels to run an egg injection system successfully (87).

**Subcutaneous or Intramuscular Injection at Day of Hatch.**

Day-old vaccination, most commonly using Marek’s disease vaccine, is generally accomplished by giving 0.2 ml of vaccine subcutaneously under the skin at the back of the neck or 0.5 ml intramuscularly in the leg. The automatic vaccination machines used in many parts of the world generally are designed for neck injection. A skilled operator can vaccinate about 1,600–2,000 chicks/hour. A 20-gauge needle generally is used, because smaller gauge needles restrict the flow in cell-associated vaccines. Needles should be changed several times during the course of the day to prevent damage from burred or bent needles. Improper positioning of the chick or a bent needle can result in damage to the neck muscles or vertebrae. A dye is frequently mixed with the vaccine to allow visualization of the vaccine under the skin after injection. A quality check of technique generally means examining each bird in several boxes, 100 to a box, after vaccination, looking for colored dye under the skin. The most frequent cause of missed birds is the operator trying to go too fast, resulting in chicks being pulled off the needle before proper deposition of vaccine.

**Spray in the Hatchery.** Spray vaccination of birds in the hatchery generally is done using a spray box that is triggered each time a box of chicks is placed inside or an in-line spray cabinet that sprays boxes as they move down a controlled-speed conveyor line in an automated hatchery. Both methods, frequently used to deliver NDV, infectious bronchitis virus, or coccidiosis vaccine, attempt to mimic eye-drop vaccination. Spray vaccination in the hatchery generally works well if the droplets generated have a particle diameter of approximately 100–150 microns. Particle size is very important. Low relative humidity may decrease the particle size by the time it reaches the bird, resulting in too fine a spray. Fine spray, generally something less than 20 microns in diameter, can travel deep into the respiratory tract, resulting in excessive vaccine
reaction if using a respiratory disease vaccine. Although there is some variability, NDV and infectious bronchitis virus vaccines are often delivered in 7 ml of distilled water/100 chicks. Coccidiosis vaccines generally use more distilled water, approximately 20–25 ml/100 chicks. Birds preening themselves and each other immediately following spray vaccination is thought to be important to the resulting vaccination response, although little data exists to support this concept.

**Spray Vaccination on the Farm.** With the increased acceptance and use of closed watering systems and the increased cost of labor required to effectively vaccinate through the drinking water, spray vaccination of respiratory vaccines, such as NDV and infectious bronchitis virus, has become increasingly popular. This method of vaccination frequently uses spray equipment adapted from insecticide and pesticide application technologies. As with the hatchery spray vaccination, the method is designed to mimic eye-drop vaccination but allows the vaccinator to avoid handling each bird in the poultry house.

Distilled water generally is used to reconstitute the vaccine(s). Although the volume of water used varies depending on the spray machine selected, 5 gallons of water/20,000 birds vaccinated is a good general recommendation. It generally is preferred to vaccinate a flock first thing in the morning. Fans should be turned off, if possible, and the lights should be as dim as the vaccinator can allow and still walk through the house. In floor houses, if another person is available, 1 person can split the flock while the vaccinator slowly sprays 1 side at a time. If possible, running fans should be minimized for the 15 minutes following vaccination.

An effective spray vaccination technique allows exposure of birds to aerosolized vaccine for approximately 5–10 seconds. This is best accomplished by spraying a relatively coarse spray, in the range of 100–150 microns, and walking slowly through the poultry house.

A visual evaluation of a spray pattern can be done with each vaccination. Look for an even distribution and consistent projection. A crude estimation of droplet size may be made using the analogies listed in Table 1.3 (78).

**Intraocular or Nasal Drop in the Hatchery or on the Farm.** Intraocular or nasal drop is a highly effective but labor-intensive method used to deliver respiratory disease vaccines for diseases such as infectious laryngotracheitis. This method generally involves depositing approximately 0.03 ml of reconstituted vaccine in the eye or nares. Both techniques generally require the vaccinator to pause briefly as the vaccine disappears in the appropriate opening. A dye-colored diluent helps to visualize the vaccine and allows a quality check on technique by looking around the nares or eye for dye. Frequently some dye can be seen by looking in the bird’s mouth around the choanal cleft or edges of the tongue.

**Drinking Water Vaccination on the Farm.** A very common and useful technique in commercial poultry has been to apply vaccine through the drinking water. Proper preparation of the watering system to be used through removal of all disinfectants, such as chlorine, should be done 2 days prior to vaccination. It is best to buffer the system by flushing it with a weak solution of powdered skim milk, generally 1 cup powdered skim milk to 50 gallons of water (23). This type of buffer generally is also used while administering the vaccine.

Best results are achieved through a process that creates a mild degree of thirst by eliminating access to drinking water for approximately 2 hours prior to the vaccination procedure. This time varies widely. Climatic conditions may necessitate longer or shorter time periods. Thirst is optimal when the time between the first and last access to vaccine is approximately 2 hours. Two hours generally allows all birds, even those lower in the social order, adequate time to get a drink of water containing vaccine. This technique requires constant adjustment as the climate changes.

**Wing Web Stab.** Wing web vaccination requires individual bird handling but can be done relatively rapidly. There are 2 commonly used wing web application tools. The first is the traditional small plastic handle, approximately 3 cm long, that has 2 solid stainless steel prongs, approximately 2 cm long, with a bevel on each prong toward the needle end. The second, newer application tool is referred to as a Grant inoculator. This tool has a self-contained reservoir for vaccine, most often used for fowlpox or fowl cholera vaccination, in which a needle passes through, loading a new dose of vaccine for each bird inoculated. Both tools are designed to deliver approximately 0.01 ml on the needles to the bird’s wing web. The wing web is an area that has relatively few feathers, bone, or muscle. The vaccinator loads the applicator and sticks the needle(s) completely through the skin on both sides of the web, originating from the underside of the wing. There is little or no bleeding, and vaccine has been inoculated through the needle holes. Wing web vaccination technique can be checked by returning to the vaccinated flock 7–10 days after vaccination and palpating the wing web area for nodular scabs or granulomas. These areas created by the vaccine are commonly referred to as “takes.” Proper vaccination technique frequently results in 95%–100% take.

**Subcutaneous or Intramuscular Injection on the Farm.** Subcutaneous and intramuscular injections are frequently used in breeder pullets and commercial egg-laying pullets prior to egg production. These vaccines are generally recommended for use at least 4 weeks prior to the onset of egg production to minimize

### Table 1.3

<table>
<thead>
<tr>
<th>Analogy</th>
<th>Diameter (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet fog</td>
<td>25–40</td>
</tr>
<tr>
<td>Visible droplets</td>
<td>50</td>
</tr>
<tr>
<td>Misty rain</td>
<td>50–100</td>
</tr>
<tr>
<td>Light rain</td>
<td>200–400</td>
</tr>
</tbody>
</table>
Vaccine Failure
Numerous factors can cause a vaccine failure. One of the most common causes of vaccine failure is the inappropriate administration of the vaccine. Certain live vaccines, such as Marek’s disease vaccine, are easily killed, and failure to follow the manufacturer’s recommended handling practices will result in the inactivation of the virus prior to administration. Likewise, viable vaccines administered in the drinking water can be destroyed before they reach the bird if they are mishandled or if water sanitizers have not been removed from the water prior to the addition of the vaccine. Vaccines that are administered by intramuscular or subcutaneous injection can also fail if vaccinators do not deliver the vaccine to the appropriate vaccination site.

Although the most common cause of vaccine failure is an inadequacy or error in vaccine delivery, numerous instances of vaccines simply not providing adequate protection have occurred. In some cases, the field strain of an organism is of very high virulence, and the vaccine strain is highly attenuated. In this situation, the flock may be effectively vaccinated, but the immunity is insufficient to protect against disease completely. Many infectious agents have several different serotypes, and vaccine failure may be the result of the antigens in the vaccine serotype being different and not providing protection against the particular serotype of the agent causing the field challenge. It is not uncommon for a vaccine break to occur with infectious bronchitis virus when the field challenge is of a serotype different from that of the vaccine used (12).

Management conditions play an important role in the prevention of vaccine failures. If infectious disease agents are allowed to build up on a farm over successive flocks without clean-out and disinfection, it is possible that the challenge dose of a particular infectious agent will be so great, or so soon, that a normally effective vaccination program will be overwhelmed. The immune status of the breeder flock also can be involved in a vaccine failure. If the breeder flock provides progeny with high levels of maternal antibodies, vaccination during the first 2 weeks of life may result in the vaccine being neutralized. The timing of the vaccination of young poultry with viable vaccines must always take the presence or absence of maternal antibodies into consideration.

Certain infectious disease agents and mycotoxins are immunosuppressive and may result in vaccine failure. Infectious bursal disease virus (Chapter 7), infectious anemia (Chapter 8), and Marek’s disease virus (Chapter 15) are examples of agents that may cause severe immunosuppression in chickens. One mycotoxin, aflatoxin, has been shown experimentally to be immunosuppressive and has been implicated in decreased resistance to disease (see Chapter 32).

Handling Disease Outbreaks
Good poultry producers watch feed and water consumption and egg production at all times, but more important, they observe normal sounds and actions of the flock. They sense immediately when any of these conditions are abnormal and interpret them as signs of abnormal health. When this happens, it should be assumed that an infectious disease has gained entry and may be tracked elsewhere during the investigation period. In a modern poultry production system, any disease creates serious disruption in the economical operation of the farm and the plant’s processing products from it. Serious infectious diseases can create havoc. The following steps should be followed when disease is suspected.

Take precautions against tracking an infectious agent that may be present, but investigate management errors immediately. A high percentage of so-called disease problems referred to laboratories for diagnosis are noninfectious conditions related to management: beak trimming errors; consumption of litter and trash; feed and water deprivation; chilling of chicks; injury from rough handling, automatic equipment, or drug injection; electrical failures; cannibalism; smothering; overcrowding; poor arrangement of feeders, waterers, and ventilators; inexpensive low-quality feed ingredients; ingredients causing feed refusal; improper particle size of feed ingredients; and rodent and predator attacks (1, 15). Zander observed a severe drop in egg production in an SPF flock after a 48-hour failure of a mechanical feeder (92). Bell (14) observed marked reduction in lay from water deprivation related to a beak trimming system that resulted in long lower beaks, making it difficult to obtain water when the level was low. These are conditions that do not require the services of a diagnostic laboratory. External parasites (mites, lice, and ticks) can be determined by producers if they examine affected birds.

Quarantine the Flock
In the event that no management factors can be found, the next step is to set up a quarantine of the pen, building, farm unit area, or entire farm, depending upon its design and programming. If this emergency was anticipated when the farm...
was laid out and programmed originally, the quarantine will be a minor problem. If the basic principle of “a single age in quarantinable units” was disregarded in original farm planning, a disease outbreak can be an economic disaster. Separate caretakers should be established for affected birds or at least sick ones should be visited last.

Submit Specimens or Call a Veterinarian
The owner or caretaker should submit typical specimens to a diagnostic laboratory or call a veterinarian to visit the farm and establish the diagnosis. Owners should seek professional diagnosis rather than trying to hide some disease because of possible public recrimination. Veterinarians and caretakers can and should help dispel this apprehension by maintaining high ethical standards and refraining from discussing one producer’s problems with others. Yet, there comes a time when all producers must be apprised of a problem. Service workers frequently are requested to examine the flock, select specimens for the laboratory, and initiate first aid procedures until the veterinarian can be called or visited. If so, they should wear protective footwear and clothing when they enter the house. No other farm should be visited en route to the laboratory.

Diagnosis
It is important to get a diagnosis as soon as possible. The course of action will be determined by the nature of the disease. A producer should not procrastinate for any reason when a disease threatens, or it may get completely out of hand before a diagnosis is made. It is not always possible to treat a disease or check its deleterious effects, but to plan effectively for the future, it is important to identify any and all diseases that occur. A veterinarian should also be aware of the owner’s economic plight at such times and render advice and assistance as quickly as information is available or a judgment can be made.

Special Precautions
In addition to causing serious losses in poultry, some diseases (chlamydiosis, erysipelas, and salmonellosis) are especially hazardous for humans. When these conditions are suspected or diagnosed, extra precautions must be taken to ensure against human infection. The proper government health authorities should be notified of chlamydiosis outbreaks, and all handling and processing personnel should be apprised of the disease, hazards, and necessary precautions.

In some states, certain diseases (mycoplasma infections, avian chlamydiosis, and infectious laryngotracheitis) must be reported immediately to the state/provincial animal disease control authorities so that proper investigation and action can be taken to protect the human population and the poultry industry. Common sense dictates that when a condition suggestive of an exotic disease, such as vNMD, fowl typhoid, or avian influenza, is encountered, the proper state and federal regulatory authorities should be informed.

Nursing Care
Nursing care plays an important role in the outcome of a disease outbreak. Additional heat should be supplied to young chicks that begin huddling because of sickness. Clean and fresh (or medicated) water should be available at close range. Temporary, more accessibly located waterers are sometimes necessary during sickness. If water founts normally are located where chickens must jump onto some raised device or turkeys must cross through hot sunlight to reach them, the sick will not have the energy or initiative to seek water. They will soon become dehydrated, an early step on the road to death.

The same principles are true for feed. Sick birds can be encouraged to eat if the caretaker will proceed through the house, stirring feed and rattling feed hoppers or adding small quantities of fresh feed. Some antibiotics appear to stimulate feed consumption when included in the diet; however, any additive that proves distasteful to the bird should be removed immediately.

Sometimes birds become so depressed and moribund that the caretaker must walk among them frequently to rouse them so that they will eat or drink.

Hopelessly sick and crippled birds should be killed in a manner to preclude or control the discharge of blood or exudates (see Diagnostic Procedures). Dead and destroyed birds should be disposed of immediately (see Dead Bird Disposal).

Drugs
Therapeutic medication in response to primary and secondary disease should be prescribed by the veterinarian after the problem has been diagnosed. Therapy is not a sustainable method of disease control and should not be considered as an ongoing part of any biosecurity program. The flock response to medication merely provides the time necessary to investigate, design, and implement further control measures to avert further need for therapeutic medication.

No drugs should be given until a diagnosis is obtained or a veterinarian consulted. If the wrong drug is given it can be a waste of money or it may be harmful or even disastrous. If an infectious disease is found and corrective drugs are indicated, they should be used very carefully according to directions.

Strict regulations govern the use of drugs in mixed feeds for food-producing animals. For information, write to the U.S. Food and Drug Administration (FDA), 5600 Fishers Lane, Rockville, MD, 20857. A handy reference is the annually updated Feed Additive Compendium published by Miller Publishing Co., Minnetonka, MN. Feed manufacturers must have FDA clearance to include drugs in mixed feeds. When treated flocks are to be marketed, a specified period (depending on the drug used) must follow cessation of treatment to allow dissipation of drug residues from tissues before slaughter. If the flock is producing table eggs when treated, the drug must be permitted for use in laying flocks, or eggs must be discarded during, and for varying lengths of time after, treatment, which is a costly alternative.
If the flock is producing hatching eggs when it becomes infected and there is danger that egg transmission of the infectious agent from dams to offspring may occur (salmonellosis, mycoplasmosis, and avian encephalomyelitis), eggs should not be used for hatching until the danger has passed. It also should be kept in mind that in fertile eggs, residues of drugs used to treat breeders occasionally may cause abnormalities in some embryos.

Disposition of the Flock
The flock should not be moved or handled until it has recovered, unless the move is to a more favorable environment as part of the therapy. After treatment, if any, has been completed and the flock appears to be completely healthy, it may be marketed or moved to permanent quarters if such a move is part of the management program. Some healthy carriers may remain. If the flock is moved to another depopulated farm, this will present no problem except that occasionally a disease may flare up from stress of handling and moving. If the recovered flock is moved to a multiple-age farm, carriers can introduce the disease into susceptible flocks already there. If the recovered flock is already in permanent quarters having multiple ages, newly introduced flocks may be exposed and contract the disease, a common occurrence, especially with respiratory and litter-borne diseases.

Acknowledgement
The author is greatly indebted to Alex J. Bermudez and Bruce Stewart-Brown for their contributions to earlier editions of this subchapter.

Antimicrobial Therapy (Including Resistance)
Charles L. Hofacre, Randall S. Singer and Timothy J. Johnson

Introduction
Successfully treating a bacterial infection without any adverse effects involves many important factors when deciding to treat and the choice of antimicrobials. One side effect from antimicrobial therapy of any food animal is the potential for increasing the level of resistance in the bacterial population of those food animals. This topic will be reviewed later in this section.

This chapter will not discuss the antimicrobials or the dosages to treat particular bacteria – that discussion will be left to the authors of the chapters on each bacterial infection. This chapter will focus on the many factors that must be taken into consideration to improve the chances of a successful treatment. Treatment of commercial poultry can be divided into 3 broad categories: prevention of infection, treatment of subclinical bacteria disease, and treatment of clinically affected birds. The prevention category includes the group of antibiotics commonly referred to as the “growth promotant” antibiotics. These are a group of drugs that at the approved doses prevent clinical enteric disease, commonly referred to as necrotic enteritis, resulting from a Clostridium perfringens infection (1). In every flock of birds exhibiting clinical signs of a bacterial infection, there are birds that are healthy but susceptible, or birds incubating the disease but showing no clinical signs and the overtly ill. The choice to treat or not treat is a decision by the veterinarian based upon the proportion of birds in each category, the age of the birds (how close to slaughter), the value of the birds (breeders vs. broilers), and many other factors that will be discussed in detail.

Routes of Medication
Commercial poultry are raised to provide a safe, wholesome protein source that is very economical to the world’s human population. To that end, we must take into account the welfare of the bird and the cost of the meat. In disease prevention, it is generally accepted that feed-grade antimicrobials are less expensive than the same drug in a water-soluble formulation. It must be emphasized that sick birds will have a decline in both feed and water consumption. However, the decline in water consumption is usually less than the decline in feed consumption. Therefore, in choosing a route to administer an antimicrobial to a clinically affected flock, especially early in the infection, water medication may be more effective than by the feed. In the event that the course of the disease lasts longer than 5–7 days, as is often the case with some diseases such as Pasteurella multocida in breeders, the veterinarian may choose to switch the route of administration after initially reducing the signs by water medication to the same drug in the feed.

Another consideration in selecting a water route of administration is the ambient temperature. Because poultry have very limited means to eliminate heat from their bodies, they utilize the cooling effect of increasing water consumption. Therefore, water consumption increases significantly as the ambient temperature increases. This must be taken into account when selecting an antimicrobial and its dosage. This is especially important when considering the use of a sulfonamide, because the therapeutic dose is close to the level that can result in toxicity (2).

Flock treatment is almost always the preferred route, and thus mass methods of administering antimicrobials are
generally used. Therefore, parenteral administration of antimicrobials to individual birds in an entire flock is cost prohibitive except when the flock is in the hatchery, i.e., *in ovo* at 18–19 days of incubation or 1 day of age. If an antimicrobial is to be administered in the hatchery, be aware of the effects some antimicrobials may have on any live vaccine that may be concurrently administered. For example, the aminoglycoside gentamicin has a highly basic pH and can damage the cells for the cell-associated Marek’s disease vaccine if used at too high a dose (greater than 0.2 mg/chick) or if the antibiotic is improperly mixed with the vaccine in the diluent (3).

Feeding, watering, and lighting schedules also must be taken into consideration. Laying hens will begin to eat, and then consume water, when the lights are turned on. In replacement birds that are under feed restriction to control body weight, both feed and water are limited to only a few hours daily. Broiler chickens and turkeys, which have continuous feed and water availability, tend to eat and then drink on intermittent intervals of 3–4 hours.

### Administration of Antimicrobials to Commercial Poultry

Antimicrobials administered in the feed must be uniformly mixed and remain stable until consumed. The prescribing veterinarian must take into consideration the length of time to have the feed manufactured and transported to the farm and then the length of time to deliver it through the farm’s feeding system (i.e., amount of nonmedicated feed currently in the feed tank).

Administering the antimicrobial in the drinking water allows for a more rapid delivery of the antimicrobial but requires several calculations to be considered:

- Freshly medicated solutions should be prepared every day.
- The volume of water consumed in 24 hours in the house to be treated must be determined.
- Bulk tank medication administration method is achieved by adding the volume of medication for that day into the total volume of water to be consumed by the flock for that day.
- The proportioner administration method is used for farms that do not have a bulk tank. A water proportioner is a device that meters the antimicrobial from a highly concentrated “stock” solution into the drinking water to achieve the appropriate concentration.
- Dosing based on body weight (i.e., mg/kg of body weight) of a representative sample of birds is much preferred to dosing based on water consumption. If the dose is calculated on water consumption, the ambient temperature must be taken into consideration or a toxic overdose may occur if the temperature rises or an underdose may occur below the therapeutic level if the temperature declines. A rule of thumb is for every 1°F increase in environmental temperature above 70°F results in a corresponding increase of water consumption by approximately 4%. In addition, younger birds consume more water daily/unit of body weight than older birds. Hens in egg production drink more water/unit of weight than nonlaying hens or roosters.

- Pulse dosing can be considered when the birds’ water consumption is limited (i.e., broiler breeder pullets) (4). This is a short intensive treatment in which all of the medication to be administered in a 24-hour period is consumed by the flock in a 4- to 6-hour period. Note: This method should only be used with bactericidal antimicrobials that have a wide margin of safety for toxicity.

### Pharmacologic Consideration

The primary goal of antimicrobial treatment is to cure the flock from the current illness. Success requires taking many interacting factors into consideration. The activity of an antimicrobial against a bacterial strain is referred to as being either resistant or sensitive. The methods used to determine this sensitivity of a particular isolate are all performed on artificial media in a diagnostic laboratory. They do not consider whether the drug can be absorbed from the birds’ intestines (i.e., aminoglycosides) or whether the drug is bound by ingredients in the feed or water (i.e., tetracyclines/calcium level) or whether the drug can reach the site of the infection (i.e., synovial fluid of a joint). It should also be remembered that the *in vitro* susceptibility is usually determined on only 1 bacterial isolate from the flock and in many infections of poultry the bacterial infection is often secondary to a viral or environmental insult. This results in a flock infection of several different bacteria which may have a wide range of antimicrobial susceptibilities. This is especially true with *E. coli* airsacculitis (5).

The immune status of the flock also must be considered when selecting an antimicrobial agent. A bacteriostatic drug, such as oxytetracycline, may be highly effective for treating *E. coli* secondary to an infectious bronchitis virus challenge. For an *E. coli* airsacculitis infection following the immune suppressive virus, infectious bursal disease, the same oxytetracycline therapy may be ineffective in curing the flock. In cases in which the immune system is compromised, it is recommended to use bactericidal antimicrobials because bacteriostatic drugs inhibit or slow the bacterial growth and require the birds’ immune system to kill the bacteria.

### Judicious Use Principles in Poultry

Judicious use of antimicrobials in poultry that are being raised for production of meat or eggs for human consumption begins with disease prevention. However, when a flock begins to exhibit the clinical signs of a bacterial disease, the veterinarian must base the decision to treat upon good professional judgment (i.e., experience), laboratory results, medical knowledge, and information about the flock to be treated. The birds...
should be physically examined, if possible, by the veterinarian or by a skilled paraprofessional (service person) which should include ante-mortem and post-mortem examination. When possible, a bacterial culture can be done to confirm the diagnosis and determine the susceptibility of the isolates. The rapid spread of disease on poultry farms often necessitates beginning treatment prior to the results of the bacterial culture and sensitivity. When laboratory results are completed, the veterinarian must use clinical judgment to decide between continuation or change in therapy. Because a flock will have birds in the 3 categories of illness (clinically ill, incubating with no outward signs of illness, unaffected susceptible), all of the birds in a house and not just the clinically affected will be treated. This strategic use of antimicrobials in anticipation of a major disease spread is justifiable under good husbandry practices. Finally, responsible antimicrobial therapy allows sufficient withdrawal time for the antimicrobial from the feed or water to ensure no drug residue in the meat or eggs for human consumption. In some instances, the veterinarian may require a longer withdrawal than is written on the drug label because of clinical judgment. For example, some sulfonamides are excreted in the birds’ droppings in an altered but active metabolite and because birds are capathic, they may have sulfonamide exposure even after the drug is removed from the feed or water.

**Antimicrobial Resistance**

Antibiotic resistance is a growing concern for human health because many of the genes encoding antibiotic resistance are transferable between bacteria, there is a concern regarding the impact of antibiotic use in food animals. Currently, there is no conclusive information regarding how much of an impact the use of antibiotics in poultry has on antibiotic resistant human bacterial pathogens.

The use of antimicrobials selects for bacteria that can survive in the presence of that antimicrobial. Therefore, development of a large number of bacteria that are resistant to an antimicrobial is greatly dependent on the level of that antimicrobial agent that is achieved at the site of infection in the bird’s body. If the dose of the antimicrobial does not reach a concentration high enough to kill or inhibit the target bacteria growth, then selection pressure exists to move the bacteria population to have greater numbers that survive in the presence of that antimicrobial. This process is simple selection and use of antimicrobials does not create resistance.

There are specific genes that give the bacterium the ability to survive in the presence of an antimicrobial. Some are genes normally present on the bacterial genome that mutate to a form that renders the antibiotic ineffective. Other genes are acquired from other bacteria, a process known commonly as horizontal gene transfer. An example of gene mutation to allow the bacteria to grow in the presence of the antimicrobial occurs when there is a mutation in the DNA gyrase gene that results in fluoroquinolone resistance. These resistant bacteria survive to reproduce and the resistance then spreads by multiplication of the resistant bacterial strain.

The rapid dissemination of most antimicrobial resistance in Gram-negative bacteria is primarily achieved through horizontal gene transfer, or the movement of genetic material between 2 unrelated bacterial cells (6). Genes encoding antimicrobial resistance are most commonly moved between bacteria via their presence on conjugative bacterial plasmids (7). Plasmids are extrachromosomal elements that are self-replicating, not essential to the bacterial host cell, and often capable of self-movement (conjugation) from one bacterium to another. They are also highly stable once established in a bacterium. Plasmids are associated with resistance to antimicrobial agents because of their propensity to acquire additional genetic material within their genome, and they come in a variety of different types, each with unique replicons and a distinct set of genetic traits (7). Plasmid type determines many phenotypic traits, such as the frequency of conjugative transfer, the range of bacterial hosts in which it can successfully replicate, and the propensity to acquire resistance genes. Among *Escherichia coli* and *Salmonella enterica* alone, there are more than 30 plasmid types identified and this number continues to grow (6). Plasmids associated with multidrug resistance are primarily a concern among *E. coli*, *S. enterica*, and *Klebsiella pneumoniae*, although numerous other Gram-negative bacteria have been shown to possess multidrug resistance-encoding plasmids (8, 9, 10).

Each plasmid type has 1 or more distinct genetic load regions where they are able to acquire accessory genes, such as antimicrobial resistance genes. Some plasmids have more of these regions than others, and some plasmids are able to acquire more genetic load within a region than others. Resistance genes are usually inserted into these genetic load regions via conjugative transposons or mobile units called integrons (11). With integrons, resistance genes are not fixed once acquired; that is, they can be discarded and/or additional genes can be acquired at any time. These flexible elements play a major role in plasmid evolution and the evolution of resistance phenotypes. The primary multidrug resistance-associated plasmid types that are a concern in poultry are known as IncF, IncI1, and IncA/C (12). Each type mentioned has signature sets of resistance genes that they commonly possess. For example, IncA/C plasmids often contain the genes *bla*<sub>KM</sub>, and *floR*, encoding resistance to third-generation cephalosporins and phenicols, respectively (9). IncI1 plasmids commonly carry extended spectrum beta lactamase genes belonging to the *bla*<sub>TEM</sub> and *bla*<sub>CTX</sub> classes. In poultry, *E. coli* and *Salmonella* spp. often harbor these genes and plasmids.

In poultry production, plasmid-associated antimicrobial resistance is of concern for several reasons. First, many plasmids are considered to be highly plastic and capable of acquiring arrays of resistance genes encoding resistance to multiple antimicrobial agents. For example, IncA/C plasmids have recently been identified among *E. coli* and *S. enterica* of poultry and encode resistance to up to 12 different classes of antimicrobial agents (12). In addition to the carriage of multiple resistance genes by a single plasmid, bacteria of poultry commonly carry multiple plasmids (12).
Finally, co-carriage of antimicrobial resistance genes with genes conferring other phenotypes routinely occurs. For example, avian pathogenic *E. coli* often carry virulence factors that co-reside with antimicrobial resistance-encoding genes (13). Furthermore, these plasmids also may possess genes encoding resistance to heavy metals and disinfectants (14). Therefore, a scenario emerges in which resistance genes may be selected for in the absence of antimicrobial pressures. This complicates the ability to control the dissemination of multidrug-resistant bacteria once they are established in an environment. Examples of these complex plasmid structures containing antimicrobial resistance and disinfectant and heavy metal resistance as well as virulence factors can be seen in Figure 1.11A, B, and C.

The dissemination of multidrug resistance in poultry has become a major concern in *Salmonella* spp. because of the potential risk that these bacteria pose to human health via food-borne transmission. It seems that certain *Salmonella* serovars have a greater propensity to acquire multidrug resistance than others, and 1 serovar of particular concern in poultry is *Salmonella* Heidelberg. *S. Heidelberg* isolates harboring multiple resistance genes have been identified in live chickens, live turkeys, humans, and retail meats, and some are identical using pulsed-field gel electrophoresis (15, 16, 17). Of particular concern are the IncA/C plasmids, which have been identified among serovars Heidelberg, Kentucky, and Typhimurium in poultry and humans (18, 19, 20, 21). Isolates harboring this plasmid are typically resistant to ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftriaxone, chloramphenicol, sulfisoxazole, tetracycline, trimethoprim/sulfamethoxazole, and gentamicin. Other plasmid types, such as IncF and IncI1, are also common among *Salmonella* spp. of poultry but do not confer such a wide array of phenotypic resistances. Also, unlike IncI1 and IncF plasmids, IncA/C plasmids have a broad host range and are likely also moved between *Salmonella* spp. and other Proteobacteria within the environment (22).

Once multidrug-resistant bacterial populations are established within an environment, they are difficult to eliminate. Certainly, plasmid dissemination from 1 bacterium to another can occur within the avian gastrointestinal tract, within poultry litter, and among beetles (23, 24, 25). There is documented evidence that antimicrobial therapy in response to disease will enhance the dissemination of plasmid-encoded multidrug resistance (26). Cessation or reduction of antibiotic usage has been suggested as a method to reduce the numbers of multidrug resistant organisms. However, the evidence for this effect is contradictory in the scientific literature. The best current practices to limit the spread of multidrug-resistant organisms are likely the same as those used to reduce disease transmission, such as thorough clean-out procedures and good biosecurity practices. In those instances in which a bacterial infection occurs and treatment becomes necessary, follow judicious use guidelines with isolation of the bacteria, determination of antimicrobial sensitivity, and use the appropriate dose and duration of therapy.

### Summary

Judicious antimicrobial therapy includes proper diagnosis, knowledge of antibiotic properties, dosage, spectrum, interactions, and early initiation of treatment. It is not as simple as offering the drug to a poultry flock. The limited arsenal of drugs available for poultry makes it imperative that we combine an accurate diagnosis with antimicrobial knowledge to result in the most efficacious and cost-effective approach to disease treatment with minimal potential risk of antimicrobial resistance development and selection.

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Public Health Significance of Poultry Diseases
Roy D. Berghaus and Bruce Stewart-Brown

Introduction

Poultry and humans have dramatically different physiologies, and consequently, many pathogens are incapable of crossing the species barrier between birds and man. Nevertheless, there are a number of diseases that humans and poultry share. These can be zoonoses (poultry to humans) or reverse zoonoses (humans to poultry). Most zoonotic diseases can be prevented through an understanding of basic disease transmission principles and the adoption of preventive practices.

Infectious organisms are transmitted by direct or indirect mechanisms. Direct routes include body-surface-to-body-surface contact, contact with soil or vegetation harboring infectious organisms, and large droplet transmission over short distances. Indirect routes include airborne transmission by small particles suspended in air, vehicle-borne transmission by objects that passively carry the organism or provide an environment for growth, and vector-borne transmission by an insect or other living carrier via mechanical carriage or biological propagation.

Common biosecurity practices including the use of gloves, eye and respiratory protection, and protective outerwear are all important elements of zoonotic disease prevention (121). Good hand hygiene and routine injury prevention are also essential. Thoroughly washing hands with soap and water after working with poultry is always recommended. When soap and water are not available, the use of antiseptic hand sanitizers may be an effective alternative. Protection of eyes, nose, and mouth helps reduce the risk of mucous membrane exposure and inhalation. Protective outerwear should include disposable or reusable overalls that can be sanitized between uses, head covers to help keep the hair and scalp free of gross contamination, and disposable or washable footwear. Skin lacerations should be kept covered, and injuries resulting from contact with animals or equipment should be promptly cleaned and protected. Eating and drinking should be done away from the poultry house.

All personnel that work around poultry should be trained and educated on zoonotic disease prevention, including who to contact if questions should arise. Persons with weakened immune systems are at increased risk for contracting many zoonotic diseases. Populations with increased susceptibility may include young children, pregnant women, the elderly, and persons who are immunocompromised due to medications or disease. Individuals with immune dysfunction are encouraged to discuss their health status with a health care professional before working around poultry or other animals.

This chapter provides a brief overview of public health issues for several infectious diseases that are common to poultry and man. It does not include all such diseases and it is not meant to serve as a human medical reference. Rather, it provides a short synopsis of the disease manifestations in humans and may serve as a starting point for further inquiry. Diseases are presented alphabetically within categories defined by the type of infectious agent (i.e., viral, bacterial, fungal, and parasitic). For each organism or disease, there is a brief description of the nature of the disease in humans, its occurrence, and reservoirs and sources of infection. Specific preventive measures are provided for some diseases.

Viral Diseases

Arboviral Encephalitis

Arbovirus is a generic term referring to viruses transmitted to vertebrates by the bite of arthropod vectors including mosquitoes, ticks, and flies. More than a dozen arboviruses are capable of causing neurological disease in humans (41, 91). However, the discussion here will be limited to 3 that cause disease in both humans and poultry: Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and West Nile virus (WNV).

Nature of the Disease in Humans

The clinical presentation varies with virus and host characteristics, but most infections are asymptomatic (41). Of those patients that do become ill, most experience a flu-like illness with sudden-onset fever, headache, and fatigue. A variable number of patients progress to develop neuroinvasive disease with typical signs of meningitis or encephalitis. Recovery from neuroinvasive disease can take several weeks to months, and sequelae such as weakness and paralysis are common. Case-fatality risks for patients with severe illness caused by EEEV, WEEV, and WNV infections have been estimated at 50%–70%, 3%–7%, and 3%–17%, respectively (86, 146).

Occurrence

The distribution of arboviruses depends on their specific reservoir hosts and vectors. EEEV and WEEV are found in both North and South America. In North America, EEEV is found primarily along the Atlantic and Gulf coasts, and WEEV is found primarily in the western United States. WNV has been identified in Europe, Africa, Asia, and North and South America. WNV was not detected in the Western hemisphere until 1999, but has since become the most commonly reported arboviral infection in the U.S. Between 1999 and 2007, the number of human WNV neuroinvasive cases reported in the U.S. was 11,125, compared to 80 cases for EEEV and 1 case for WEEV (114). In North America, approximately 90% of human cases are identified between July and September.
Neuroinvasive disease is more likely in organ transplant recipients and the elderly (86).

Reservoirs and Sources of Infection
The principal mode of transmission to humans is from the bite of an infected arthropod vector. For all 3 of the viruses discussed here, the primary enzootic transmission cycles occur between birds and mosquitoes. Transmission is not limited to ornithophilic mosquito species, however, as several other genera of mosquitoes have the potential to act as bridging vectors between birds and humans (17, 146). The incubation period is typically 5–15 days (91). Humans do not produce a sufficient viremia to serve as an amplifying host; however, nonvector-borne transmission has been documented via blood transfusion, organ transplantation, breast feeding, and needle-stick injury (17). An increased incidence of seroconversion was reported in goose farmers and veterinarians during an outbreak of WNV in Israel during 1999–2000, although it is not clear whether this may have been attributable to contact with infected birds or concurrent transmission from infected mosquitoes (13).

Avian Influenza
Avian influenza (AI) is caused by type A influenza viruses which are classified by their hemagglutinin (H1-H16) and neuraminidase (N1-N9) subtypes (110). Human disease caused by direct infection with poultry-adapted viruses is rare, but has been reported with specific virus lineages in some subtypes: H5N1, H7N2, H7N3, H7N7, H9N2, and H10N7 (77, 93). Pathogenicity in poultry is not indicative of the pathogenicity in humans.

Nature of the Disease in Humans
Clinical manifestations of human infections with AI viruses vary from mild to severe and depend on the subtype. H7 subtypes have mainly been associated with conjunctivitis and mild influenza-like illness, whereas H5N1 viruses have been associated with more severe respiratory disease (1, 55). Common presenting signs include fever, cough, dyspnea, pneumonia, and myalgia. In some cases gastrointestinal signs have been reported. Acute respiratory distress syndrome is a common complication of H5N1 virus infections, which have a case-fatality risk of approximately 60%.

Occurrence
AI viruses are found worldwide, although the distribution of specific subtypes varies. Most cases of human infection reported to date, and all deaths, have been caused by either the H5N1 or H7N7 subtypes (77, 93, 110). Between 2003 and 2011, 574 human illnesses and 337 deaths due to H5N1 AI viruses (Guangdong lineage) were reported to the World Health Organization (WHO) from 15 countries across Asia, Africa, and the Middle East (145). The H7N7 subtype was associated with 89 human cases and 1 fatality in a single large HPAI outbreak in the Netherlands during 2003 (55). All other AI virus subtypes combined accounted for approximately 20 documented human infections between 1959 and 2009 (77).

Reservoirs and Sources of Infection
Wild aquatic birds belonging to the orders Anseriformes and Charadriiformes are the natural reservoirs for AI viruses (77). Influenza viruses are shed in the feces and respiratory secretions of infected birds, and direct contact is believed to be the most important route of transmission to humans (1, 110). Mucous membrane exposures and the inhalation of potentially infectious aerosols should be avoided. Ingestion is of theoretical concern, although AI viruses are readily destroyed by cooking and to date there have been no documented cases of human infection due to the consumption of infected poultry. The incubation period is typically 2–5 days, but may be as long as 9 days (1). Person-to-person transmission of AI viruses is uncommon, but has been reported among relatives having close contact with persons infected with the H5N1 subtype (137). Viral RNA of H5N1 viruses has been identified in the respiratory secretions of infected persons for as long as 3–4 weeks after onset (1).

Preventive Measures
The U.S. Centers for Disease Control and Prevention (CDC) recommends that persons involved with AI outbreak control and eradication procedures wear appropriate personal protective equipment including disposable gloves, protective outer garments, shoe covers, and a fitted respirator of class N-95 or higher (27). CDC also recommends that workers receive a seasonal influenza vaccination and prophylactic antiviral drugs while handling potentially infectious materials.

Newcastle Disease
Newcastle disease, also known as Ranikhet disease, avian pneumoencephalitis, and pseudo-fowl pest, is caused by avian paramyxovirus serotype 1 (APMV-1) (31, 131). The virulence of strains varies widely, with the severity of disease in poultry ranging from inapparent to near 100% mortality. However, virulence in birds is not a predictor of the potential for human infection.

Nature of the Disease in Humans
In humans, NDV typically causes a transient, unilateral, acute follicular conjunctivitis with no involvement of the cornea. Swelling of the preauricular lymph nodes is common. Conjunctivitis typically lasts for 3–4 days but may persist for as long as 3 weeks (101). Mild generalized signs of illness such as low-grade fever, chills, headache, and pharyngitis are uncommon but may be more likely following an aerogenous exposure (64). Patients typically make a complete spontaneous recovery.

Occurrence
NDV has been reported in every poultry-producing region of the world. Vaccination is widely practiced, although periodic outbreaks still occur in countries where virulent strains of the virus are no longer endemic. Human infection following contact with infected live birds is uncommon (131). Most reported
cases have been in diagnostic and vaccine laboratory workers, veterinarians, and processing plant workers (31).

Reservoirs and Sources of Infection
Birds are the natural reservoir for NDV; more than 240 species have been reported to be susceptible to infection (75). Transmission between birds occurs by inhalation of respiratory droplets or the fecal-oral route (3). Transmission to humans occurs by splashing contaminated liquids in the eye, or by touching the eyes after contact with contaminated tissues or feces. The incubation period in humans ranges from 1–4 days, but 1–2 days is typical (64). Secondary transmission from 2 infected mothers to their children was suspected in 1 study of broiler plant workers, but was not confirmed (135).

Preventive Measures
Eye protection should be worn when working with NDV in the laboratory or when handling live vaccines or infected tissues. Wearing disposable gloves and washing hands with soap and water after handling infectious materials is also advisable. Wearing a respirator or mask reduces the risk of aerosol inhalation, although human infection by this route is believed to be uncommon.

Bacterial Diseases

Botulism
Botulism is a paralytic intoxication caused by botulinum toxin, which prevents acetylcholine release from motor neuron synaptic terminals. Botulinum toxin is produced by Clostridium botulinum as well as related species C. baratii and C. butyricum (124, 127). Although C. botulinum is considered a single species, different strains can be distinguished by the type of toxin they produce. There are 7 recognized toxin types (A–G), but only types A, B, E, and rarely F cause human illness. Toxin types C and D are the most common causes of botulism in wild birds and poultry but are not associated with human disease (26). Cattle and sheep are susceptible to type C and D toxins, however, and several outbreaks in these species have been linked to exposure to poultry litter (107).

Nature of the Disease in Humans
Four naturally occurring forms of botulism are recognized in humans: food-borne intoxication, wound botulism, infant botulism, and adult intestinal toxemia (toxicoinfection). Regardless of the form, the clinical presentation is characterized by flaccid symmetric descending paralysis that begins with cranial nerve palsies and may progress to respiratory arrest. The availability of antitoxin along with improvements in supportive care and mechanical ventilation has decreased the case-fatality risk in the U.S. to 3%–5% (127).

Occurrence
Clostridium botulinum has a worldwide distribution. The median annual number of cases in the U.S. between 1973 and 1996 was 24 for food-borne botulism, 3 for wound botulism, and 71 for infant botulism (124). Most food-borne intoxications result from the consumption of improperly preserved home-canned foods. Wound botulism is typically associated with deep tissue injuries such as open fractures, or in recent years with the nonintravenous injection of black tar heroin (127). Infant botulism is believed to result from a toxicoinfection rather than the ingestion of preformed toxin, and has been associated with the consumption of honey in up to 15% of cases (124). Adult intestinal toxemia is rare, and occurs in patients with a history of abdominal surgery, gastrointestinal abnormalities, or recent disruption of the normal flora as a consequence of antibiotic administration.

Reservoirs and Sources of Infection
Clostridium botulinum is found in soils throughout the world. Heat-resistant C. botulinum spores are capable of surviving many food preparation methods, and germination occurs when they are exposed to a warm anaerobic environment with nonacidic pH (greater than 4.6) and low salt and sugar concentrations. The incubation period for food-borne intoxications is typically 12–36 hours, but may range from 6 hours–10 days (26). Contact transmission from animal to person or person to person does not occur.

Clostridium perfringens Infection
Clostridium perfringens causes 2 different types of food-borne disease as well as gas gangrene in humans (22). Food-borne disease is usually caused by enterotoxin-producing strains of C. perfringens type A, and rarely by C. perfringens type C.

Nature of the Disease in Humans
C. perfringens type A food poisoning results when enterotoxin is produced during sporulation of vegetative cells in the intestine. Typical symptoms include acute abdominal pain and cramping, nausea, and diarrhea. Most cases are self-limiting and resolve without treatment in 24 hours (128). C. perfringens type C food poisoning is primarily mediated by betatoxin and is associated with necrotic enteritis in humans. Symptoms include acute abdominal pain and distension, bloody diarrhea, and sometimes vomiting (22). The case fatality risk for type A food poisoning is less than 0.1%, while that for type C food poisoning is 15%–25% (22, 120).

Occurrence
C. perfringens type A is one of the most common causes of food-borne disease worldwide. In the U.S., it causes an estimated 1 million illnesses annually (120). C. perfringens type C food poisoning is rare, and is usually limited to patients with abnormally low intestinal protease production who are unable to inactivate beta-toxin (60). Young children and the elderly
are at increased risk for severe illness due to type A food poisoning, whereas malnourished individuals and diabetics are at increased risk for developing necrotic enteritis due to type C food poisoning (22, 128).

**Reservoirs and Sources of Infection**

*C. perfringens* is a common inhabitant of soil and intestinal tracts of animals and humans, and is commonly isolated from retail meat products including poultry. Food-borne illness caused by *C. perfringens* results from improper food handling techniques, especially the inadequate cooling and reheating of dishes containing meat (7). Spores survive the initial cooking, and after germination can propagate rapidly under inadequate refrigeration. The incubation period for type A food poisoning ranges from 6–24 hours, but is most commonly 10–12 hours (122). Direct exposure to infected persons or animals does not constitute a disease risk, but colonized individuals may serve as a source of contamination during food preparation and handling (65).

**Campylobacteriosis**

Campylobacteriosis is an enteric infection caused by members of the genus *Campylobacter* (5, 69). Most human infections are caused by the thermophilic species *C. jejuni* or *C. coli.*

**Nature of the Disease in Humans**

*Campylobacter* causes an acute gastroenteritis characterized by fever, abdominal pain, and profuse diarrhea that is frequently bloody (5, 15). Most patients recover within 1 week without antimicrobial treatment. Bacteremia and other extraintestinal infections are uncommon complications (16). Reactive arthritis may occur as a sequela of enteric *Campylobacter* infections in 1%–5% of patients, and Guillain-Barré syndrome occurs in approximately 0.1% of patients (111, 132).

**Occurrence**

*Campylobacter* is one of the most commonly reported causes of bacterial gastroenteritis worldwide (5, 69). In the U.S., *Campylobacter* causes an estimated 845,000 cases of food-borne illness and 76 deaths each year (120). Most cases are sporadic; outbreaks are uncommon, but have been linked to unpasteurized milk, contaminated water, and the ingestion of undercooked poultry (15). In developed countries there is a male predisposition, a seasonal peak in cases during the late spring and summer, and a bimodal age distribution with infection being most common in children less than 1 year of age and in young adults from 15–44 years old (5). In developing countries there is less evidence of a seasonal pattern, and infection is common in children younger than 2 years of age but uncommon in adults (38).

**Reservoirs and Sources of Infection**

*Campylobacter* species are normal intestinal inhabitants of wild and domesticated animals and birds. Colonization of broiler chickens is common, and contaminated poultry meat is considered to be the most important source of human infections (69). Transmission occurs by ingestion of the organism, and approximately 80% of domestically acquired infections in the U.S. are considered to be food-borne (120). Transmission to poultry processing plant workers has been well documented (56). Contact with colonized animals or drinking untreated water are additional potential sources of exposure. The incubation period ranges from 1–10 days, but is most commonly 2–5 days (106). Person-to-person transmission can occur but is uncommon. The duration of fecal shedding can range from 2–7 weeks, although the median duration of shedding is less than 3 weeks (15, 106).

**Preventive Measures**

Cook poultry to a minimum internal temperature of 74°C (165°F) and avoid cross contamination between raw poultry and other foods (106). Avoid drinking unpasteurized milk and wash hands after contact with poultry or other animals.

**Chlamydioidosis (Psittacosis)**

Psittacosis, also known as ornithosis or parrot fever, is a respiratory disease of humans caused by *Chlamydophila psittaci* (126). The corresponding disease in birds is referred to as avian chlamydioidosis (6). *C. psittaci* is an obligate intracellular pathogen.

**Nature of the Disease in Humans**

Psittacosis is primarily a respiratory disease in humans that ranges from a mild flu-like illness to severe pneumonia with respiratory failure and death (10, 126). Typical symptoms include fever, chills, headache, and myalgia. A nonproductive cough is common and may be accompanied by respiratory difficulty. Occasional signs include a nonspecific rash and enlarged spleen. Complications include endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, and encephalitis. Most cases respond well to antibiotic therapy.

**Occurrence**

Psittacosis and avian chlamydioidosis have a worldwide distribution. Psittacosis is a reportable disease in most countries, but the number of reported cases is likely an underestimate of the true incidence because many cases are mild, the symptoms are nonspecific, and diagnosis can be difficult. Sixty-six cases were reported to the U.S. Centers for Disease Control and Prevention between 2005 and 2009. Beeckman and Vanrompay summarized reported cases of psittacosis from 24 countries between 1996 and 2007, with Australia, Germany, Japan, The Netherlands, and Great Britain having comparatively high numbers of reported cases (10). Most cases are sporadic, but outbreaks have occurred in groups of people exposed to infected pet birds and poultry (49). There is no evidence to suggest that immunocompromised individuals are at increased risk for infection (8).
Reservoirs and Sources of Infection
Birds are the natural reservoir for C. psittaci, and Chlamydia spp. have been identified by culture or serology in 467 bird species from 30 different orders, including all of the major domestic poultry species (76). Human infections are most frequently associated with exposure to psittacines, pigeons, turkeys, and ducks, although psittacosis is rare in modern poultry production systems. Different serotypes have been identified more frequently in certain bird species, although all serotypes are considered to be potentially infectious to humans (6). Humans become infected by inhalation of aerosolized organisms shed in the feces or respiratory secretions of infected birds, or by direct contact with infected carcasses or tissues. Intermittent shedding by subclinically infected birds is common. The incubation period in humans ranges from 1–30 days, although 5–14 days is typical. Possible secondary transmission of C. psittaci has been reported in a hospital setting but has not been confirmed (68).

Preventive Measures
Wearing gloves, protective eyewear, and a properly fitted respirator with an N95 rating or higher are recommended when working with potentially infected birds (126). Loose-fitting surgical masks may not provide adequate respiratory protection. Necropsies on potentially infected birds should be performed in a biological safety cabinet and carcasses should be moistened with a disinfectant solution to minimize the generation of aerosols during the procedure.

Erysipelothrix rhusiopathiae Infection
The most common clinical manifestation of infection with Erysipelothrix rhusiopathiae in humans is called erysipelas, a condition that is usually caused by Streptococcus pyogenes (113).

Nature of the Disease in Humans
Three forms of E. rhusiopathiae infection are typically recognized in humans (141). Erysipelas is the most common and is characterized by a localized cutaneous lesion, usually on the hand. Pain and swelling may be severe, although there is no suppuration or pitting edema. Systemic illness is uncommon and the condition usually resolves without treatment in 3–4 weeks, or within 48 hours after beginning antibiotic therapy (61). Diffuse cutaneous and systemic forms of the infection are much less common, although endocarditis has been reported in approximately 90% of systemically infected patients with a corresponding case fatality risk of 38% (58). Other clinical manifestations of infection, including arthritis and peritonitis, also have been recognized (46).

Occurrence
E. rhusiopathiae has a worldwide distribution although the incidence is unknown. Persons with exposure to animals or animal products, including processing plant workers, butchers, fish handlers, food handlers, farmers, and veterinarians are at increased risk of infection (58). Transmission from infected quail and laying chickens to processing plant employees and animal caretakers has previously been reported (99, 100).

Reservoirs and Sources of Infection
E. rhusiopathiae is a pathogen or commensal organism in a wide variety of animal species. Swine are the most commonly affected domestic animal and are considered to be the most important reservoir, although several poultry species including turkeys, chickens, ducks, and emus are also susceptible (141). E. rhusiopathiae can survive weeks to months in farm and marine environments, and is commonly found in the mucoid slime coating of fish (21). Transmission occurs by inoculation of the organism into an abrasion, cut, or puncture wound when working with infected animals or in contaminated environments. The incubation period for erysipelas is 2–7 days (61). Person-to-person transmission of E. rhusiopathiae infection has not been documented.

Escherichia coli Infection
Most strains of Escherichia coli are commensal inhabitants of the lower intestinal tracts of warm-blooded animals; however, some strains possess virulence traits that allow them to cause disease. Strains that cause intestinal pathology are categorized as belonging to 1 of 6 pathotypes: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), or diffuse-adherence E. coli (DAEC) (54). Strains with recognized extraintestinal virulence factors or that exhibit enhanced virulence in an animal model of extraintestinal infection have been designated as extraintestinal pathogenic E. coli (ExPEC) (118). Of the 6 intestinal pathotypes, only the EHEC strains (e.g., O157:H7) are considered to be zoonotic pathogens (142). Avian pathogenic E. coli (APEC) have not been associated with human intestinal infections, but some APEC strains are indistinguishable from human ExPEC strains (72, 138). The discussion here will be limited to the potentially zoonotic EHEC and ExPEC strains.

Nature of the Disease in Humans
Intestinal infection with EHEC serotypes of Shiga toxin producing E. coli (STEC) such as O157:H7 typically causes abdominal cramps with an initially watery diarrhea progressing to bloody diarrhea in 1–4 days (109, 115). Approximately 10%–15% of patients develop hemolytic uremic syndrome (HUS) with thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure within 5–13 days after the onset of diarrhea. Extraintestinal E. coli infections are associated with a variety of illnesses including urinary tract infection, newborn meningitis, and septicemia (118).

Occurrence
Enterohemorrhagic E. coli strains are recognized as an important problem in North and South America, Europe, Japan, and southern Africa (54). Extraintestinal E. coli infections are
an important worldwide problem. Shiga toxin producing *E. coli* cause an estimated 176,000 illnesses and 20 deaths in the United States each year (120). Extraintestinal *E. coli* infections are estimated to account for 85%–95% of approximately 130–175 million cases of uncomplicated cystitis globally each year (117).

**Reservoirs and Sources of Infection**

EHEC strains of Shiga toxin producing *E. coli* have previously been identified in retail chicken samples and in fecal samples collected from turkeys and pigeons (42, 63, 67). Chickens are also readily colonized with *E. coli* O157:H7 in experimental trials (123). Cattle and other ruminants are the most important source of human EHEC infections, however, and avian species are not considered to be an important reservoir (53, 142). Transmission of EHEC strains occurs via contaminated foods, person-to-person contact, or contact with colonized animals. Human infections with ExPEC strains typically originate from the person's own intestinal tract, although poultry may potentially serve as a reservoir for human colonization (72, 138). The incubation period for EHEC strains ranges from 2–10 days, with a median of 3–4 days (54). Secondary transmission of EHEC strains is common, especially among children in daycare centers (112). The duration of shedding is typically 1 week or less in adults, but may be 3 or more weeks in children (54).

**Listeriosis**

Listeriosis is caused by *Listeria monocytogenes*. Thirteen serotypes have been described (51), although 3 are most frequently associated with human disease: 1/2a, 1/2b, and 4b (4, 83).

**Nature of the Disease in Humans**

*L. monocytogenes* can result in a variety of clinical syndromes, ranging from febrile gastroenteritis to severe invasive disease (4). Septicemia and meningoencephalitis are most frequently reported in pregnant women, neonates, and immunocompromised adults. Focal infections may include brain and hepatic abscesses, cholecystitis, conjunctivitis, endocarditis, joint infections, skin infections, and osteomyelitis (4, 51). Infection of pregnant women may result in fetal infection or death (71, 83).

**Occurrence**

*L. monocytogenes* has a worldwide distribution, and has been estimated to cause approximately 1,500 hospitalizations and 250 deaths in the United States each year (120). Populations at increased risk of systemic infection include pregnant women and neonates, immunocompromised adults, and the elderly (130). Veterinarians and farm workers are at increased risk for developing cutaneous infections, particularly following large-animal obstetric procedures (94). Listeria conjunctivitis has previously been reported in 2 poultry processing workers (52). Most cases of listeriosis are sporadic, but common source outbreaks are frequently identified (4, 130). The prevalence of asymptomatic intestinal carriage has been estimated at 1%–5% in animals and 5%–10% in humans (51).

**Reservoirs and Sources of Infection**

*L. monocytogenes* is widely distributed in nature and can be identified in soil, water, silage, and animal feces. *Listeria* is commonly found on poultry farms and in processing plants (34, 37). It is an important source of environmental contamination in processing plants because of its ability to grow at low temperatures and form biofilms that are resistant to routine sanitation procedures (134). Most cases of human listeriosis result from food-borne transmission (4, 71). Several outbreaks have been associated with ready-to-eat delicatessen meats and soft cheeses made from unpasteurized milk (130). The incubation period ranges from 3–70 days, with a median of 3 weeks (4). Transplacental transmission from pregnant mothers to the fetus is common, but transmission from infected patients to household contacts has not been reported.

**Mycobacteriosis**

Mycobacterial species other than *M. tuberculosis* and *M. leprae* that are associated with human disease are commonly called nontuberculous mycobacteria (NTM). More than 125 NTM species have been recognized, but only around 20 have been associated with human illness (62). The discussion here will be limited to members of the *Mycobacterium avium* complex (MAC), which along with *M. genavense* are responsible for most cases of avian mycobacteriosis (133).

**Nature of the Disease in Humans**

The most common clinical syndromes associated with MAC infections are pulmonary disease, lymphadenitis, and disseminated infection. Common signs of pulmonary disease include chronic cough, fever, chills, night sweats, dyspnea, and weight loss (139). Lymphadenitis frequently manifests as a painless unilateral swelling of the cervical, submandibular, submaxillary, or preauricular lymph nodes. Disseminated infection is characterized by intermittent fever, sweats, weakness, anorexia, and weight loss (70).

**Occurrence**

Members of the *M. avium* complex cause disease in humans worldwide. Exposure is common but disease is rare in immunocompetent persons. Pulmonary MAC infections are typically identified in men with pre-existing lung disease, in elderly women with no history of underlying lung disease, and in adolescents with cystic fibrosis (70, 139). Lymphadenitis is most common in children from 1–5 years of age, and disseminated infections are usually recognized in severely immunocompromised persons, especially those with advanced AIDS.

**Reservoirs and Sources of Infection**

Nontuberculous mycobacteria, including MAC, are ubiquitous in the environment and are commonly isolated from soil and water (70). Humans become infected by ingestion or inhalation of MAC organisms from the environment.
Infected animals and birds commonly shed mycobacteria in their feces, but are not considered to be an important source of human infections (133). While birds may serve as an important reservoir of some *M. avium* strains, molecular studies have suggested that bird-type *M. avium* isolates are genetically distinct from those that are typically isolated from humans and swine (95, 136). Person-to-person transmission of MAC has not been documented and is not believed to occur (57, 70).

**Salmonellosis**

Nontyphoidal *Salmonella* infections may be caused by any of the nonhost-specific *Salmonella* serotypes that commonly affect both animals and humans. Based on World Health Organization estimates, *S. Enteritidis* and *S. Typhimurium* were the 2 most common nontyphoidal serovars isolated from humans between 2001 and 2007 (66). *Salmonella Gallinarum* and *Salmonella Pullorum* are rarely isolated from humans.

**Nature of the Disease in Humans**

Nontyphoidal salmonellosis typically manifests as an acute enterocolitis or gastroenteritis with sudden-onset headache, abdominal pain, diarrhea, nausea, and sometimes vomiting. Fever is usually present (11). Most cases are self-limiting, with diarrhea resolving without treatment after 3–7 days. Bacteremia is a potentially serious complication that occurs in approximately 5% of cases (125). Possible sequelae of bacteremia include endarteritis and disseminated focal infections.

**Occurrence**

*Salmonella* has a worldwide distribution. It has been estimated that there are 93.8 million human cases of nontyphoidal salmonellosis and 155,000 deaths globally each year, with 80.3 million of these cases resulting from food-borne transmission (92). Children, the elderly, and people with compromised immune systems are more likely to develop severe disease (59, 125). Poultry and eggs are frequently identified as sources of infection in food-borne salmonellosis outbreaks. Of the 40 salmonellosis outbreaks identified in the U.S. during 2008 that could be attributed to a single food commodity, 11 were attributed to poultry (28).

**Reservoirs and Sources of Infection**

Nontyphoidal *Salmonella* are capable of colonizing the gastrointestinal tracts of a broad range of wild and domesticated animal hosts including poultry. Transmission occurs by the fecal-oral route and can be achieved by direct contact with infected animal feces or indirectly by the consumption of contaminated food products. The incubation period is typically 12–36 hours, but can range from 6–72 hours. Secondary transmission can occur but is uncommon with appropriate hygiene. The median duration of fecal shedding is approximately 5 weeks after infection, although *Salmonella* can still be identified 1 year post infection in 5% of children younger than 5 years of age and in 1% of adults (23).

**Staphylococcus aureus Infection and Foodborne Intoxication**

*Staphylococcus aureus* frequently colonizes the skin and mucous membranes of humans and animals, including poultry. It is both a commensal organism and a frequent cause of clinically important infections. Antibiotic-resistant strains, especially methicillin-resistant *S. aureus* (MRSA), have become increasingly common in recent years (82).

**Nature of the Disease in Humans**

*S. aureus* causes a wide variety of clinical manifestations ranging from minor skin pustules to septicemia and death (18, 88, 143). Common cutaneous infections include impetigo, cellulitis, folliculitis, carbuncles, furuncles, and abscesses. Most superficial infections respond well to cleaning and topical antibiotics. Hematogenous spread of localized infections can lead to serious complications including arthritis, endocarditis, osteomyelitis, pneumonia, meningitis, and sepsis. Staphylococcal food-borne intoxication is mediated by the production of heat-stable enterotoxins in uncooked or inadequately refrigerated foods (18). Signs include acute onset of nausea, abdominal cramps, vomiting, and often diarrhea. Most cases of food-borne intoxication resolve without treatment in 1–2 days. *S. aureus* is also the causative agent of toxic shock syndrome in humans (88).

**Occurrence**

*S. aureus* has a worldwide distribution and is 1 of the most common pathogens associated with skin and soft-tissue infections. *S. aureus* is the second most common cause of hospital-acquired bloodstream infections (143), and causes approximately 240,000 foodborne intoxications in the U.S. each year (120). Newborn infants and the chronically ill are at increased risk for developing *S. aureus* skin infections (18).

**Reservoirs and Sources of Infection**

The anterior nares are the most common site of human colonization. Approximately 20% of persons are persistent carriers, 30% are intermittent carriers, and 50% are noncarriers (143). Transmission is by direct or indirect contact. Hands are the most important vehicle for transmission, and at least one-third of skin infections are believed to result from autoinfection (18). Airborne transmission is uncommon but may occur as a consequence of sneezing by nasal carriers. Retail chicken meat is frequently contaminated with enterotoxigenic *S. aureus* strains, although colonized food handlers are believed to be responsible for most cases of food-borne intoxication (81, 122). Signs typically appear within 2–4 hours after ingesting staphylococcal enterotoxins (122). Person-to-person transmission of *S. aureus* from asymptomatic carriers or persons with draining skin lesions is common.

**Fungal Diseases**

**Cryptococcosis**

Cryptococcosis is a fungal infection caused by members of the genus *Cryptococcus*. There are at least 37 species belonging to this genus, although only *C. neoformans* and *C. gattii*...
are considered to be major human and animal pathogens (85). Cryptococcosis is not considered to be a zoonosis; rather, humans and animals both acquire infection from environmental sources (14).

**Nature of the Disease in Humans**
The central nervous system (CNS) and lungs are the most frequently recognized sites of *Cryptococcus* infection. Meningitis is the most common presentation in immunocompromised patients, while pulmonary disease may be more common in immunocompetent patients (35). Even with appropriate anti-fungal treatment, the 3-month case fatality risk for cryptococcal meningitis in HIV-infected patients remains approximately 20% (87).

**Occurrence**
*Cryptococcus neoformans* has a worldwide distribution and occurs most frequently in immunocompromised persons. *Cryptococcus gattii* has historically been limited to tropical and subtropical regions, although it has recently been recognized in British Columbia, Canada, and in the Pacific Northwest region of the United States (39). In contrast to *C. neoformans*, *C. gattii* causes disease in both immunocompromised and immunocompetent individuals. It has been estimated that there are approximately 720,000 cases of cryptococcal meningitis in HIV-infected persons in Sub-Saharan Africa each year, with approximately 500,000 fatalities (105).

**Reservoirs and Sources of Infection**
Humans are infected with *Cryptococcus* by inhalation of desiccated encapsulated yeast cells or basidiospores from the environment (84). Pigeon guano from old pigeon lofts or roosts is an important environmental source of *C. neoformans*, while *C. gattii* is frequently found in the hollows of *Eucalyptus* and other tree species (39, 102). The incubation period is unknown, but CNS disease may be preceded by a pulmonary infection acquired months or years previously. Person-to-person transmission has been reported but is believed to be rare (140).

**Dermatophytosis (Favus)**
*Microsporum gallinae* is a contagious zoophilic fungus that is responsible for causing dermatophytosis in poultry and also in humans (19, 43). This condition is alternatively referred to as favus, dermatomycosis, or ringworm.

**Nature of the Disease in Humans**
Like other dermatophytes, *M. gallinae* affects keratinized areas of the body including the hair, nails, and skin (20). Lesions begin as small circumscribed areas of erythema, crusting, and scaling, and subsequently spread peripherally. Skin lesions are not associated with systemic illness, although treatment with topical and/or systemic antifungal medications for 4–8 weeks may be required to eliminate the infection (33).

**Occurrence**
*M. gallinae* possibly has a worldwide distribution, although it is rarely reported as a cause of disease in either poultry or man. Miyasato et al. identified a total of 44 human cases that had been reported in the literature as of 2010 (96). Thirty-four of these cases were reported from 2 studies conducted in Nigeria and Iran (79, 103). Young children, the elderly, immunosuppressed persons, and those with diabetes may be at increased risk of infection.

**Reservoirs and Sources of Infection**
Gallinaceous birds are considered to be the most important reservoir for *M. gallinae*. Transmission occurs by direct contact with infected animals or humans, or indirect contact via contaminated fomites (36). The incubation period is unknown, but for other dermatophytes has been reported as 4–14 days (20). Dermatophyte infections are easily transmitted from person to person while lesions are present. Infectious materials may remain viable in the environment or on contaminated objects for months to years (36).

**Histoplasmosis**
Histoplasmosis is caused by the fungus *Histoplasma capsulatum* (78). Two varieties cause disease in humans: *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*. The discussion here will focus on the more widely distributed and well known *H. capsulatum* var. *capsulatum*. *Histoplasma* is not considered to be either a contagious or zoonotic pathogen.

**Nature of the Disease in Humans**
Histoplasmosis is associated with a wide spectrum of clinical illness, with as many as 95% of sporadic infections in endemic areas being asymptomatic (25, 78). Acute pulmonary histoplasmosis is characterized by fever, myalgia, nonproductive cough, dyspnea, and chest pain. The infection is usually self-limiting, but immunocompromised patients or those exposed to a large inoculum may progress to acute respiratory distress syndrome. Chronic pulmonary histoplasmosis is a progressive infection characterized by the formation of cavitary lesions in patients with pre-existing emphysema. Progressive disseminated histoplasmosis is a systemic manifestation that typically only occurs in individuals with inadequate T-cell immunity. Complications of histoplasmosis include mediastinal granuloma, fibrosing mediastinitis, pericarditis, and broncholithiasis.

**Occurrence**
*H. capsulatum* var. *capsulatum* causes histoplasmosis across the Americas, parts of Africa, eastern Asia, Australia, and rarely in Europe (25). In the United States, *H. capsulatum* is endemic in the Ohio and Mississippi River valleys. In a study of U.S. naval recruits conducted from 1958–1965, the overall prevalence of histoplasmin sensitivity was 20%, and recruits from endemic areas in the east-central part of the country had sensitivity prevalences greater than 80% (48). Young children, the elderly, and immunosuppressed persons are at increased risk for developing histoplasmosis. While most cases are sporadic, large outbreaks have been reported, often in association with construction projects or other activities that involve disturbing soil in the vicinity of bird roosts (25).
Reservoirs and Sources of Infection
Histoplasma grows in the soil, particularly in areas contaminated with undisturbed bird and bat droppings such as wild bird roosts, old chicken coops, and bat caves (2). Humans become infected by inhalation of airborne microconidia. The typical incubation period is between 4 and 14 days (25). Transplacental transmission has been reported, but contact transmission from animal-to-person or from person-to-person does not occur (25, 144).

Parasitic Diseases

Avian Mite Dermatitis
Avian mite dermatitis is most frequently caused by Dermanyssus gallinae (the poultry red mite or chicken mite) or Ornithonyssus sylviarum (the northern fowl mite) (89, 104). Synonyms include gamasoidosis, acariasis, and fowl mite dermatitis.

Nature of the Disease in Humans
Typical clinical signs in humans include pruritic erythematous papules, vesicles, and dermatitis on exposed skin (12, 89). Excoriation of lesions is common. Avian mites also have been associated with occupational asthma and otitis externa in poultry workers (90, 116). Symptoms are alleviated when the source of mites is removed. Mites are not typically observed on human skin because they leave quickly after biting. Not all exposed persons develop bite reactions (89).

Occurrence
Avian mites have a worldwide distribution. Avian mite dermatitis is not a reportable disease and the incidence is unknown. Most published case reports result from proximate exposures to abandoned bird nests in an urban setting (9, 24, 104). Mite infestation of layer and breeder flocks is common, however, and is recognized as a frequent cause of discomfort in poultry workers (98, 129).

Reservoirs and Sources of Infection
A broad variety of avian species serve as the natural hosts for Dermanyssus and Ornithonyssus, although mites from these genera can survive at least 5 months and 3 weeks, respectively, without a host (104). When no birds are available, the mites will seek out alternative food sources, including humans and other mammals. Humans are an accidental host and do not serve as a reservoir of avian mites (104).

Cryptosporidiosis
Cryptosporidiosis is caused by intracellular protozoan parasites belonging to the genus Cryptosporidium. Three avian Cryptosporidium species are currently recognized: C. baileyi, C. galli, and C. meleagridis. Of these three, only C. meleagridis is considered to be a zoonotic pathogen (119).

Nature of the Disease in Humans
Cryptosporidiosis is primarily associated with enteric disease in humans. Watery diarrhea, abdominal cramping, and increased gas production are the most common clinical signs, and may be accompanied by vomiting, fever, and loss of appetite (29, 32, 40). The median duration of illness in immunocompetent persons is 10–14 days, and signs may persist for up to 1 month. Immunocompromised persons may experience severe chronic diarrhea, and are at increased risk for complications including pancreatitis, cholangitis, bronchial involvement, and death.

Occurrence
Cryptosporidium species have a worldwide distribution and are 1 of the most common causes of protozoal diarrhea in humans. More than 95% of human infections are caused by C. hominis or C. parvum. Cryptosporidium meleagridis is the third most commonly identified species in humans, accounting for approximately 1% of infections in the U.K. (50, 108). Young children, immunocompromised persons, and people with occupational exposure to infected animals are at increased risk of infection.

Reservoirs and Sources of Infection
Cryptosporidiosis is caused by infection with the obligate intracellular protozoan Toxoplasma gondii. Cats are the definitive host for T. gondii. Mammals and birds serve as intermediate hosts.

Nature of the Disease in Humans
Postnatal infection with T. gondii is asymptomatic in approximately 90% of immunocompetent children and adults (97). In the remaining 10%, mild transient cervical or occipital lymphadenopathy is the most common clinical presentation. Immunocompromised persons frequently experience severe disease including encephalitis, chorioretinitis, and pneumonitis. Infection of pregnant mothers is usually asymptomatic, but may lead to abortion or infection of the fetus. Congenital infections can result in chorioretinitis, hydrocephalus, intracranial calcifications, and mental retardation (73).

Occurrence
Toxoplasma gondii has a worldwide distribution, and exposure to the organism is common. The overall seroprevalence in the adolescent and adult U.S. population has been estimated at 23%, and the prevalence among women from 15–44 years
of age was estimated at 15% (74). There are an estimated 400–4,000 cases of congenital toxoplasmosis in the U.S. each year (73). Immunocompromised persons and infants with congenital infections are at increased risk for severe disease.

**Reservoirs and Sources of Infection**

Cats become infected after ingesting an infected intermediate host, and will shed oocysts in their feces for 1–3 weeks following their first infection. Oocysts do not become infective until 1–5 days after excretion, although they can remain viable in the environment for several months. Humans become infected by ingesting oocysts shed by cats or by consuming infected intermediate hosts. Historically, undercooked lamb and pork have been common sources of human infection (80). Commercial poultry raised in confinement is rarely infected but has been common sources of human infection (80). Toxoplasma can be transmitted from mother to fetus, although this typically occurs only when the mother’s first exposure to the parasite occurs during gestation (73). Person-to-person transmission does not occur by direct contact, but transmission by organ transplantation or blood transfusion has been documented.

**References**

**Principles of Disease Prevention, Diagnosis, and Control Introduction**

SECTION I  General Concepts of Poultry Diseases


Antimicrobial Therapy (Including Resistance)


Public Health Significance of Poultry Diseases


