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Principles of Disease Prevention, Diagnosis, and Control

Introduction

Stephen R. Collett

In an effort to cost-effectively meet demand, producers have increased 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, also increase the risk and impact of disease challenge. The close proximity of susceptible hosts increases the chance and rate of infectious disease spread. Replication of mutable viruses (including live virus vaccines) in large populations mathematically increases the probability of the emergence of variants, and reliance on immunization to control these diseases selects for antigenically dissimilar mutants that escape adaptive immunity. Diseases previously recognized as unimportant, because they have been adequately controlled, have now re-emerged as significant concerns. Many of today's disease challenges are not new problems, they have merely expanded their geographic distribution or re-emerged primarily because of management techniques and production system design constraints.

Disease control priorities have evolved with intensification of the industry. While initially focused on diseases of catastrophic nature, attention has shifted from defined, clinical disease at the individual house or farm level, to less well-defined sub-clinical disease, performance shortfalls, and bird welfare. Disease prevention, diagnosis, and control strategies have changed to prevent physiological, nutritional, and agent-induced pathologies from affecting performance.

Since the production system is profit driven, decisions regarding management of disease challenge can no longer be made based solely on biological grounds. Unless a disease poses a specific risk to human health, animal welfare, productive efficiency, or product quality, 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 first, an epidemiologic and economic analysis to determine and quantify the production effect of the disease challenge, and second a proposed intervention strategy and the costs thereof.

Recently, regulatory changes in the United States have eliminated growth-promoting and other non-therapeutic uses of antibiotics, but have continued allowance for most therapeutic uses under increased veterinary supervision. However, market-driven restrictions on all uses of antibiotics in poultry production have created challenges in the control of bacterial and protozoal infections and maintaining welfare of farmed poultry. This has led to new challenges in confronting increased early mortality, coccidiosis, and necrotic enteritis (NE) in broilers; coccidiosis and bacterial infections such as *Bordetella avium* in turkeys; and colibacillosis and NE in table egg layers. Solutions for these health problems will require changes to management and diet, use of non-antibiotic medications such as chemically synthesized coccidiostats, and alternative products such as probiotics. However, even with these changes, performance and health problems may exceed those found in conventional production schemes with unrestricted access to approved medications.

In conventional production schemes, appropriate antimicrobial uses includes proper pathogen diagnosis, knowledge of antibiotic properties, dosage, spectrum, interactions, and early initiation of treatment. The limited drug availability for poultry makes it imperative to 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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Several poultry diseases have zoonotic potential. Some zoonotic diseases are rarely reported and others are commonly associated with human illness. The most high profile zoonotic diseases include H5N1 Gs/GD lineage and H7N9 Anhui lineage of avian influenza viruses, and foodborne pathogens such as *Salmonella* and *Campylobacter*. Educating poultry workers with respect to zoonotic pathogens and their modes of transmission is an important step toward disease prevention.

Principles of Disease Prevention, Diagnosis, and Control

Stephen R. Collett and John A. Smith

Summary

Disease challenges have evolved in step with the evolution of industrial-scale poultry production, and the principles of disease prevention, diagnosis, and control have, and must continue to, evolve as well. Disease management has shifted from classically recognized acute diseases of individual animals to management of both clinical and subclinical disease in populations. Morbidity and mortality are no longer the primary metrics monitored, and the emphasis has shifted to economic performance through the entire production chain, product quality, and animal welfare. Environmental considerations, food safety, marketing claims, and the like increasingly impact decision-making. Recognition of the roles of management, environmental stressors, and population ecology have been added to the traditional medical disciplines, and biosecurity and risk management have assumed equal importance to practical diagnosis and treatment in the job of the poultry practitioner.

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 classed 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 non-specific 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 (33). Stress is cumulative and only impacts performance measurably once the aggregate of each individual stress exceeds the host's coping mechanisms. An interesting study (48) 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 where animals are expected to produce at genetic potential, the definition of health needs to be expanded to freedom from "dis - ease" or stress.

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. In intensive animal agriculture environmental disease determinants often decide 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 also 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 in order 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. Since stress negatively impacts health it also negatively impacts resistance. Ironically the process of mounting an effective immune response is itself a stress because of the demands made on the immune system, and the consequence of the resulting fever response. An immune response, adequate to contain disease, can be considered as the cost of health. There is a delicate balance between too little and too much since 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 (48). While immune response is crucial to maintaining health, the consequence of an immune response 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 at the same time, 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. Since 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 they act and produce as an equivalent non-stressed sibling would do in a laboratory situation. This approach unfortunately takes little cognizance of the flock variance, since flock performance indicators are based on flock averages. Population variance or range is a much better indicator of flock health.

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 (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

Since 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 potential for *feed conversion* is governed primarily by intrinsic or genetic determinants, in an intensive production system it is the extrinsic determinants, including nutrition, minimization of stress by management, and disease 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 agent 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 two 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 re-emerging viral diseases arise when novel or immunologically distinct viruses are introduced into naïve populations (45). 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 (42). Under such conditions virus replication and spread occurs rapidly with potentially devastating consequences (24). 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 (81). Although controlled environment housing and good biosecurity practices have been highly effective in preventing the introduction of novel viruses, increased population density and vaccination have likely enhanced the emergence of variant strain viruses. The high population densities provide the opportunity for antigenic shift through gene mutation and recombination, while vaccination creates positive selection pressure for the variant strain viruses (73).

Bacteria and protozoa are, in contrast to viruses, 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 and chemotherapeutics have remained the primary means of control (26); a point well illustrated by the continuing difficulties experienced in the EU with the systematic withdrawal of in-feed antibiotics (32). It is no coincidence that the downward trend in prophylactic (in-feed) antibiotic usage has been matched by an increase in therapeutic use (54). 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 (44). 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 re-emergence of previously controlled bacterial and protozoal diseases will likely continue. The industry must adapt in order to remain competitive. Refer to the section of this chapter on Disease Prevention and Control in Antibiotic-free Production for further discussion.

The Principles

Disease prevention and control involves the three 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, and *preventing dissemination* of the disease causing agent (70). There are three 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, control effort is focused on the complete elimination of the agent from the environment (70). 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.

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, since resource allocation must match risk. 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 (37).

No disease control or prevention/eradication program would be successful without diligent diagnostic surveillance. 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 since this requires frequently testing a large sample of the population. The heavy economic burden of such intense surveillance is difficult to carry especially when

the probability of a disease outbreak is low. Potentially devastating diseases such as highly pathogenic avian influenza (HPAI) can be effectively eradicated provided adequately robust bioexclusion, surveillance, and biocontainment programs are in place. The fact that some strains of the virus have 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, active and passive surveillance via laboratory testing, and necropsy findings. The sample size and frequency constraints of these procedures severely limit sensitivity, thus emphasising the need for careful sample selection and attention to detail. The focus should be on identifying and eliminating subtle disease challenge since 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 downregulated the clinical signs of intestinal disease; i.e., 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, may be 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 preventative measures must be practical, enforceable, and cost-effective and thereby form an integral part of the production system. Since 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 preventative 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

This 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. Biosecurity should be considered 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 at 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 each 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. They 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

This 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. 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.

$$\textit{Risk} \text{ of infection} = \textit{probability} \text{ of the event causing infection} \times \textit{frequency} \text{ of the event.}$$

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.

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

The probability of infection occurring can thus be reduced by improving host resistance through immunization and stress reduction, reducing the challenge dose through biosecurity, 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 amongst 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, non-specific disease challenge, or disease of the immune system, will reduce both individual immunity and flock immunity. Since 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 and flock immunity drops dramatically because of the presence of highly susceptible individuals within the population.

Disease Challenge

(Dose × Virulence × Frequency)

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 infection 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 one 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. Since 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) in order to design an effective flock health or biosecurity program.

For flock health management purposes, each disease must be analyzed first in terms of its *relative risk*, 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 the agent, 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 where 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, sub-acute, 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. Any environmental stress could for example 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 while 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. This term is used to describe 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 while the morbidity tends to be 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 what course of action it is best to take to limit the current and future financial risk of that particular disease.

Disease Prevention: Bioexclusion

Preventing or reducing *disease challenge* requires that there is 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. The World Trade Organization (WTO) is an intergovernmental organization that regulates international trade. The WTO's Sanitary and Phytosanitary Agreement seeks to harmonize sanitary and phytosanitary measures on as wide a basis as possible, and references the World Organization for Animal Health as the relevant organization for animal health. The World Organization for Animal Health was formerly known as the Office International des Epizooties and still goes by that acronym (OIE). The OIE currently represents 181 member countries, including the United States of America, and is led by the World Assembly of Delegates, consisting of representatives from each member country. It is important to understand the workings of the OIE as it pertains to disease prevention, diagnosis, and control. In order to trade internationally in poultry and poultry products, control measures implemented at farm level must ultimately comply with the organization's stipulated requirements. The reader is referred to the official OIE

website (http://www.oie.int/standard-setting/terrestrial-code/access-online/) for details of the rules and regulations as laid out in the OIE Terrestrial Animal Health Code (Terrestrial Code), but in summary the objectives of the OIE are to:

- Ensure transparency in the global animal disease situation by reporting detected disease.
- Collect, analyze, and disseminate veterinary scientific information on animal disease control.
- 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.
- 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.
- Improve the legal framework and resources of national veterinary services.
- To provide a better guarantee of food of animal origin and to promote animal welfare through a science-based approach. The OIE works in conjunction with the Codex Alimentarius Commission (CAC) 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 international agreement on the application of sanitary and phytosanitary measures. This so-called Sanitary and Phytosanitary (SPS) Agreement of the World Trade Organization 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*.

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 (7). The following avian diseases are included in the OIE List (8): avian chlamydiosis, avian infectious bronchitis, avian infectious laryngotracheitis, avian mycoplasmosis (*Mycoplasma gallisepticum*), avian mycoplasmosis (*M. synoviae*), duck virus hepatitis, fowl typhoid, highly pathogenic avian influenza and H5 and H7 low pathogenic avian influenza in poultry, infection with influenza A viruses of high pathogenicity in birds other than poultry including wild birds, infectious bursal disease (Gumboro disease), Newcastle disease, pullorum disease, turkey rhinotracheitis, and West Nile fever.

Region or State Perspective: Zoning and Compartmentalization

Due to the difficulties in controlling the disease status and management practices of poultry flocks across the vast expanse of large countries like the United States, the Terrestrial Code makes allowance for zoning and compartmentalization. Compartment, as defined by the Terrestrial Code, means *an animal subpopulation contained in one or more establishments 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* (6). 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 what is required to establish these subpopulations will vary according to the disease in question and the requirements of the trading partners. These details are ideally decided prior to the disease outbreak. Of particular interest is 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.

Since 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, documented, and evidence of compliance documented.

The plan must demonstrate adequately 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.

Disease Status: Classification of Diseases for Biosecurity Purposes

The allocation of resources to the prevention of diseases that have a major biological and financial impact is relatively easy. First, the control measures are the *cost of doing business*; freedom from the disease in question is a perquisite to doing business. Second, through eradication the cost of the disease is usually totally recoverable. In contrast, designing a disease control strategy for diseases that are likely to occur with a high degree of certainty but have less of a financial impact, is a lot more difficult (37). The process begins with clearly defining the estimated cost that the disease presence may incur and the potential benefits that the options for control may provide. Unfortunately there are several unknowns in health related matters, and it is consequently impossible to perform detailed and accurate cost-benefit analysis to ensure optimum resource allocation. Instead, partial farm budgeting is commonly used to compare the economic efficiencies of the various control options, including nonaction. In such instances immunization and its related biological and financial impact is the *cost of health*. One of the more difficult but important factors to quantify is the degree of productivity recovery that the control option provides, since disease losses are in this case seldom if ever totally recoverable.

For biosecurity purposes, diseases should initially be grouped into those that are exotic and those that are endemic to the region as this helps to optimize resource allocation to biosecurity. In the case of foreign diseases the emphasis is on reducing the risk of disease through prevention and eradication. In the case of endemic diseases the emphasis is on limiting the consequence of the disease.

The success of an eradication program hinges on good biosecurity and early detection of disease. In the United States the following diseases are usually prevented by eradication:

- Bacterial diseases: pullorum disease (*Salmonella enterica* serovar Pullorum), fowl typhoid (*Salmonella enterica* serovar Gallinarum), salmonellosis (*Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium), avian mycoplasmosis (*M. gallisepticum*, *M. synoviae*, *M. meleagridis*, and *M. iowae*), avian chlamydiosis (*Chlamydophila psittaci)* and avian tuberculosis.
- Viral diseases: highly pathogenic avian influenza (HPAI), low pathogenicity avian influenza (LPAI) of H5 and H7 types, velogenic viscerotropic Newcastle Disease (vvND), West Nile fever, duck virus hepatitis, and duck virus enteritis.

The National Poultry Improvement Plan (NPIP) is a voluntary cooperative United States federal–state–industry program initially developed to control and eventually eradicate pullorum and fowl typhoid. Over the years this program has expanded to provide established programs with specific procedures for not only pullorum and fowl typhoid, but also *M. gallisepticum*, *M. synoviae*, *M. meleagridis*, *S. enteritidis*, and avian influenza (HPAI and H5 or H7 LPAI), as well as other programs for general sanitation monitoring. Compliance with these programs provides official certification of control of these specific diseases by a company. In order to be eligible for indemnity in the case of depopulation for reportable avian influenza, commercial producers must participate in the NPIP Avian Influenza Monitored program. Among other monitoring requirements and response plans, this program now requires documented and audited minimum biosecurity practices. The NPIP biosecurity principles include requirements for a biosecurity coordinator; documented training of personnel; functional lines of separation for each house; perimeter buffer areas for each farm; personal protective equipment; wild bird, rodent, and insect control; equipment and vehicle sanitation; mortality disposal; manure and litter management; replacement poultry; feed, water, and litter supplies; monitoring; and auditing. Refer to the official NPIP website (http://www.poultryimprovement.org/documents/StandardE-BiosecurityPrinciples.pdf) for details on these programs.

Biosecurity program design 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 share 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, 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.

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 "downtime" is possible between successive placements. After placement the emphasis shifts to first limiting the *frequency* of any house perimeter crossing (event), and second at 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, overemphasizes 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 since stress of any kind stimulates a cascade of physiological and biochemical changes which erode host resistance and productivity (69). 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 (70). The influence of the house environment on viral disease of poultry has been reviewed (3).

Turnaround-time and Downtime. Turnaround time is the time lapse from the start of depletion/transfer to the start of subsequent placement. Downtime, 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 pathogen free. The risk of pathogen carry over from one production cycle to the next is directly linked to the time interval between the removal of one flock and the subsequent placement of the next flock. Pathogen attrition occurs with time and the chance of pathogen carry-over from one grow-out cycle to the next is reduced by extending downtime. 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 turnaround time can be used to substitute for clean-out and disinfection.

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

Decontamination: Clean-out and Disinfection. This procedure 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 turnaround 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 five steps: removal of debris, detergent application, washing with water, drying, and disinfecting (56).

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 physicochemical properties 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 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 is done, one must be aware 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 one complete flock cycle.

Washing begins with *blow-down*, a process by which water (preferably hot) and detergent sprayed though high-pressure nozzles is used to wet the surfaces and remove most of the dirt and dust from the house. The detergent helps to dissolve the organic biofilm and aids the cleaning process. This is followed by *cleaning* with water (preferably hot) sprayed at high pressure to remove residual dust and dirt. If washing is not possible, dry-cleaning must be thorough and includes 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. The house should be allowed to dry to prevent dilution of the applied disinfectant by residual water. 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 volumes 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 a variety of trade names; follow the manufacturers' recommendations. A disinfectant is a physical or chemical agent that destroys vegetative forms of harmful microorganisms, usually on inanimate objects but sometimes on the animals (10).

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 be applying disinfectants must read the label on the disinfectant closely and look specifically for references to the WPS. 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 (10, 17, 46, 56). Additional references on disinfectants and their use (28) 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 EPA (United States) (5).

After disinfecting the house, all subsequent processes and movements should be controlled to prevent recontamination. This requires that "clean" and "dirty" areas are clearly demarcated. Stringent sanitary practices are frequently ineffective because disease is 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 minera1 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) (41, 55).

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 per farm permit complete depopulation at the end of each brood. Cleaning and disinfecting of houses is in such instances reserved for disease outbreak control.

While 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 as 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 flock health. The carcass undergoes decomposition with the production of millions of decomposition bacteria and potentially pathogenic organisms 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 at least daily and categorized per house according to the likely cause of death. If daily mortality is abnormally high (more than 1/1000 in broilers and more than 0.3/1000 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 in order to prevent soiling of nests, nest material, and eggs. Table-egg breeding stock are traditionally raised on slatted or sloping wire-floor houses which greatly reduces the number of dirty eggs. Broiler and turkey 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, the shavings must be dry, 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 two weeks to keep it filled to a depth of 5–10 cm and ideally replaced (and the nest box disinfected) monthly. Any extraneous material should be removed from the nest box as soon as possible—broken eggs, fecal material, and so on.

The house environment is from a biosecurity point of view, classed 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 four 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 which 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.

Properly handled, nest-clean hatching eggs will produce suitable chicks and fumigation or sanitation may not be necessary. If a sanitation procedure is desired, in order to derive maximum benefit from any disinfection procedure the eggs need to be sanitized or fumigated within two hours of being laid, that is, immediately after collection. 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 a lot more arduous. Stringent health and safety regulations apply even in the United States where the EPA has classed formalin as a probable human carcinogen (9, 28).

Egg washing is routine practice in the commercial egg industry. These table-eggs are washed with warm (43–51.8 °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 Mackenzie (56) and Scott and Swetnam (67).

After fumigation the eggs should be transferred to the egg storeroom. 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 15–20 °C with a relative humidity 75%. Movement of eggs in and out of the storeroom should be done as quickly as possible to avoid excessive fluctuation in temperature and humidity.

Feed and Drinking Water. The potential for feed or waterborne 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 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 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 or slatted dropping pits are common in floor-laying and breeder hen houses to keep chickens away from their feces (Figure 1.1). 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

Figure 1.1 Slat floors aid in the control of intestinal diseases and parasites. Droppings fall through open spaces and out of reach of the flock.

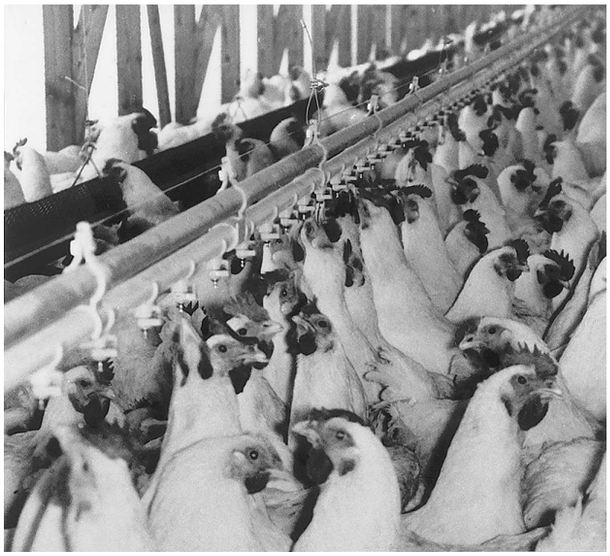

Figure 1.2 Nipple drinkers are effective in preventing microbiological contamination of clean water and help maintain dry litter conditions.

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 two basic categories: those that provide a constant reservoir of water, which is maintained automatically (troughs, cups, and hanging plastic bells), and nipple drinkers (Figure. 1.2), 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 one 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, as 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 of plastic, or lined with some 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. These reduce 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 and Equipment. People and 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 disease agent, but rather because they track in infectious diseases, use contaminated equipment, or manage their flocks in such a way that spread of disease is inevitable. At least one avian disease pathogen (Newcastle disease virus) has been found to survive for several days on the mucous membrane of the human respiratory tract and has been isolated from sputum. 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 diseases. 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. Shower-in facilities with dedicated clothing and footwear are the optimum solution, but are rarely available in commercial production in the United States. As an absolute minimum any person entering the house should don coveralls, a hair net, gloves, and boot covers to reduce the risk of disease 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 as they are the most likely means of infectious agent transfer. Any equipment brought into the house while birds are present or after cleaning and disinfection should follow similar rules. In particular, shared or borrowed equipment should be avoided if possible, and if not, it must be thoroughly cleaned and disinfected prior to entry.

When moving from one house to another it is best to change boot covers or use house dedicated footwear. Footbaths might work well when the boots or boot covers are clean and the disinfectant is clean and at the correct concentration. Footbaths are however notoriously difficult to manage and frequently end up enhancing disease transmission not 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 surgical gloves is the best option but hand washing and disinfection between houses is acceptable. It is preferable to have hand washbasins 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 be meticulous in following procedural biosecurity practices to prevent spreading disease. For such personnel a "no shoes touch the ground" policy is recommended. The vehicle should be parked in a secure area away from exhaust fans and as far as practical from the houses. Shoe covers are carried in the vehicle, donned before exiting the vehicle, and doffed as the vehicle is re-entered. Outer boots or shoe covers are used to enter the houses as described above. This practice, coupled with the use of hairnets, coveralls, and exam gloves that are doffed prior to re-entering the vehicle will help control contamination of the vehicle interior. 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 disease 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. Personnel traveling internationally should be advised to use clothing and footwear on the trip which will not be worn to farms upon return home. Requiring a waiting period of several days before international visitors or those returning from international trips are allowed to visit farms is a prudent practice.

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 so as 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 are allowed to roam among the poultry, 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 diseases 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, and to exclude free-flying wild birds from the houses. Poultry raised on range or with access to the outdoors are especially vulnerable to infections 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 like HPAI.

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 disease 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 destined for pet stores have been found infected with a virulent exotic form of Newcastle disease virus, which in at least one 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 can also be a source of dangerous strains of Newcastle disease virus.

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. 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, elimination of tall grass and other harborages in the vicinity of the houses, 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 entitled, *A Practical Guide for Managing Risk in Poultry Production* (78).

House Access: Insect, Mite, and Tick Control. Many insects act as transmitters of disease. Some are intermediate hosts for blood or intestinal parasites, others are mechanical carriers of disease through their biting parts. They also act as reservoirs of disease, in that they can transfer an infectious agent from one flock to the next. Litter beetles are not only a major pest in the poultry house environment but can play a vital role in the spread or carry-over of disease. The control of litter beetles is based primarily on the spraying of insecticide between flocks and during the clean-out process. The beetles migrate into the walls and out of the house as soon as the birds and feed are removed, so applications should be made as soon as possible after depletion. Insecticide resistance is a problem, so frequent assessment of efficacy and rotation of products is necessary. Flies and mosquitoes can be a problem in disease transmission in layers, breeders, and birds on range. Mites and ticks 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 the preventing, destroying, repelling, or mitigating of 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, their active chemical properties, limitations, tolerances, and various applications, are discussed in detail in Chapter 26. See also 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 (64).

Building Construction. An apron of concrete at the entrance to a poultry house helps prevent tracking of disease into the unit. Rain and sunshine help keep the apron cleaned 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 diseases 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.

Optimally, all surfaces inside the building should preferably be of impervious material (such as finished concrete) to permit thorough washing and disinfection. It is impossible to sterilize a dirt floor! Unfortunately, broiler and broiler breeder houses in the United States are typically constructed with dirt floors and porous walls such as unfinished concrete blocks or plywood, with many cracks and crevices, making disinfection difficult.

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 will remain pathogen-free only if it hatches in a clean hatcher, is put in a clean box, and held in a clean room where it can breathe clean air, and is then hauled to the farm in a clean delivery van.

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 chick-loading area. The cleanup 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. Hatcheries with poor floor designs and faulty traffic patterns are highly contaminated compared with those with one-way flow (36).

Importance of Good Sanitation. Techniques have been devised for evaluating the sanitary status of commercial hatcheries by culturing fluff samples (83), detecting microbial populations in hatchery air samples (27, 36, 50), and culturing various surfaces in the hatchery (52). To minimize bacterial contamination of eggs and hatching chicks, hatchery premises must be kept free of reservoirs of contamination, which readily become airborne (51). Trays used for hatching should be thoroughly cleaned with detergent and hot water and then disinfected before eggs are placed in them. This can be done by dipping in a tank of suitable disinfectant (see Disinfectants), 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. 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 rapidly (36). 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. Keeping chicks of different breeder codes separate ensures that all have more nearly the same level of maternal antibodies against the same diseases, which may permit a more uniform response to vaccines applied to chicks the first two to three weeks of life when maternal antibodies have a protective effect. Segregating chicks by breeder sources also contributes to better size uniformity and reduces the impact of any vertically transmitted pathogens.

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 one 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. 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 at least 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 like *Staphylococcus aureus* or primary pathogens like *Erysipelothrix rhusiopathiae*. Similarly, other surgical procedures, such as removing wattles, combs, or toenails of certain toes, must be done as aseptically as possible.

Storage Facilities. Hatching eggs are frequently stored in a cool room (about 15–20 °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 (82). 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. Cold eggs should, therefore, 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 caretakers and 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 (non-fenced) line of access control to the secondary contro1 zone. The farmer should enforce full biosecurity with no uncontrolled access from the start of the disinfection process—site is *closed*. The farmer should enforce general biosecurity from the start of transfer/depletion with access only granted to necessary vehicular traffic—site is *open*. The farmer should enforce routine control from the point of last bird removal—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 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, 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 to solving the problem. This is even more successful if coordinated area depopulation and restocking is practiced.

One Age of Fowl per 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 one 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 its disposal. Depopulated premises are then cleaned out, washed, and disinfected, and left idle for at least two 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 1.3). 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 one quarantinable segment.

Farm Environment

The farm or site must be maintained so as to minimize the breeding areas and any overt protection given to wild birds (especially waterfowl), 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 clean-out. 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 in such a way as to avoid offering shelter or protection to unwanted creatures.

Farm Access Control

The site should ideally be completely fenced with sufficient deterrents to access by predators, vermin,

Figure 1.3 This isolated breeding farm benefits from several fundamental disease prevention and control principles. It is isolated from other poultry farms, is surrounded by forest land, and is divided into quarantinable sections separated by woods as well as distance.

and unauthorized people. There should preferably be only one 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 two days prior to entering the farm. Only essential visits by authorized personnel such as mechanics, managers, working crews, and so on should be allowed on site. They must not have had contact with other non-company poultry or domestic birds for at least two days and must observe the prevailing biosecurity procedures for house access if entering a poultry house.

All non-essential visits by company employees and all non-company 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 two 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, that is, 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 they 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 policy, 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 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 can also 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 one site per 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 with regards following biosecurity protocols.

Vehicle Access. To reduce the risk of disease agent transmission it is best to prohibit vehicle access to the site. Unfortunately, it is often necessary for vehicles to drive onto site, for example to deliver feed, propane, or egg collection supplies (trays, racks, boxes) and to pick up eggs. If possible, 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 the production cycle pass through this dip. Any vehicle that carries live birds, non-wettable 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 whilst 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 reentry.

Equipment. All equipment entering a site should be suitably decontaminated by a detergent 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 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 cleanout 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, and so on 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 are 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 buggies. It is preferable to wheel the egg buggies 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 buggies, 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 buggies 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 straight after eggs are dispatched to the hatchery, that is, when the room is empty.

Fumigation Room

Fumigation is the process of decontamination of an object through the use of a gas compound. Since 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 two accesses – 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 site. The site access must be used for receiving fumigated goods onto site and for dispatching potentially contaminated goods from site. Only one access must be open at any given time. Nothing should be allowed to be taken onto site unless it has been showered (soap wash), disinfected, or fumigated. Certain exceptions do however exist and include live birds and non-wettable 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) so as 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 1.4A), composting (Figure 1.4B) or burial, or off site through burial, composting, central depots, or rendering plants (61), 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.

On-site disposal is generally preferable due to the hazards associated with entry of disposal vehicles to the site and to other farms associated with transport of the carcasses. Transport vehicles should be completely sealed to prevent leakage of liquids and to exclude insects, rodents, and scavengers. On-site disposal areas must also be secure from insects, vermin, and scavengers.

Tertiary Control Zone: Complex

An epidemiological unit may also refer to groups of birds that share a communal animal handling facility. The sites/farms within a complex will for example 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. Grandparent stock are for example substantially more valuable than broiler 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 disease transmission. The tertiary control zone is seldom fenced so the access control boundary is usually imaginary.

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

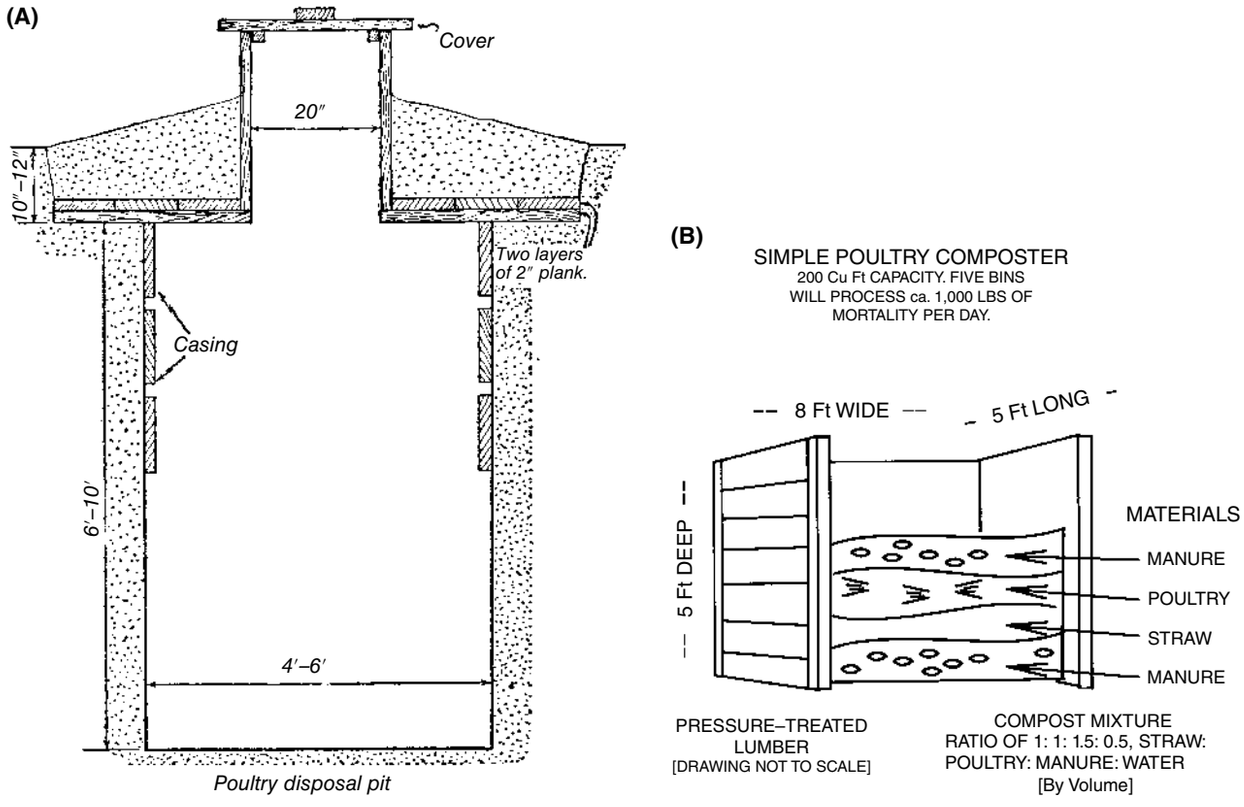

Figure 1.4 (A) Poultry disposal pit. Such a pit can be made any size that is convenient. (B) A simple above-ground poultry carcass composter of 200 ft^3 (5.7 m^3) capacity. Five such bins will process 1000 lb (455 kg) of carcasses per day. (Courtesy Poultry Science Dept., University of Maryland).

be "operational" in nature. Such a complex does not merely exist because two 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 so as 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, and so on should be allowed onto a complex. All non-essential visits by company employees must be authorized by the veterinarian. All non-company 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.

Diagnosis: Monitoring, Surveillance, and Confirmation

Judicious use of cost items like antibiotics and non-antibiotic feed additives makes both scientific and economic sense and begins with *accurate* and *early* diagnosis.

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. A *surveillance* program is in contrast 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 disease 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 so as 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 the significance of this in program design.

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). So 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 does however automatically invalidate 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. 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, and so on.

Poultry flock health tracking requires that flocks 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 one diseased animal, in a finite population of birds to be greater than a predetermined confidence level (commonly 95%).

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

Where: n = sample size, N = flock size, p = probability of selecting at least one 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 test used is 100% sensitive and 100% specific, sampling is performed with replacement, and the data is collected by simple random sampling) renders the estimate inaccurate (21, 23). A more accurate determination of sample size is given with a computer program like "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 (21).

Sample frequency must be calculated based on the epidemiology of the disease under consideration. With *M. gallisepticum* 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 (39, 40, 66). After flock exposure to *M. gallisepticum* there is a latent phase of 12–21 days in which less than 5% of the flock has a detectable antibody response (57). 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. To prevent infected eggs from entering the hatchery it would be necessary to sample flocks every two weeks (assuming 100% sensitivity for the test system). The testing interval can be extended by two 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 percent hatch, 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), and government reporting services such as the poultry slaughter reports published monthly by the National Agricultural Statistics Service (NASS), Agricultural Statistics Board, US 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 for evaluating overall health and vaccination program efficacy.

Examination of Field Birds

Health surveys (11, 47) that include extensive gross and microscopic evaluation of necropsy specimens, and controlled challenge studies (59) to measure a relative protection level, are both useful in assessing vaccine program effectiveness. Perhaps the most frequent controlled challenge work done is measurement of passive protection of broiler chicks from hens hyperimmunized to infectious bursal disease (59). Trends in program efficiency may be identified over time if sufficient groups of chicks are sampled.

Serologic Monitoring

Serologic monitoring (71) is only useful in production medicine if adequate samples have been analyzed over time in order 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 they 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 versus 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 or concentration of antibody in the serum) reported from different laboratories may be confusing. It is best to use one 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.

There is value in correlating ELISA profiling data with flock performance (71), and with gross and microscopic pathology data (53). 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 1.5) is combined with the diagnostician's veterinary skills and experience in assimilating medical information and establishing a plausible diagnosis.

Diagnostic Procedures

Many satisfactory diagnostic and necropsy methods exist. 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.

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. The techniques and procedures necessary to make an accurate diagnosis and identify specific disease agents are found in succeeding chapters of this book and in reference manuals such as *A Laboratory Manual for Isolation Identification and Characterization of Avian Pathogens* (80), *Avian Disease Manual* (18), *Avian Histopathology* (1), and *Color Atlas of Diseases and Disorders of the Domestic Fowl and Turkey* (62). *Avian Hematology and Cytology* (22) should be consulted for detailed information on avian blood elements and methods for preparation and study. New information is continually being presented in journals such as *Avian Diseases*, *Avian Pathology*, and *Poultry Science*, in the proceedings of several regional poultry disease conferences, and in other avian pathology and science journals.

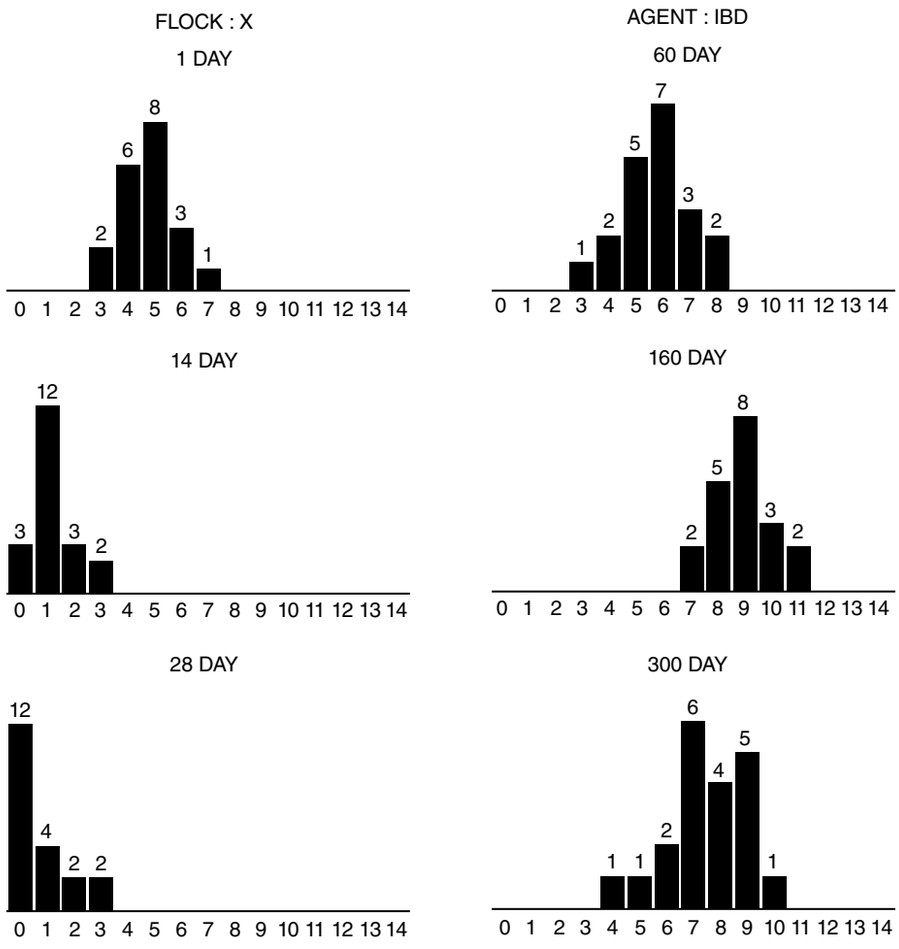

Figure 1.5 Temporal graphic distribution of infectious bursal disease (IBD) enzyme-linked immunosorbent assay (ELISA) group titer levels at 1, 14, 28, 60, 160, and 300 days of age for an IBD-vaccinated broiler breeder flock. Numbers on the X-axis represent group titer levels obtained by ELISA. Titers of 0 are group 0; 1–350 are group 1; 351–1,500 are group 2; 1,501–2,500 are group 3; 2,501–3,550 are group 4; etc., with titers of 12,500 comprising group 14. Numbers above each bar represent the number of samples reacting at each level on the indicated day of age.

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. 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.

External Examination

Look for external parasites. Lice and northern fowl mites (*Ornithonyssus silviarum*) can be found on the affected chicken. If red mites (*Dermanyssus gallinae*) or blue bugs (*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. Venipuncture of the brachial vein is usually the simplest and best method for obtaining blood from turkeys, chickens, and most fowl 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 1.6). The needle should be inserted opposite to the direction of blood flow. 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 10–12-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 also

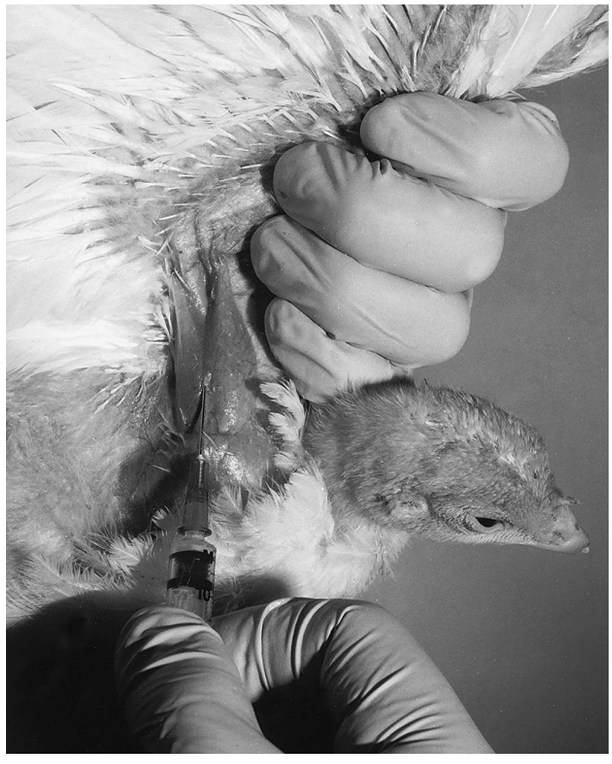

Figure 1.6 Obtaining a blood sample from the wing vein.

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, as this will hinder the clotting process. Sera should not be frozen if agglutination tests are to be performed as 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 gently shaken. 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 EDTA can also 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 (22). 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

Several methods can be used to kill fowl, and each has certain advantages. The objective is to kill the bird instantaneously so it will not suffer in the process. Cervical dislocation and decapitation are considered humane methods of poultry euthanasia by the American Veterinary Medical Association (AVMA) (4). For large breeders and turkeys in which cervical disarticulation is not feasible, a purpose-designed captive bolt gun may be used. Specimens selected for diagnosis may also be killed by intravenous injection of euthanasia solutions or by placing the bird in a chamber filled with carbon dioxide (CO_2) or a mixture of 30% CO_2 and either nitrogen or argon. Local availability of a source of these gasses may limit utilization of this technique. Other methods of euthanasia can be found in a report of the AVMA (4). The method selected will depend upon the existing situation: species, size, and number of birds to be necropsied or sacrificed; tissues, fluids, and cultures to be taken; and so on.

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. 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 safety glasses and 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 (35). 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 1.7A).

The skin is cut between the two 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 1.7B). Hemorrhages of the musculature, if present, can be detected at this stage.

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 1.7C). Bone shears are used to cut the rib cage and then the coracoid and clavicle on both sides (Figure 1.7D). With some care, this can be done without severing the large blood vessels. The process may also be done equally well in reverse order, cutting through the clavicle and coracoid 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 1.7E, 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 uncontaminated 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.

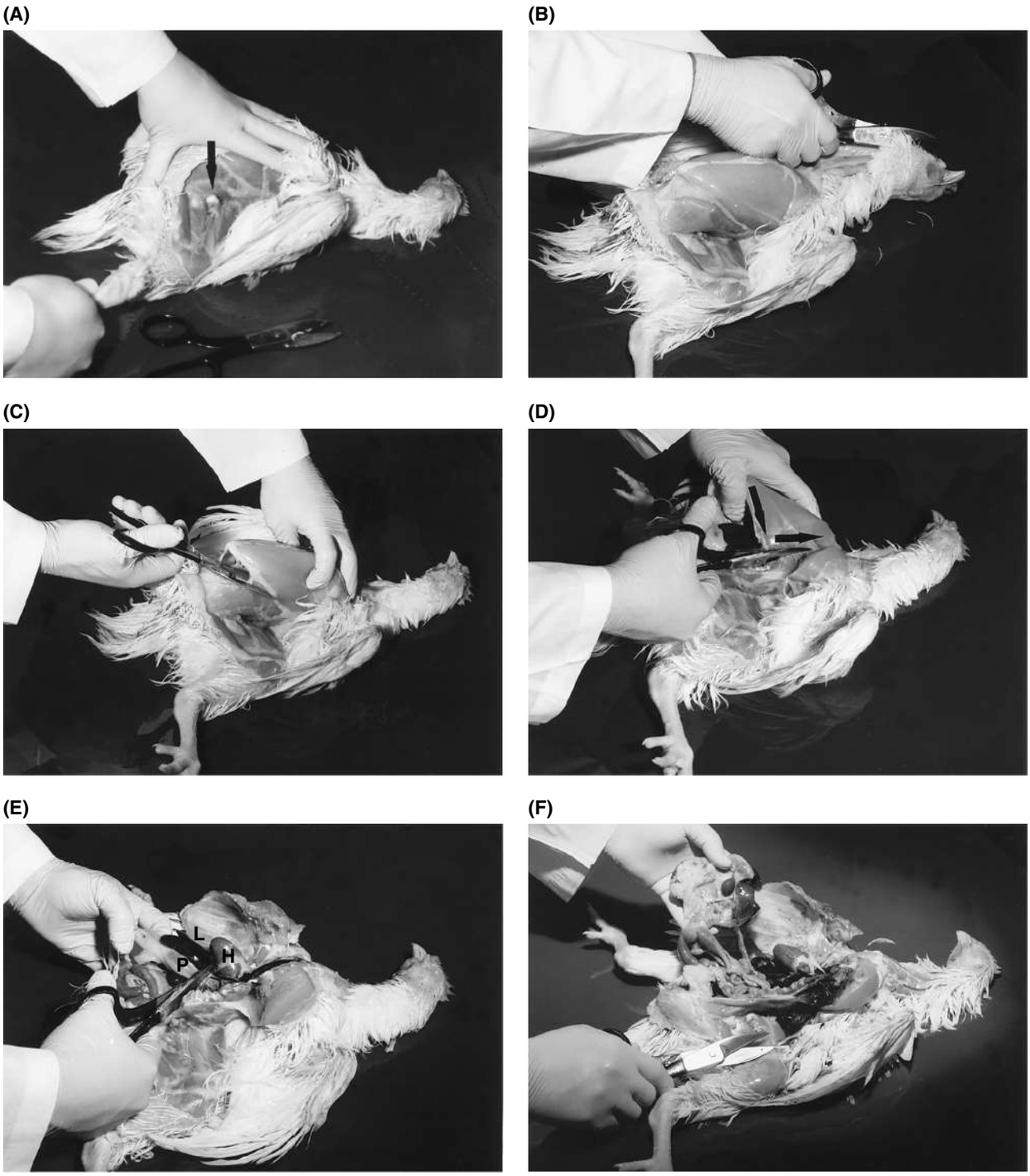

Figure 1.7 Each pathologist will develop their own systematic technique for conducting a necropsy. A sturdy pair of poultry shears is usually sufficient to conduct a necropsy. Other instruments such as scissors, forceps, and scalpel may be helpful in collecting small or delicate samples. A knife may be needed to cut through joints and bone. 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.

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* (80) 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 costochondral 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 sampled 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 the Brain

Removing the intact brain is not easy, since 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 atlantooccipital 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 one 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 1.8A). Lift off the cut portion of bone and expose the entire brain (Figure 1.8B).

If a portion is needed for culture or animal inoculation (e.g., avian encephalomyelitis virus suspect) and also one 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 one 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 1.8C). 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 may also be removed in reverse order (Figure 1.8D). If all of the brain is required for either purpose, proceed with proper precautions for the

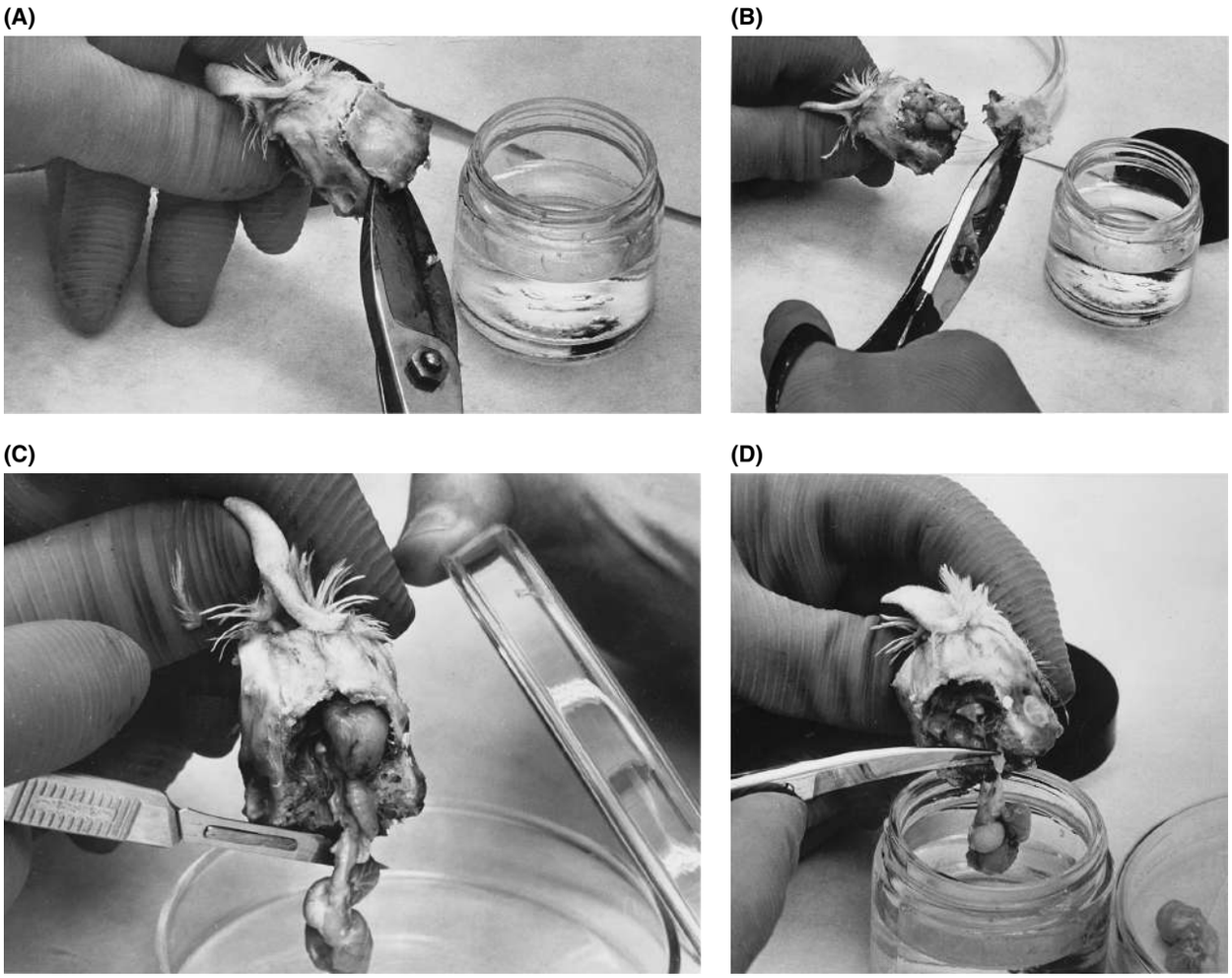

Figure 1.8 With a little practice, the brain can be removed with a minimum of trauma. (A) Incise bone all the way around the periphery of the cranial cavity with heavy bone shears. (B) Remove loosened portion of the bony skull. (C) Incise brain longitudinally with sterile, sharp scalpel and remove one-half for sterile culture technique. (D) Remove second half by dropping it into 10% formalin for histologic techniques.

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.

Tissues for Histopathologic Examination

For good preservation, the tissue pieces from killed birds should be saved immediately after death, especially gut, 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 × their own volume of 10% formalin or other fixative. Bone pieces should be sawed with a sharp bone saw unless thin or soft enough to cut with scissors or scalpel.

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.

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 (60, 61, 74) should be consulted for detailed procedures.

Disposing of the Specimen

If a disease infectious for humans is suspected, the carcass should be autoclaved, incinerated, or otherwise rendered incapable of causing infection 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 they 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 their 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 disease into a country, region, zone, or compartment it is essential that potentially infectious material is kept in isolation until they have been shown to be clear of the disease(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. First, the movement of anything into, onto, from, or through the area of control must be restricted and monitored. The extent of the control zone will depend 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 ten kilometer radius around the affected farm. It is important to establish the extent of the disease outbreak through disease monitoring, first within the quarantine zone 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 will have a predetermined emergency response plan carefully designed to handle all 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. For example, chemoprophylaxis is used routinely in the control of coccidiosis worldwide. Judicious use principles dictate that chemoprophylaxis with antibiotics for bacterial diseases should be reserved for situations in which a specific bacterial disease is judged highly likely to occur in the absence of chemoprophylaxis, and other measures such as biosecurity and immunization have proven inadequate.

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 in order 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. Some 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 progeny. Chicks frequently receive up to three 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 IBD, many areas of the world have found maternal antibodies a very useful tool in IBD prevention and control. The effect of maternal antibodies on the efficacy of modified live virus vaccines in young chicks must be considered, and varies with the disease. For example, maternal antibody is highly effective in blocking active immunization with homologous IBD, moderately effective in blocking lentogenic Newcastle disease virus vaccines, and much less effective in blocking infectious bronchitis virus vaccines.

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. 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 (20). 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 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 laryngotracheitis, fowl pox, and Marek's disease.

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

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

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

Table 1.2 Methods of generating live vaccine candidate.

Method	Example
Virulent organism inoculated to a less susceptible target tissue or at a controlled dose	Laryngotracheitis–cloacal route
Naturally occurring mild pathotype	*Mycoplasma gallisepticum* F strain
Egg passage of virulent parent	Infectious bronchitis—Arkansas strain
Temperature-sensitive mutant of virulent parent	Turkey coryza vaccine—*Bordetella avium*
Chemically derived mutants of virulent parent	M-9 Fowl cholera vaccine
Tissue culture/passage of virulent parent	Laryngotracheitis
Combination of egg passage and tissue culture passage of virulent parent	Infectious bursal disease—Lukert virus
Plaque selected "clones" of parent virus	Newcastle disease virus—cloned Lasota vaccines
Selection of subpopulations or organisms based on replication characteristics *in vivo*	Precocious strains of *Eimeria* spp.
Relatively virulent organisms given at an age that minimizes disease	Avian encephalomyelitis

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 dating. 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, pH, 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 a specific pathogen free (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 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 fowl pox virus vaccines expressing genes to protect against H_5N_2 avian influenza (14), Newcastle disease virus (19), and IBD virus (12), and baculovirus-expressing IBD virus (77). Commercially licensed live virus-vectored vaccines currently available and widely used in the United States include herpesvirus of turkeys (HVT) expressing IBD virus antigens, Newcastle disease virus antigens, infectious laryngotracheitis virus antigens, fowl pox expressing Newcastle Disease virus antigens, infectious laryngotracheitis virus antigens, and *M. gallisepticum* antigens. There are also licensed HVT-avian influenza and pox-avian influenza constructs available, but use of these vaccines is only under permit from the USDA (United States Department of Agriculture). Bacteria-vectored vaccines described in poultry include bacteria such as *E. coli* (43) and *Salmonella* spp. (63) expressing antigens from coccidia and *E. coli*, respectively. Vaccines to reduce *Salmonella* infection, made from a gene deletion mutant of *Salmonella typhimurium* (30), and an *E. coli* gene-deleted vaccine are 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. 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 utilized in eradication programs such as 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 (58).

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 two 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. Injection of humans that are administering these inactivated vaccines should be avoided. 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 a 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. DNA vaccines have been used successfully experimentally in poultry for avian influenza and Newcastle disease in chickens (34, 65) and duck hepatitis B in ducks (76). 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 hatcher. 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, 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 (38). The most common vaccine administered *in ovo* in the United States is Marek's disease vaccine; IBD vaccine, reovirus vaccine, and the various Marek's-vectored vaccines are also commonly given by this route. The original experiments on *in ovo* vaccination with Marek's disease vaccine showed that chicks were protected earlier than those vaccinated after hatch (68). 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 (75). Using an egg injection system (Embrex Inovoject® Egg Injection System, Research Triangle Park, NC), one machine with three people generally inoculates 20,000–30,000 eggs per hour (Figure 1.9). 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 hatcher. Hatcheries must be acutely aware of their aspergillosis levels to run an egg injection system successfully (79).

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 the neck injection. A skilled operator can vaccinate about 1,600–2,000 chicks/hour. A 20-gauge needle generally is used, as 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

Figure 1.9 A modern hatchery with an egg injection system for *in ovo* vaccination.

cabinet that sprays boxes as they move down a controlled speed conveyor line in an automated hatchery. Both methods, frequently used to deliver Newcastle disease virus, 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, Newcastle disease virus and infectious bronchitis virus vaccines are often delivered in 7 mL of distilled water per 100 chicks. Coccidiosis vaccines generally use more distilled water, approximately 20–25 mL per 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 Newcastle disease virus 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, five gallons of water per 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, one person can split the flock while the vaccinator slowly sprays one 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 (72).

Table 1.3 Visual analogy to droplet size diameter.

Analogy	Diameter (microns)
Wet fog	25–40
Visible droplets	50
Misty rain	50–100
Light rain	200–400

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 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 two days prior to vaccination. It is best to buffer the system by flushing it with a weak solution of powdered skim milk, generally one cup powdered skim milk to 50 gallons of water (25). 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 two hours prior to the vaccination procedure. This time varies widely. Climatic conditions may necessitate longer or shorter time periods. Emptying the drinking system and then charging the water lines with vaccine-laden water ensures that the first birds to drink receive a dose of vaccine. The total time required to administer the vaccine is a balance between the gradual deterioration of vaccine titers in the water system against adequate time for all birds to get a drink. 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 two commonly used wing web application tools. The first is the traditional small plastic handle approximately 3 cm long that has two solid stainless steel prongs, approximately 2 cm long, with a bevel on each prong toward the needle end. The prongs are dipped into an open container of vaccine between each bird. The second is a Grant inoculator, a syringe-like tool with a self-contained reservoir for vaccine, most often fowl pox or fowl cholera, through which a needle passes 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 four weeks prior to the onset of egg production to minimize any adverse effect the handling or the vaccine may have on egg production performance. Subcutaneous vaccination is most frequently performed using a ½inch, 18-gauge needle, in the neck. The area halfway between the head and the shoulder is optimal and allows the vaccinator to lift the skin away from the neck muscle and insert the needle, pointed toward the body of the bird, into the subcutaneous area and deposit the vaccine. Intramuscular injection generally is performed using a ½ inch, 18-gauge needle to inject vaccine into the breast or leg muscle. Breast muscle injections are safest when the vaccine is deposited in the superficial pectoral muscle 2–3 cm lateral to the keel bone. If the needle is kept at a 45-degree angle to the bird, any accidental injections into the body cavity or liver can be avoided (49). Leg vaccination generally is done in the lateral gastrocnemius muscle. Both intramuscular injection sites may result in residual emulsion being present for an extended period of time (31). A residual deposit in muscle depends on many factors including the antigen and the adjuvant found in the vaccine. Care should be taken to determine the intended use of meat before injecting intramuscularly.

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. Viable vaccines administered in the drinking water can, likewise, 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 (13).

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 two 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. 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. In a modern poultry production system, any disease creates serious disruption in the economical operation of the farm and the plants processing products from it. The following steps should be followed when disease is suspected.

Take precautions against tracking an infectious disease 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 (2, 16). Bell (15) 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 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.

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 laryngotracheitis) must be reported immediately to the state 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 vvND, fowl typhoid, or avian influenza is encountered, the proper state and federal regulatory authorities should be informed immediately.

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. 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, if appropriate, 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. Beginning in 2017 in the United States, all medically-important drugs given to food animals by feed or water must be used under a veterinary prescription (for water) or a Veterinary Feed Directive (for feed). See Antimicrobial Therapy Including Resistance for a discussion of the types of drugs included in these categories. 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. A handy reference is the annually updated *Feed Additive Compendium* published by Miller Publishing Co., Minnetonka, MN. Feed manufacturers must have Food and Drug Administration (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 one 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 (for example, salmonellosis, mycoplasmosis, reovirus, inclusion body hepatitis, and avian encephalomyelitis), eggs should not be used for hatching until the danger has passed. It should also 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 or for emergency slaughter if permitted. 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.

Disease Prevention and Control in Antibiotic-Free Production

John A. Smith, Martine Boulianne, Robert L. Owen, and Eric Gingerich

Summary

Regulatory changes in the United States have restricted non-therapeutic uses of medically important antibiotics, but continue to allow most therapeutic uses under increased veterinary supervision. More importantly, market-driven restrictions on all uses of antibiotics in poultry production have created challenges in the control of bacterial infections. Resulting problems in broilers are increased early mortality, coccidiosis, and

necrotic enteritis (NE); in turkeys, coccidiosis and bacterial infections such as *Bordetella avium*; and in table egg layers, colibacillosis and NE. Management of these problems may require adjustments to management and diet, use of non-antibiotic medications such as chemically synthesized coccidiostats, and alternative products such as probiotics. Even with these interventions, performance and health problems may exceed those found in conventional production schemes with unrestricted access to approved medications.

Introduction

Current United States Regulatory Environment

In an effort to address the emergence of antibiotic-resistant bacterial pathogens in the human population, government authorities in numerous nations have enacted restrictions on agricultural uses of antibiotics. In 2017 the United States rescinded all growth promotion and feed conversion clearances for all medically important antibiotics. The Food and Drug Administration (FDA) list of critically important, highly important, and important classes of antibiotics includes all currently approved poultry drugs except the ionophores, bacitracin, bambermycins, and avilamycin (43). Therapeutic uses of currently approved drugs (including medically important antibiotics) are still permitted, albeit with increasing restrictions and oversight. For example, in the United States all over the counter (OTC) clearances for medically important antibiotics administered by feed or water were withdrawn in 2017. All such uses must now occur under a veterinary feed directive (VFD) for in-feed treatments, or by veterinary prescription for water treatments, and duration of use restrictions are being tightened. Clearances for prevention and control of disease are considered therapeutic uses and remain available in the United States with the same requirements and restrictions for a VFD or prescription. Non-medically important drugs remain OTC except for avilamycin, a non-medically important drug which was nevertheless cleared as a VFD drug. In operations where marketing schemes permit access to all remaining clearances, chemically synthesized anticoccidial drugs and non-medically important antibiotics can be used without restriction to control coccidiosis and NE, and approved antibiotics, including medically important drugs, can be used judiciously under veterinary supervision to treat, prevent, and control NE and other diseases. Consequently, these new regulatory restrictions should have only modest impacts on bird health and efficiency and should be manageable with minor changes to management practices. Industry veterinarians are called upon to address production shortfalls and these changes may impact that aspect of practice. Veterinarians are expected to observe judicious principles of use in employing these remaining clearances.

Marketing Restrictions on Antibiotics

A larger emerging issue is marketing strategies that more severely limit or entirely prohibit the use of all antibiotics in food animals. These marketing campaigns are becoming increasingly common in developed countries and can have major impacts not only on productive efficiency but also on poultry health. In the United States, these campaigns are conducted via product labeling and advertising strategies. Many are initiated by retailers who demand these restrictions of their supplier–producers, frequently under pressure from activist groups and social media, while others have been initiated by the producers themselves in an effort to distinguish their products and capture a niche market of affluent customers. These marketing strategies are quite varied in their restrictions, and the resulting impacts and necessary responses therefore vary as well. Regardless of the scientific merit or prudence of such marketing strategies, they have become a fact of life in many countries and veterinarians will be called upon to address the resulting health problems. The veterinarian must therefore be fully aware of and understand the restrictions imposed by a given marketing program in order to know what substances are prohibited or allowed and how to manage that specific program.

Levels of Restriction

In the United States, all food labeling and marketing claims are governed by federal regulation. All claims regarding production, product attributes, and so forth on labels of animal products must be approved by the United States Department of Agriculture (USDA), Food Safety and Inspection Service, and cannot be false or misleading. Perhaps the most restrictive labeling claim permitted is "certified organic production".

The next most restrictive category commonly seen on labels in the United States is "raised without antibiotics" (RWA) or "no antibiotics ever" (NAE) and similar claims. Regulators in the United States generally disallow claims such as "antibiotic free", "residue free", or "chemical free" as such claims generally cannot be conclusively proven and are considered misleading. If RWA/NAE statements are made on labels or in advertising, no antibiotics of any sort, whether deemed important in human medicine or not, can be used at any point in the life of the bird, by any route, including *in ovo* or at hatch. If a sick flock must be treated, the produce from that flock cannot be labeled as RWA/NAE and must be diverted to commodity product; the producer must be able to demonstrate to the regulator that systems are in place to reliably and completely segregate such treated produce. In the United States ionophores are considered antibiotics for labeling purposes.

Consequently, marketing programs in the United States making RWA/NAE claims cannot use ionophores and the only options for coccidiosis control are coccidiosis vaccines or chemically synthesized non-antibiotic coccidiostats (i.e., those not derived from fermentation or other biological processes). Canada formerly did not allow chemically synthesized coccidiostats in "raised without antibiotics" programs and therefore Canadian producers making these production claims were restricted entirely to coccidiosis vaccines and nonpharmaceutical measures for the control of coccidiosis and NE. These vaccine-only programs have been difficult to manage on a commercial scale, and in August 2016 the Canadian restriction on chemically synthesized coccidiostats in RWA/NAE labeled production was rescinded. While there have been vaccine-only, completely nonpharmaceutical programs in the United States similar to the original Canadian program, these appear at present to be uncommon outside of organic production. As consumers and activists become more demanding, such extremely restrictive vaccine-only marketing programs may become more common in the future.

An intermediate level of antibiotic restriction has been adopted by a major international fast food retailer based in the United States (27) and many competitors have promulgated similar programs. This program cites the World Health Organization (WHO) definitions of critically important, highly important, and important antibiotics for human medicine. In practical terms, the three WHO categories together include essentially all agricultural antibiotics except ionophores, avilamycin, and bambermycins (49). This intermediate program prohibits any use of WHO critically important human antibiotics that have no veterinary approvals (i.e., no extralabel use of critically important antibiotics). It requires veterinary oversight (a veterinarian–client–patient relationship) for use of any so-called "dual use" human/veterinary drugs for treatment or prevention. It prohibits the use of WHO medically important antibiotics for growth promotion. Producers adhering to this program can use ionophores, avilamycin, bambermycins, and chemical coccidiostats, and the program allows treatment of sick flocks and prevention/control uses of veterinary-labeled drugs under veterinary supervision, without requiring diversion of the product. As with the new US federal restrictions on antibiotic use, programs such as this may have minor effects on productive efficiency, but animal health issues should be manageable with only minor deviations from standard commercial practices. Other programs continue to emerge, creating a complex and confusing situation for regulators, consumers, producers, and veterinarians.

At present most antibiotic-based marketing campaigns have not reached back beyond the hatching egg. Broiler hatching egg production has generally been less reliant on antibiotic use, and recent changes in regulation of antibiotics in the United States are not anticipated to have major impacts in this area. More severe restriction could increase issues however. Broiler production with RWA/NAE restrictions often entails increased neonatal mortality, and hatching egg quality and hygiene are important aspects of controlling this problem. Further restrictions on the use of antibiotics in breeder flocks could compromise health management and therefore hatching egg quality, exacerbating neonatal health problems in RWA/NAE broiler operations.

Economic Impacts and Metrics of RWA/NAE Programs in Broilers

It is common for RWA/NAE programs to include additional marketing claims such as an all-vegetable diet or bird density restrictions. Due to the wide variations on the theme of antibiotic restriction itself and these additional ancillary claims, it is difficult to precisely separate the results of varied restrictions on antibiotics from the effects (positive or negative) of these other differentiating attributes, and to cite conclusive figures for the impacts and costs of antibiotic restriction alone. One index of the impacts can be gleaned from a reporting service widely used by the United States broiler industry. AgriStats, Inc. is a United States-based company that gathers production information from over 95% of United States broiler producing companies to produce reports that benchmark individual companies' performance to industry average performance levels. Individual company numbers are confidential as companies see their information identified but are unable to identify the information from individual competitors. Subsets of the data can be created to analyze average results for items such as breed comparisons and nutrition and feeding programs, such as whether or not birds were fed an antibiotic. Before 2013, approximately 90% of birds grown in winter months were fed an antibiotic with a growth promotion claim. In late spring and through fall, the use of these medications typically declines to roughly 70% of broilers (15). By early 2017 fewer than 50% of all birds processed were fed antibiotics important in human medicine. The percentage of birds grown under RWA/NAE programs has also increased significantly, from 13.9% of all birds in December of 2015 to 38.7% of all birds in February of 2017 (15). Historically, broiler performance has shown a steady improvement over the last thirty years due to continually improving genetics, management technology, scientific feeding, and veterinary care. The anticipated annual gains have been tempered in recent years coinciding with the growth in antibiotic-restricted and RWA/NAE programs. For instance, in April 2017, for bird weights between 2.36 and 3.18 kg

(5.2–7.0 pounds, the most common weight range for RWA/NAE production), the 21 complexes producing RWA/NAE birds suffered a 2.9% increase in caloric conversion (2,595 calories per pound versus 2,522) and 0.68% higher mortality (15).

Managing RAW/NAE Programs in Broiler Production

Primary Issues

The major issues in RWA/NAE programs in broilers are coccidiosis, NE/dysbacteriosis, secondary issues related to gut health and gut barrier function, and the removal of therapeutic doses of antibiotics from Marek's disease vaccines which may lead to increased neonatal infections. In addition, and especially when all-vegetable diet claims are included, litter moisture control becomes more difficult, which can lead to foot and hock burns, skin lesions, air quality issues (and concurrent respiratory system problems), as well as increased coccidial and bacterial challenges from the wet litter (4). Finally, although RWA/NAE producers and retailers almost universally claim that sick flocks must be treated for welfare reasons, the produce from treated flocks cannot be marketed as RWA/NAE. This situation creates very real pressure on managers and veterinarians not to treat sick flocks in these programs until losses become severe, resulting in economic losses, compromised animal welfare, and a serious moral and ethical dilemma for the veterinarian.

Early Mortality

Increases of about 0.5–1% above the normal average seven-day mortality of 1.4% (3) can be expected at the onset of RWA/NAE production. There are no simple nonpharmaceutical solutions to address this problem, but strict attention to details from the hen house through brooding can recoup the majority of this loss. Proper hen house lighting, ventilation, litter management, nest space, nest placement, nest management and hygiene, egg collection practices, gut health management, and pest control will help to minimize floor eggs and produce a nest-clean hatching egg. Farm egg coolers should be managed to provide clean storage with constant temperature and humidity, and egg pickup and transport procedures should be designed to minimize egg sweating. Egg disinfection is controversial in broiler production and should not be necessary for dry nest-clean eggs. Refer to the section of this chapter on nest and egg hygiene for further discussion. Hatchery cleanliness and maintenance become paramount. Hatcher and setter halls should have adequate ventilation capacity to supply the needs of the machines, and hall temperature and humidity should be regulated to maximize machine ventilation while minimizing the work that the machines must perform to maintain stable incubation parameters. Machine maintenance is critical to provide uniform target incubation conditions throughout the box, to minimize the hatch window and produce the highest quality chicks. The machine manufacturer should be consulted for advice and assistance on ventilation, maintenance, and operation of their specific machines. There should be careful and continuous monitoring of set and pull times to produce a clean, dry chick with a healed navel, that is neither "green" nor "overdone" and dehydrated. Scrupulous cleaning and disinfection of the egg trucks, egg coolers, incubator and hatcher halls, incubators, hatchers, egg trays, racks, hatcher baskets, processing equipment (including *in ovo* injection machines, separators, processing belts, reusable chick boxes, vaccination equipment, etc.), and chick delivery vehicles are essential. Refer to the section of this chapter on disinfectants for further information. Wash water temperatures and water, detergent, and disinfectant flow rates and usage in tray and basket washers should be monitored. The hatchery water supply should be monitored for bacterial contamination, especially for humidification in incubators and hatchers, as should the room ventilation systems. Fumigation of eggs and fumigation of hatchers may be necessary, especially in the early stages of transition to RWA/NAE production. Formaldehyde is effective but controversial, and there are other alternatives for fumigation such as hydrogen peroxide that can be helpful. Bacterial monitoring of hatchers, *in ovo* injection machines, spray vaccination equipment, vaccine mixing rooms, and all chick contact surfaces (trays, boxes, belts, etc.) after cleaning by means of swabs and contact plates is a useful means to check the effectiveness of cleaning procedures.

Proper brooding management becomes even more critical in RWA/NAE production. A useful acronym for assessing brooding practices is "FLLAWSS", for feed, lights, litter, air, water, sanitation, and security. Starter feeds are best crumbled for easy prehension, and multiple shallow supplementary feeders easily accessible by the chicks should be placed throughout the thermal comfort zone. Light levels of 30–40 lux are recommended throughout the brooding period, and spotlights on the feed and water sources can be an added advantage. Litter should be clean, warm, dry, absorbent, friable, and deep enough to insulate chicks from the floor. The air must be of proper quality in terms of dust, ammonia (less than 20 ppm), humidity (60–70% relative humidity [RH]), and carbon dioxide, and must be of the proper temperature (28–30 °C). Zoned heating units such as radiant heaters are useful to provide a range of temperature zones in which chicks can seek their own

comfort levels. Clean water should be easily accessible near the feed and during brooding should be warmed to avoid chilling the chicks. Drinkers should be adjusted to proper height for access by the chicks and in the case of nipples the pressure must be regulated so chicks can easily trigger the nipples. Biosecurity and sanitation are as critical during brooding as at any time during rearing. Monitoring of proper brooding conditions is important. Tools such as infrared thermometers to check floor and air temperatures, rectal thermometers to check chick body temperature, light meters, and ammonia and carbon dioxide meters are useful not only to inspect conditions but also to illustrate to growers and caretakers where they are doing a good job and where improvement is needed. Assessing crop fill at 24 hours of age by palpation, with a target of at least 90–95% of chicks having detectable feed in the crop, is a good means of assessing overall preparation for brooding and early brooding conditions. The primary breeders have excellent rearing guides with extensive advice on brooding management.

Coccidiosis

Coccidiosis Vaccines and Vaccine-Only Programs

Live coccidiosis vaccines used in RWA/NAE programs, whether in vaccine-only programs or in rotation with chemical coccidiostats, should have high numbers of viable oocysts of at least the three major species for broilers (*Eimeria acervulina*, *E. maxima*, and *E. tenella*) and probably should contain *E. mitis* as well in many areas (47). *E. maxima* is especially critical (35). Proper refrigerated storage and usage within the expiration date is therefore important. Unattenuated and attenuated or precocious strains are both available, but neither is clearly superior under United States conditions. Methods of administration should be used that maximize initial uptake by the greatest number of chicks possible. Administration of the vaccines has been problematic, from both logistical and efficacy standpoints, as evidenced by the continued evolution of methods adopted to attempt uniform uptake. Feed and water administration were generally abandoned many years ago as logistically difficult and marginally effective. Administration via spray cabinets at the hatchery has been the standard since the 1990s, typically using large spray volumes up to three or more fold greater than those used for respiratory vaccines (often 21–24 mL per box of 100 chicks or even higher). Mechanisms such as stir bars or aerators are necessary to maintain oocysts in uniform suspension during spray administration. Wetting and chilling of the chicks with these high spray volumes must be managed, especially if used in tandem with a respiratory vaccine spray. Ancillary measures to increase preening of the sprayed vaccine, such as inclusion of dye in the spray, increasing light intensity, and maintaining quiet conditions and warm temperature (33–35 °C) in the chick holding area post-application prior to transportation have been recommended (7, 8). Nevertheless, spray vaccination still results in wide variability in uptake and subsequent shedding after the first cycle (34, 35). Gel discs containing the coccidiosis vaccine and sometimes nutritional supplements have been administered for consumption by the chicks in transport boxes, and eye spray and *in ovo* administration have been utilized, with none of these techniques significantly more efficacious than spray cabinet administration. Recently commercialized colored gel droplets that are preened by the chicks in the transport box show promising results (35). Excessive dosing is generally not the problem; rather it is the chicks not receiving an initial immunizing dose that are at much higher risk for coccidiosis or NE (35). An optimum vaccine preparation and a logistically simple administration method delivering a viable immunizing dose to a large percentage (more than 95%) of chicks on initial administration would greatly improve the efficacy of these vaccines, but such a method has yet to be devised.

A single infection will induce immunity to reinfection, although the extent varies depending on the species and dose (11). However, due to marginal initial immunization, and to the fact that better immunity develops from repeated low doses, coccidiosis vaccines rely on natural cycling of the administered *Eimeria* vaccine strains and possibly wild strains resident in the litter to develop immunity before the cycling of the wild strains reaches clinical levels (47). Some vaccine manufacturers recommend holding the chicks in a restricted space (the "brood chamber", usually representing one-third to one-half of the total space eventually allocated to growing the flock) for a specified period (typically about 12 days) to encourage early cycling. Exact recommendations vary with the brand of vaccine, and the manufacturer's directions should be followed. Excellent brooding conditions, especially proper temperatures and light levels that encourage chick foraging, and control of litter moisture are critical. It is conventional wisdom that excessively dry litter will decrease oocyst cycling and timely development of immunity, and that wet litter can result in excessive cycling and clinical disease. Managers in arid or very cold areas with low environmental humidity levels and ensuing dry litter (Western Canada) even add moisture to litter to encourage cycling. However, it has been shown that sporulation was optimum in dry litter (16% moisture), intermediate in moist litter (42% moisture), and worst in wet litter (62% moisture) (45). Nevertheless, the association of wet litter with diseases is probably accurate and litter moisture should be controlled. Proper cycling can be difficult to manage on a large commercial scale without some measure to control NE (i.e., prophylactic antibiotics or coccidiostat treatment programs),

and problems can be expected on a percentage of farms when these additional controls cannot be used (21).

The risk of problems with coccidiosis and NE in vaccine-only programs often varies among farms within a production complex (21, 37). When RWA/NAE marketing needs are considerably less than the total capacity of the production complex, it is possible over time to identify lower and higher risk farms via trial and error and concentrate RWA/NAE production on the low risk farms, decreasing the incidence of issues (37). This process is part of the institutional learning curve and can be painful for several years, especially when a pathogenic *Clostridium perfringens* clone is present on the premises and persists from one production lot to the next (32). Some clinicians have noted a curious phenomenon in which performance on vaccine-only RWA/NAE farms tends to decline over time, even on low-risk, well-managed farms. Again, when part of the production complex's output consists of conventionally raised poultry production, placing a conventional flock (e.g., with ionophores or chemical coccidiostats and perhaps prophylactic non-medically important antibiotics) on those RWA/NAE farms results not only in excellent performance in that conventional flock, but also in several subsequent RWA/NAE flocks. The excellent performance in the first conventional flock is likely due to the fact that the farm environment has been seeded with vaccine strains of coccidiosis, which are sensitive to coccidiostats. The phenomenon of vaccine use restoring coccidiostat sensitivity in a flock is well documented (47, 48). The renewed performance in the subsequent RWA/NAE flocks is more difficult to explain; perhaps reducing resident coccidial challenge and/or alteration of the litter flora may be involved. When RWA/NAE production comprises the majority or all of a complex's output, these selective placement and rotational programs are not available and long-term management of vaccine-only programs can be difficult.

Since immunity from coccidiosis vaccination currently depends on controlled cycling of vaccine strains before wild challenge builds to critical levels, one might suppose that placement of vaccinated chicks on new, clean litter would be optimum, but this does not always appear to be the case under United States conditions, where reuse of litter is common. Clinicians in the United States have for years recognized "new house syndrome", in which coccidiosis and NE seem to be more prevalent and severe in new (or cleaned out) houses. In one report on risk factors for NE in a large vaccine-only program over a 30-week period, the odds of a NE outbreak were 2.6 times higher on new litter compared to built-up litter (38). Possibly, new litter is not as conducive to adequate cycling of the vaccine, or perhaps normal flora in the used litter is beneficial, or perhaps resident coccidial populations contribute to development of solid immunity.

A subunit coccidiosis vaccine has been registered in several countries outside the United States for administration to breeder pullets to provide maternal immunity to progeny. The vaccine is based on subunits forming the oocyst wall, and is therefore directed against the final sexual stages of the life cycle. It reportedly suppresses oocyst production while still allowing schizogony of any oocysts ingested from live vaccines or the environment, and is therefore hypothesized to allow the development of immunity via schizogony while controlling the early buildup of challenge in the house (36).

Chemically Synthesized Coccidiostats

In programs allowing chemically synthesized coccidiostats, control of coccidiosis and therefore NE becomes more feasible but is still not without difficulties. The number of chemically synthesized coccidiostats available in the United States is limited, most are old compounds, and some are relatively expensive compared to other options such as vaccines and ionophores. The currently approved list in the United States includes amprolium, clopidol, decoquinate, diclazuril, halofuginone, nicarbazin, robenidine, and zoalene. As of 2017, clopidol and halofuginone were not being marketed, and the supply of some of the others has been problematic at times due to limited sources and fluctuating demand. Although sulfonamides are chemically synthesized and can be used to control coccidia, they are classified as antibiotics and cannot be used in RWA/NAE programs. Chemical coccidiostats have more issues with development of resistance than ionophores or vaccines. The older chemicals in this order are listed from most to least likely to develop rapid resistance: quinolones (decoquinate), clopidol, robenidine, amprolium, zoalene, and nicarbazin (10). Indeed, the last three on the list have proven most durable, at least in terms of continued use in industry. Diclazuril and halofuginone were not marketed yet when this list was proposed, but most would place them fairly high on that list (i.e., likely to rapidly develop resistance). It appears that the more effectively the drug suppresses oocyst excretion, the greater the selection pressure applied to the population, the less the immune system is engaged, and the more rapidly resistance appears to emerge (46). Consequently, careful design of shuttle programs (using two or more drugs in one growing cycle) and rotational plans (changing drugs seasonally) over the long term is necessary to preserve drug efficacy. The producer may need to utilize less potent drugs more frequently, accepting some decline in productive performance in exchange for prevention of overt clinical problems with coccidiosis and NE, and resisting the typical response to change the coccidiostat whenever a modest rise in feed conversion is detected. Occasionally a failed drug must be abandoned for an extended period in the hope that prolonged rest and the use of unrelated

compounds or vaccine in the interim will result in some restoration of sensitivity. While there is evidence that vaccine use can help restore drug efficacy (47, 48) the evidence for shuttle and rotational programs is less robust (10). Coccidiostat sensitivity tests in batteries may be helpful, but are expensive, time-consuming and, due to the limited number of isolates that can be tested, may not always be representative of the overall field situation (33). Nicarbazin, one of the more durable options, causes heat intolerance and can be used safely only in cooler seasons, and it must be initiated in the starter feed, as toxicity is likely if introduced in grower and later feeds, further limiting shuttle options. Nicarbazin can have severe impacts on egg production and egg quality in breeders and layers, so it must be used with care to prevent cross-contamination in mills that manufacture both broiler and breeder or layer feeds. Amprolium and zoalene are two less potent drugs that may compromise performance to some degree, but this aspect can also be used to some advantage as the coccidial leakage engenders some premunity. With proper management these "leaky" drugs are less likely to result in catastrophic failures, which have been known to occur with most of the other drugs excepting nicarbazin. These leaky drugs are generally best positioned in the earlier feeds, to take advantage of any immunity that might be generated and because early coccidial leakage has less economic consequences than late (40). Chemical coccidiostats, especially amprolium, can be used in shuttle programs or as scheduled prophylactic treatments with vaccines to reduce vaccine-associated problems, as was commonly practiced in the early days of coccidiosis vaccination (48). Prior to the use of spray cabinets for vaccine administration, it was common to treat vaccinated flocks with a low dose of amprolium approximately 10–12 days post-vaccination. However, it can be difficult to determine the dose and timing of such treatment to control vaccine issues without also circumventing immunity development and incurring NE, or creating performance issues related to delayed cycling. Development of immunity requires about 3–4 weeks whereas problems with NE tend to occur at 16–21 days, making timing of chemical treatment problematic.

Necrotic Enteritis and Dysbacteriosis

Nutritional Risk Factors

Necrotic enteritis is typically though not always associated with coccidiosis, so adequate control of coccidiosis will preclude most clinical problems with NE. Necrotic enteritis and dysbacteriosis may still be an issue in both vaccine-only and chemical coccidiostat programs even in the absence of clinical coccidiosis. Limiting substrates (both protein and energy) reaching the terminal ileum and cecum limits the proliferation of *C. perfringens*. Accordingly, use of well-balanced diets formulated with high quality ingredients is an important adjunct to success with RWA/NAE programs (5). Least-cost formulation, the use of lower priced, lower quality ingredients, sourcing of less commonly used alternative ingredients to which the birds are not accustomed, and frequent changing of raw materials to realize buying opportunities may all be problematic in these programs. Successful RWA/NAE programs are expensive, and the cost of a quality diet is a major factor in the increased expense. Since high protein levels in particular predispose to NE, nutritionists formulating for RWA/NAE programs may consider limiting total protein levels and targeting high biological value, highly digestible, high quality proteins (26, 30, 41). High inclusion rates of animal proteins, especially fish meal, have been used experimentally to reproduce NE, so it has been posited that rendered animal byproducts per se may contribute to NE, and that all-vegetable diets may therefore be an aid to RWA/NAE programs. Poor quality rendered products, with biogenic amines, rancid fats, indigestible substances, and clostridial spores are a risk in conventional production and probably even more so in RWA/NAE programs, and should be avoided in either case. However, if rendered animal protein sources are of high quality with high digestibility and high biological value, and are used in moderation, they should be safe to use if permitted by the marketing scheme. Since fish meal is especially high in zinc, glycine, and methionine, which appear to be risk factors for clostridial proliferation and toxin production, it should be used with caution (16, 30). Crystalline amino acids to help limit crude protein levels while still attaining ideal amino acid ratios and levels, and enzymes that improve digestibility and therefore promote better protein absorption in the upper gut may be beneficial (5, 26, 28). Animal fat appears to increase *C. perfringens* counts compared with vegetable oil, so there may indeed be some advantage to vegetable fats (25). Small grains (wheat, rye, oats, and barley) with high levels of non-starch polysaccharides are predisposing factors compared to corn theoretically because they increase viscosity of the digesta and slow peristalsis, favoring *C. perfringens* colonization (16, 26, 28, 30, 41). There is evidence that unknown factors in digested corn suppress proliferation *in vitro* while factors in digested wheat and barley may enhance proliferation (2). In one study, barley-derived distillers dried grains with solubles (DDGS) increased the effects of a coccidiosis/*C. perfringens* challenge model in a wheat/barley/sorghum diet (4). With small grains, it appears that a fine grind predisposes to NE compared to a coarser grind (6), but pelleting may negate the effect of grind size (17). Addition of whole small grains to the diet may also be beneficial by increasing gizzard activity, acidity, and digestion in the upper gut (18, 39). Any factor that results in irritation of the

gut, secretion of mucus, or decreased protein digestion and absorption may predispose to NE and should be avoided. Such factors may include feed outages, sudden changes in diet composition, mycotoxins, under- or overcooked soybean meal, tannins, and so forth (26, 30, 41).

Environmental Risk Factors

Wet litter is strongly associated with NE (22), so management of the diet and ventilation to maintain dry litter is important. Clostridia do not thrive in acidic environments (20) or high salt concentrations, and acidification or salting of the litter has been used to good effect for gangrenous dermatitis and NE. Sodium bisulfite, alum, salt, or Glauber's salt (sodium sulfate) at roughly 0.25 Kg/m^2 (50 pounds per 1000 $feet^2$), and other mineral and organic acids have been used commercially. Multiple applications can be made, such as before placement and again at the anticipated time of risk for NE. Many clinicians believe that they realize benefits from acidification of the water, although controlled research to support this is lacking. Various combinations of short-chain volatile fatty acids (typically acetic and propionic), mineral acids (sodium bisulfite), methionine, and iodine are used. Care must be taken that concentrations do not restrict water intake or result in wet litter.

High stocking density is commonly accepted as a predisposing factor for NE (26). In the one paper to date directly examining the impact of density on NE, increased density failed to increase the effects of challenge at 17 days on mortality, weight gain, and feed conversion at the final 21-day weighing, but did increase lesion scores and cecal *C. perfringens* counts at 24 days, when the birds were necropsied (42). The reduced density in this study (15 birds/m^2, 0.64 $foot^2$ per bird) was approximately half of current National Chicken Council standards and the high density pens would exceed current industry standards only at market weights above 2.9 Kg (6.35 pounds) (31). Profit for the farmer is usually maximized at higher stocking densities (19), and current commercial stocking densities generally reflect the tipping point between health, welfare, performance, and profit. Whether stocking densities can be reduced enough to significantly impact the incidence of NE in RWA/NAE production while remaining commercially feasible is unknown. At present, recommendations for minor reductions in stocking density as a tool to control NE are difficult to support. Breed may influence susceptibility to NE (22, 24). Significant differences in apparent susceptibility to NE between crosses of two different male lines on the same female line were documented in a spontaneous outbreak in the field (38).

Vaccination for Necrotic Enteritis

An alpha toxoid NE vaccine administered to breeder pullets to provide maternal antibody to the progeny is licensed in the United States and has been shown to offer protection under field conditions (12). While this and other studies have demonstrated protection from alpha toxin-based preparations, it should be borne in mind that alpha toxin has been shown not to be critical in the pathogenesis (41). A limitation of any maternal vaccination strategy is that the peak age of occurrence of NE (usually at 2–4 weeks of age) coincides with the decline in maternal antibody. Multiple immunizations are necessary for a good immune response to toxoids and immunogenic proteins of *C. perfringens* and a single vaccination at day of hatch is not sufficient (29). Multiple parenteral boosters are not practical in broilers. Advancing knowledge about the virulence factors of *C. perfringens* (such as NetB and collagen adhesin) and pathogenesis may lead to more successful immunization methods in the future.

Alternative Control Measures

A variety of non-drug substances have been promoted as aids to enhance gut health and to treat or control coccidiosis and NE in antibiotic-free production. These products include probiotics, prebiotics, organic acids, botanical extracts, yolk immunoglobulins, bacteriophages, yeast products, and others (13). Probiotics and competitive exclusion (CE) cultures are available for hatchery application, usually by spray, and most of the alternatives are available for feed or water administration. Because of logistical issues, integrators usually prefer feed-administered products, especially if intended for large-scale application for prevention. Any feed-administered product must be able to survive typical pelleting conditions. Water-administered products are more commonly used for treatment and metaphylaxis. Grower compliance in administration of products in drinking water can be problematic, as can issues with fouling of drinker lines and nipples with some products. Any product used in commercial production should be approved as a food additive by the FDA, or generally recognized as safe by the FDA (GRAS), or listed by the American Association of Feed Control Officials (AAFCO). Most if not all of these alternative products are not formally cleared as new animal drugs by the FDA, and hence are not subject to the same scrutiny and regulation for purity, safety, potency, and efficacy as an approved drug.

Probiotics have been defined as live microbial feed supplements which beneficially affect the host by improving its microbial balance (30). They range from defined single-organism products to complex mixtures of undefined normal gut flora, the latter usually understood as CE cultures. The proposed modes of action include competition with pathogens for nutrients and binding sites, production of inhibitory short-chain volatile fatty acids, alteration of gut pH, production of

antimicrobial substances such as bacteriocins, and alteration of gut immune responses (30). Competitive exclusion cultures are administered early in life, typically at day one, to promote early colonization with beneficial complex flora to exclude pathogens. Products administered in feed typically are composed of one to several organisms, and are usually spore-formers such as *Bacillus* species so they can survive pelleting. Many are not normal flora, do not permanently colonize, and must be fed continuously; these are often referred to as direct-fed microbials (DFM). Water-administered products may contain these same DFM organisms and/or non-spore-formers such as *Lactobacillus*, *Streptococcus*, and *Enterococcus* species. In a cross-sectional survey of risk factors for NE in the United Kingdom, the use of CE products did not appear to lower the risk of NE (22). Complex, undefined CE cultures generally have fared better in experimental studies than simpler, defined DFMs (13), but the CE cultures are not readily available in the United States and are difficult to mix and administer.

Prebiotics have been defined as indigestible feed ingredients that selectively stimulate the growth or activity of beneficial normal gut flora, to the detriment of pathogens (13, 30). They include carbohydrates such as lactose, lactitol, inulin, pectin, stachyose, raffinose, arabinogalactans, mannan-oligosaccharides (MOS), malto-oligosaccharides, fructo-oligosaccharides (FOS), galacto-oligosaccharides, gluco-oligosaccharides, glycol-oligosaccharides, xylo-oligosaccharides, lactulose, and lactosucrose. MOS is extracted from yeast cell walls, and in addition to its role as a carbohydrate source for beneficial bacteria, it and other yeast products are purported to bind bacterial fimbriae and act as pathogen-associated molecular patterns to stimulate the innate immune system (33). They also are reported to increase villus development (30).

Organic acids such as formic, acetic, propionic, butyric, lactic, malic, tartaric, sorbic, fumaric, caprylic, capric, lauric, myristic, and oleic acids have been suggested to improve growth and feed conversion and reduce pathogen colonization. One proposed mode of action is diffusion of the non-dissociated molecule into the bacterial cell, where it dissociates, reducing intracellular pH and exhausting the proton-ATPase pump (30). Microencapsulation of acids to preserve the non-dissociated form into the lower gut is a recently developed delivery strategy. Two reviews (1, 30) have indicated that these acids have not demonstrated the effectiveness against NE that they have against gram negative enteropathogens such as *Salmonella* and *E. coli*.

Botanical extracts such as essential oils, terpenes, flavonoids, phenolics, and saponins with purported selective antimicrobial and/or anticoccidial properties have been investigated for control of bacteria, coccidiosis, and NE, and include artemisinin, betaine, citric extracts and organic acids, *Echinacea purpurea*, gentian violet, mushrooms and their extracts, oregano (carvacrol), thyme (thymol), cloves (eugenol), mustard (allysothiocyanate), garlic (allicin), curcumin, piperine, turmeric, Persian lilac, bitter melon, green tea, cinnamon (cinnamaldehyde), capsaicin, *Yucca schidigera* extracts, eucalyptus, cabbage tree extracts, golden wattle tree extracts, seaweed extracts, marjoram, rosemary, sage, yarrow, hops, grape pomace, pennyroyal, and others (14, 33).

It is becoming more commonplace to see combinations of products, such as probiotics and prebiotics or essential oils and organic acids, in a so-called "synbiotic" approach (33). A field study showed no significant difference between the essential oils-based alternatives used in RWA flocks (21). In summary, a large volume of literature is accumulating concerning various alternative products, but as observed by at least three reviewers, there is often a publication bias toward positive results, the results overall are frequently variable or conflicting, and the alternatives to date only partially compensate for the loss of antibiotics, with slow adoption in the field (1, 23, 33).

Gut Barrier Function and Associated Issues

In Europe, cholangiohepatitis is a recognized consequence of gut barrier disruptions associated with antibiotic restriction programs. It is hypothesized that bacterial showers from the damaged intestinal epithelium escape the filtering action of the liver and may serve as the source of other systemic problems, such as bacterial arthritis, osteomyelitis (chondronecrosis with osteomyelitis), and vertebral osteoarthritis. Control of coccidiosis and NE should lessen the incidence and severity of these issues, but may not totally eliminate them. The alternative control measures listed above are also promoted to help address these issues.

Litter Moisture Issues

Deep, used litter is more absorbent than new litter and can therefore be an advantage where used litter is permitted and can be successfully managed. As indicated previously, under United States conditions used litter does not appear to be a risk factor for coccidiosis and NE, and in some cases appears protective (38). Its greater absorbency may be one reason for this observation. Control of ammonia is a greater challenge with used litter. The litter acidification amendments discussed under NE are actually used primarily to control ammonia, with any effects on clostridial proliferation in the litter being a secondary advantage. Other than these amendments and the diet, the control of both ammonia and litter moisture, which go hand in hand, is entirely dependent on

proper ventilation. Removal of caked litter between flocks when litter is reused is important to maintain proper absorptive characteristics, to lessen footpad and skin issues, and to control pathogen pressure. While excellent production and poultry health have been achieved in the United States on used litter, disease carry-over from flock to flock can become an issue. All-in, all-out systems with adequate downtime between flocks are a must with reused litter. Other strategies such as windrowing and composting the litter between flocks or heating the houses at 38 °C for 4 days will help reduce pathogens in used litter. If disease carry-over becomes an issue in used litter systems, then a total clean-out, washing, and disinfection is necessary.

General Disease Prevention Measures

Coccidia and *C. perfringens* are ubiquitous (30, 44) and would be difficult to exclude from production facilities. Nevertheless, in a cross-sectional survey of risk factors for NE in the United Kingdom, a number of variables related to hygiene and biosecurity were associated with lower NE prevalence, including use of dedicated clothing and footwear, hand washing, downtime greater than 14 days, and cleaning and disinfection (22). Additionally, as antibiotic treatment must be minimized, general disease prevention becomes more important than ever. Biosecurity practices should be designed, promoted, enforced, and monitored to minimize not only catastrophic but also local endemic diseases. Refer to the section on biosecurity in this chapter for more guidance. Ventilation, water, litter, light, and feed presentation should be managed to decrease stressors, respiratory challenges, and challenges to the skin barrier. A water sanitation program is highly recommended. Vaccination programs for both the breeders and the broilers, particularly for immunosuppressive diseases (Marek's disease, infectious bursal disease (IBD), and chicken infectious anemia) and respiratory diseases should be robust, and proper administration and immune response carefully monitored. Adequate downtime between flocks is even more critical for RWA/NAE programs than conventional programs. More robust disease monitoring to allow more rapid detection and response is important to minimize the number of houses that must be treated and diverted to conventional markets.

Managing the Impacts of Antibiotic Restrictions in Turkey Production

While the challenges of commercial turkey production without antibiotics are similar to those for broilers, the importance of some of the challenges is different. Control of coccidiosis is very important but NE, while it can develop, occurs much less frequently. Bacterial challenges with agents such as *Bordetella avium* and *Ornithobacterium rhinotracheale* are much more prevalent in turkey production. Success in RWA/NAE turkey production requires close attention to detail, including biosecurity, water sanitation, brooding, poult quality, feed presentation, animal welfare, vaccination, ventilation, supportive care, and coccidiosis control.

Control of Coccidiosis

Coccidiosis control is much more problematic in RWA/NAE turkey production due to the lack of currently available preventative tools. As with RWA/NAE broiler production, ionophores are not permitted, and the list of available chemicals approved for use in turkeys includes only diclazuril and zoalene. Clopidol is also sometimes used for control of coccidiosis but its approval in turkeys is only for prevention of leukocytozoonosis. As in broilers, overuse of any of these compounds rapidly results in development of resistance and loss of efficacy. Because turkeys are slaughtered at older ages than broilers, coccidiosis vaccination is a good choice to control this organism. Unfortunately, there is only one vaccine marketed for turkeys in the United States and its efficacy, due to a variety of factors, has been somewhat variable.

Water Sanitation

Because bacterial infections are more prevalent in turkeys than in broilers, consistent and effective sanitation of closed drinker systems is of paramount importance. A variety of products including various forms of chlorine dioxide, chlorine, hydrogen peroxide, and iodine are available and a variety of application technologies are currently marketed. When selecting a chemical and water sanitation application system an important factor to consider is a frequent and easy monitoring system for the active ingredient. All water sanitation systems should be monitored at least weekly and more frequently in high risk flocks and high risk areas.

Ventilation

In today's modern enclosed poultry houses the role of proper ventilation to maintain proper humidity, temperature, and air quality cannot be overemphasized. The respiratory defense mechanisms of a bird are different than those of mammals and depend heavily on proper mucociliary clearance. This is especially important in turkey production where bacterial challenges are frequent. Noxious gases such as ammonia, excessive dust, and high humidity all can have a significant detrimental effect on the respiratory defense mechanisms. The result

is bacterial-induced respiratory diseases that are sometimes not amenable to alternative therapies other than antibiotics.

Supportive Care

The ancillary and alternative products discussed under broiler production have also been advocated for use in turkeys.

Animal Welfare

Unfortunately, one discussion point that is frequently overlooked or ignored in discussions of RWA/NAE production is the welfare and comfort of the animals. Even with the best husbandry, animals will occasionally develop disease. Pressures to preserve the RWA/NAE status of a flock create a serious ethical dilemma for the veterinarian. The issue of the welfare of the animals needs to be brought to the forefront and thoroughly discussed by all parties involved. The responsibility of all participants in the food chain is not to preserve an artificial marketing status but rather the welfare and comfort of the animals.

Managing the Impacts of Antibiotic Restrictions in Table Egg Production

The VFD, prescription status for water medications, and marketing campaigns that restrict antibiotic usage have had minimal impacts on table egg production because the egg industry has essentially been working in an antibiotic-free climate for many years due to the gradual removal from the market of approved antibiotics for egg-type chickens. The list of antibiotics affected by the new regulations and labeled for egg-type pullet or layer use is limited to four clearances for pullets in feed (chlortetracycline, oxytetracycline, neomycin and oxytetracycline, and tylosin), five for pullets in water (chlortetracycline, oxytetracycline, sulfadimethoxine, sulfamethazine, and tylosin), one for layers in feed (chlortetracycline), and one for layers in water (sulfamethazine). Antibiotic usage in pullets is minimal due to good control of the most common problem, NE associated with coccidiosis, through vaccination and management. In layers, the main reason for antibiotic usage is *E. coli* infections and to a lesser degree, NE. Both of these diseases are likewise well controlled by vaccination for *E. coli* and management for both diseases. A majority of egg producers use a progressive team approach to layer health management utilizing a team involving veterinarians, a vaccinologist, a nutritionist, and a management specialist in order to accomplish an antibiotic-free program. This team must address nine main components in order to be successful in antibiotic-free (ABF) egg production.

Vaccination Programming and Administration

The veterinarian should formulate a comprehensive vaccination program that addresses all common bacterial and viral diseases for which effective vaccines exist. The second member of the team should be an expert in poultry vaccine administration. This could be a veterinarian or vaccine company representative that has expertise in the proper handling and administration of vaccines to optimize the response. It is critical to reap the maximum benefit from vaccines to avoid even mild outbreaks of disease. This person, through training of staff, observations of vaccination crews, and sampling of vaccinated flocks, should continually work toward the goal of administering the vaccines in the right condition and proper site to 100% of the birds in a flock. The increase in the number of pullet aviaries and floor growing systems has created issues in achieving optimum immunity from vaccinations due to bird movement in those systems.

Biosecurity Program

Eliminating the introduction of disease pathogens for which there are no vaccines (avian influenza or *Gallibacterium anatis* for example) and antigenic variants of existing vaccine strains is the backbone of the ABF program, with the goal of ensuring that a reduced infectious dose of pathogens will reach the flock compared to current conventional programs. Outdoor access required by some welfare programs and organic production increases the risks of disease introduction. Control measures to minimize disease introduction from wild bird or animal sources will need to be developed for each specific site. Table 1.4 lists the minimum components of biosecurity that should be included in the plan (9).

Water Sanitation

Water sanitation is a critical component of ABF programs to preclude pathogens from entering via water. *E. coli* water contamination can lead to high mortality from colibacillosis. Chlorine-based continual sanitizing systems appear to do the best job with chlorine dioxide systems being popular.

Nutrition

The third team member is a qualified nutritionist who can optimize the ingredient mix and use of enzymes to prevent bacterial diseases. The general nutritional principles discussed above to reduce NE in RWA/NAE broiler systems are largely applicable to table egg production.

Table 1.4 Minimum components of a biosecurity plan for table egg layers (9).

- Designate a biosecurity officer for each farm
- Establish the line of separation (LOS) and perimeter buffer area (PBA) for each facility
- Establish employee parking and entry procedures into facilities; Danish entry system with dedicated farm clothing and footwear and hand sanitation at LOS recommended
- Define employee actions off farm to minimize introduction of disease (outside employment, ownership of private fowl, waterfowl hunting, etc.)
- Control employee and other personnel movement while within the LOS; the LOS may not be crossed without re-entering through the established entryway
- Establish bird movement procedures including crews and bird moving vehicles
- Establish procedures for egg pickup
- Establish procedures for manure removal
- Establish procedures for visitors and pre-visit requirements
- Establish dead bird disposal procedures
- Establish ongoing rodent, free-flying bird, and insect monitoring and control programs
- Clean water supply
- Bird source requirements
- Feed supply and delivery requirements
- Litter supply requirements
- Developing steps for proper cleaning and disinfection (C&D) of equipment, floors, walls, and ceilings between flocks

Preventative Use of Non-Antibiotic Products

The non-antibiotic alternatives discussed under broilers may be useful to decrease the risk of disease that would require antibiotic intervention. Organic acids are not used routinely in layers for either treatment or prevention of enteric disorders since over usage may affect shell quality.

Bird Management, Environment, and Housing

The fourth component in the equation for ABF production is bird management and housing, with the goal of reducing the level of stress on the bird that can be immunosuppressive. Managers should frequently consult the primary breeders' technical manuals and staff for assistance in developing programs suited to each specific breed, as well as equipment suppliers for optimal management of equipment. Optimal feed presentation, light levels and hours, air quality and temperature, water availability, space allocations, and sanitation should be assured at all times.

Non-Antibiotic Treatments for Disease

There are means of managing outbreaks of disease due to bacterial infections without the use of antibiotics. Examples include vaccination in the face of a viral or bacterial disease outbreak, additional iodine or chlorine added to water during an outbreak, increasing heat and ventilation to dry the litter during a NE outbreak, or increasing the dosage of probiotics, fermentation metabolites, prebiotics, and so on during an outbreak.

Disease Surveillance

A good disease surveillance program is needed to assess the efficacy of control programs and identify adjustments.

Training of Employees

Employee training can pay many benefits in improved disease prevention by improving their observation abilities and skills in managing flocks. Regularly scheduled training sessions put on by management and the team members in regard to biosecurity, disease recognition, vaccinations, and bird and environment management are suggested. Investing in sending key people to meetings and layer health management schools is also recommended.

In summary, an increase in management inputs will be needed to be successful in producing eggs without the use of antibiotics. Enlisting the services of a qualified veterinarian, nutritionist, vaccine expert, and management consultant on your ABF consulting team is a must to provide advice in regard to vaccination programs, vaccine administration, biosecurity, nutrition, water sanitation, use of non-antibiotic feed or water additives, bird management, use of non-antibiotic interventions during outbreaks of disease, and disease surveillance. Investments in continual training of personnel on these subjects by ABF consulting team members, industry meetings, or health management schools should be a high priority.

Antimicrobial Therapy (Including Resistance)

Randall S. Singer, Timothy J. Johnson, and Charles L. Hofacre

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 to 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.

Introduction

Successfully treating a bacterial infection without any adverse effects involves many important factors, including the choice of antimicrobial, the route of administration, and the dose and duration of treatment. One possible 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. Antimicrobial resistance can lead to decreased effectiveness of future antimicrobial therapy in the food animal population, but can also pose a potential risk to human health. This topic will be reviewed later in this section. This section will not discuss the antimicrobials or the dosages to treat particular bacteria—that discussion will be left to the authors of chapters 16–24 (Section III—Bacterial Diseases) that discuss each individual bacterial infection. This section 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 three broad categories: prevention of infection, treatment of subclinical bacterial disease, and treatment of clinically affected birds. A common application of antimicrobials in the prevention category targets clinical enteric disease, commonly referred to as necrotic enteritis (NE), resulting from a *Clostridium perfringens* infection (14). Disease prevention antibiotics are commonly given in the feed of broilers and turkeys. In contrast to disease prevention, treatment of the clinically affected birds is based on the observation of birds in the flock exhibiting clinical signs of a bacterial infection. When some birds are demonstrating clinical illness, there will be many other birds in the flock that are healthy but that have likely been exposed to the infection and are possibly incubating the disease. This entire flock will typically be treated, and thus is often described as a treatment and control administration of antimicrobials. Antimicrobials for treatment and control of disease are most commonly given in the water. The decision of whether to treat should be made 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.

Antimicrobial therapy in US poultry production changed dramatically in January 2017 due to key regulation and policy revisions. In 2012 the US Food and Drug Administration (FDA) published *Guidance for Industry* (GFI) #209 which described a broad policy shift regarding antimicrobial drugs used in animal agriculture. This document was intended to limit medically important antimicrobial drugs to uses in food-producing animals that: (1) are only considered necessary for ensuring animal health, and (2) include veterinary oversight or consultation (29). This document was followed in 2013 with GFI #213 (30), which provided more detail on implementing the key principles in GFI #209. Specifically, GFI #213: (1) defined the term "medically important", (2) voluntarily removed claims relating to production uses (growth promotion/feed efficiency), and (3) brought remaining therapeutic uses under veterinary oversight by changing the marketing status from over the counter (OTC) to Veterinary Feed Directive (VFD) or prescription (Rx). Finally, the VFD regulation was updated, and these new regulations went into effect in October 2015 (31). The VFD describes requirements relating to the distribution and use of VFD drugs (feed-use drugs that require supervision of a licensed veterinarian) and is considered a critical step for facilitating the transition of antimicrobial therapy in animal agriculture to veterinary oversight.

A point of confusion for many in the general public was related to the term "voluntary" in GFI #213. The only aspect of this policy that was voluntary was the request by FDA to have the animal pharmaceutical companies remove indications for growth promotion/feed efficiency from all labels of medically important antibiotics. Once these changes were made, the veterinarian would be required to follow the label instructions, as there is no permitted extralabel use of in-feed antibiotics. All of the animal pharmaceutical companies complied with GFI #213, and therefore, as of January 1 2017 there are no longer approved medically-important antibiotics for growth promotion/feed efficiency in the United States. Tables 1.5 and 1.6 show the currently

Table 1.5 Approved feed medication of US poultry that requires a Veterinary Feed Directive (VFD). Table courtesy of Dr. Steven Clark.

VFD medications	Chicken	Turkey
Albamix (Novobiocin)	√	√
Aureomycin® (Chlortetracycline)	√	√
ChlorMax® (Chlortetracycline)	√	√
Inteprity™ (Avilamycin)	√	—
Lincomix® (Lincomycin)	√	—
Neo-Oxy® (Neomycin + Oxytetracycline)	√	√
Neo-Terramycin® (Neomycin + Oxytetracycline)	√	√
Pennchlor® (Chlortetracycline)	√	√
Pennox® (Oxytetracycline)	√	√
Pharmastatin (Nystatin)	√	√
RofenAid® (Sulfadimethoxine + Ormetoprim)	√	√
Stafac® (Virginiamycin)	√	—
Terramycin® (Oxytetracycline)	√	√

Table 1.6 Approved feed medication of US poultry that does not require a Veterinary Feed Directive (VFD). Table courtesy of Dr. Steven Clark.

NonVFD medications	Chicken	Turkey
Albac® (Bacitracin Zinc)[a,b]	√	√
Amprol® (Amprolium)	√	√
Avatec® (Lasalocid)	√	√
Aviax® (Semduramicin)	√	—
Bio-Cox® (Salinomycin)	√	—
BMD® (Bacitracin Methylene Disalicylate)[b]	√	√
Clinacox® (Diclazuril)	√	√
Coban® (Monensin)	√	√
Coyden® (Clopidol)[a,c]	√	√
Deccox® (Decoquinate)	√	—
Flavomycin® (Bambermycin)[b]	√	√
Hygromix® (Hygromycin B)[a]	√	—
Maxiban® (Narasin + Nicarbazin)	√	—
Monteban® (Narasin)	√	—
Nicarb® (Nicarbazin)	√	—
Robenz® (Robenidine HCL)	√	—
Sacox® (Salinomycin)	√	—
Stenorol® (Halofuginone)[a]	√	√
Topmax™ (Ractopamine)[a]	—	√
Zoamix® (Zoalene)	√	√

[a] Not currently marketed.
[b] Includes label claim for improved weight gain and feed conversion.
[c] As an aid in prevention of leucocytozoonosis caused by *Leucocytozoan smithi*.

Table 1.7 Approved water soluble medication of US poultry that requires a prescription. Table courtesy of Dr. Steven Clark.

Prescription medications	Chicken	Turkey
Aureomycin® Soluble (Chlortetracycline)	√	√
Di-Methox® (Sulfadimethoxine)	√	√
Lincomycin Hydrochloride Soluble (Lincomycin HCL)	√	
Neo-Sol® (Neomycin)	—	√
NeoMed® (Neomycin)	—	√
Oxytet® Soluble (Oxytetracycline)	√	√
Pennchlor 64® (Chlortetracycline)	√	√
Pennox 343® (Oxytetracycline)	√	√
PoultrySulfa® (Sulfamerazine, Sulfamethazine, Sulfaquinoxaline)	√	√
TetraMed® 324 HCA (Tetracycline)	√	√
Tetroxy® HCA Soluble (Oxytetracycline)	√	√
Tet-Sol™ 324 Soluble (Tetracycline)	√	√
Tylan® Soluble (Tylosin Tartrate)	√	√
Tylovet® Soluble (Tylosin Tartrate)	√	√

Table 1.8 Approved water soluble medication of US poultry that does not require a prescription. Table courtesy of Dr. Steven Clark.

Non-prescription medications	Chicken	Turkey
Amprol (Amprolium)	√	√
BMD® Soluble (Bacitracin Methylene-Disalicylate)	√	√
Safe-Guard® AquaSol (Fenbendazole)	√	—

approved feed medications for US poultry, and Tables 1.7 and 1.8 show the currently approved water soluble medications for US poultry.

Routes of Medication

Commercial poultry are raised to provide a safe, wholesome protein source that is economical for the world's human population. To that end, the welfare of the bird and the cost of the meat must be accounted for. 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* infection 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 (12).

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, that is, *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 (21).

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 each day. 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 under dose 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 non-laying hens or roosters.
- Pulse dosing can be considered when the birds' water consumption is limited (i.e., broiler breeder pullets) (3). 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 the targeted strain 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 one 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 (11).

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 (IBD), 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 antemortem and postmortem 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 three 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 coprophagic, they may have sulfonamide exposure even after the drug is removed from the feed or water.

There are many sets of guidelines globally for the judicious use of antimicrobials. The American Veterinary Medical Association (AVMA) has a general set of principles (1), and these include: "Therapeutic exposure to antimicrobials should be minimized by treating only for as long as needed for the desired clinical response"; "Regimens for antimicrobial treatment, control, or prevention of disease should be based upon current scientific and clinical principles, such as microbiological and pharmacological tenets"; and "Antimicrobial use should be confined to appropriate clinical indications. Inappropriate uses such as for uncomplicated viral infections should be avoided." Judicious use principles specific to the poultry industry have also been developed, for example by the American Association of Avian Pathologists (AAAP) in conjunction with the AVMA (2). In general, veterinarians should strive to optimize therapeutic efficacy and minimize resistance to antimicrobials to protect public and animal health.

Antimicrobial Resistance

Antimicrobial resistance is a growing concern for human health because of the increasing incidence of bacterial infections that are refractory to antimicrobial therapy. Many of the genes encoding antimicrobial resistance are transferable between bacteria, and therefore, resistance genes that are present in bacteria of animals can be transferred to bacteria that cause human disease. Of course, the reverse is also true. All uses of antimicrobials in animal agriculture have the potential to increase the prevalence, distribution, and spread of resistant bacteria and resistance genes, again highlighting the need to observe judicious use principles when antimicrobial therapy is needed. Currently, the degree to which the use of antimicrobials in poultry impacts antimicrobial resistance in human bacterial pathogens remains uncertain but is definitely non-zero (28).

All uses of antimicrobials have the potential to select 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 contacts the bacterial population, such as 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 bacterium, then a selection pressure exists that can shift the bacterial population toward a population that can survive in the presence of that antimicrobial. Even if the dose is high enough to kill or inhibit the target bacterium, those bacteria in the population that possess mechanisms capable of resisting the action of the antimicrobial will survive, and these resistant bacteria can then spread.

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 two unrelated bacterial cells (10). Genes encoding antimicrobial resistance are most commonly moved between bacteria via their presence on conjugative bacterial plasmids (16). 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 often 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 (16). 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 *E. coli* and *Salmonella enterica* alone, there are more than 30 plasmid types identified and this number continues to grow (10). 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 (6, 9, 32).

Each plasmid type has one 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 (23). 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 (18). Each type mentioned has signature sets of resistance genes that they commonly possess. For example, IncA/C plasmids often contain the genes $bla_{\text{CMY-2}}$ and *floR*, encoding resistance to third-generation cephalosporins and phenicols, respectively (6). IncI1 plasmids commonly carry extended spectrum beta lactamase genes belonging to the bla_{TEM} and bla_{CTX} 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 (18). In addition to the carriage of multiple resistance genes by a single plasmid, bacteria of poultry commonly carry multiple plasmids (18). 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 (17). Furthermore, these plasmids also may possess genes encoding resistance to heavy metals and disinfectants (15). 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 1.10A, 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 foodborne transmission. It seems that certain *Salmonella* serovars have a greater propensity to acquire multidrug resistance than others, and one 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 (13, 20, 26). Of particular concern are the IncA/C plasmids, which have been identified among serovars Heidelberg, Kentucky, and Typhimurium in poultry and humans (7, 8, 24, 25). Isolates harboring this plasmid are typically resistant to ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, 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 (19).

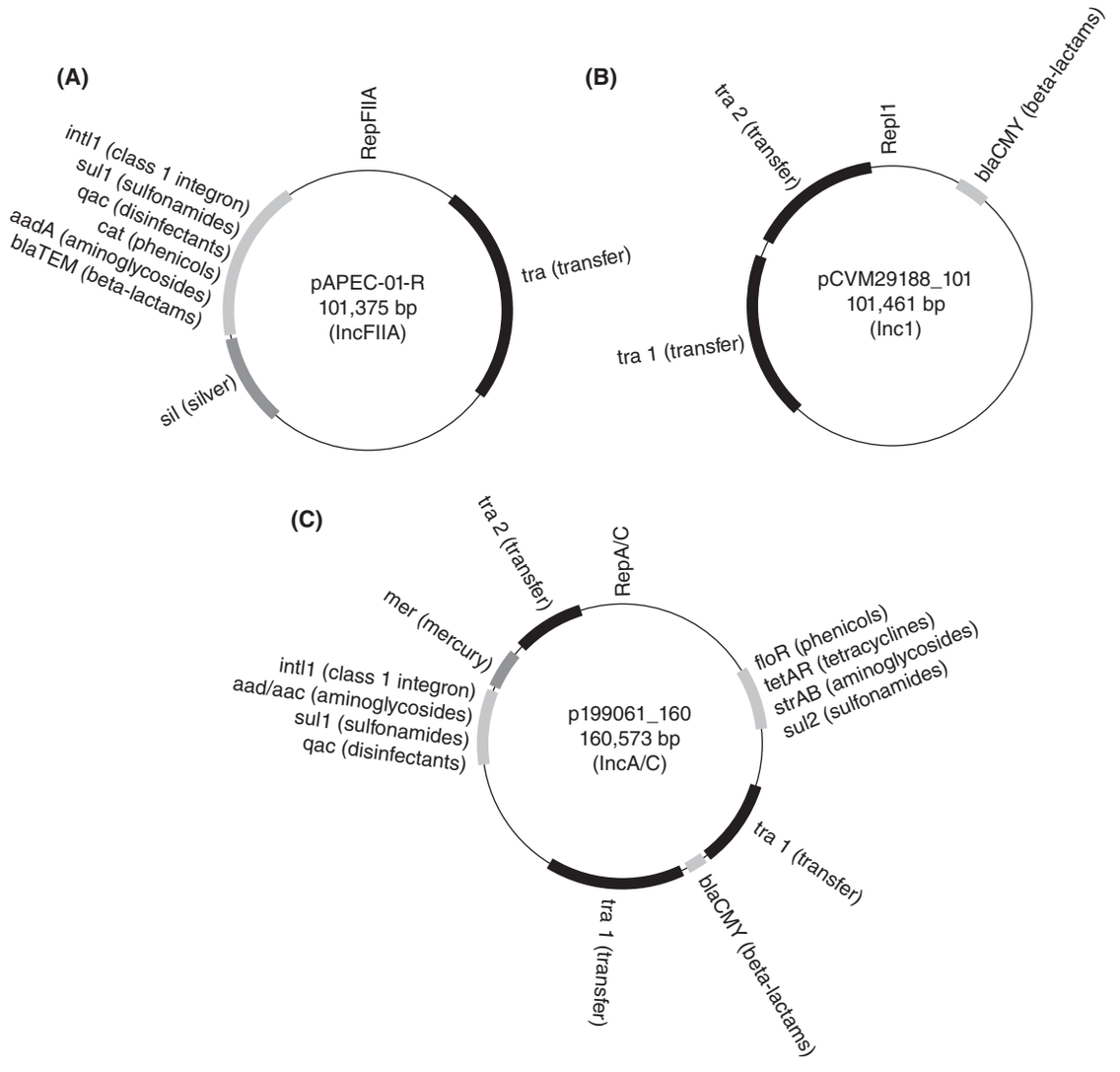

Figure 1.10 Circular maps of transmissible resistance-encoding plasmids isolated from poultry bacteria. Transfer regions are shaded in black, antibiotic resistance genes are shaded in light gray, and heavy metal resistance genes are shaded in dark gray. (A) Map of pAPEC-O1-R, an IncFIIA plasmid isolated from avian pathogenic *E. coli*. (B) Map of pCVM29188_101, an IncI1 plasmid isolated from S. Kentucky. (C) Map of p199061_160, an IncA/C plasmid isolated from avian pathogenic *E. coli*.

Once multidrug-resistant bacterial populations are established within an environment, they are difficult to eliminate. Certainly, plasmid dissemination from one bacterium to another can occur within the avian gastrointestinal tract, within poultry litter, and among beetles (4, 22, 27). There is documented evidence that antimicrobial therapy in response to disease will enhance the dissemination of plasmid-encoded multidrug resistance (5). 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.

Acknowledgement

The authors wish to acknowledge Steven Clark for providing the information in Tables 1–4.

Public Health Significance of Poultry Diseases

Roy D. Berghaus and Bruce Stewart-Brown

Summary

Several poultry diseases have the potential to impact human health. Some diseases are rarely reported in humans but are of theoretical concern. Others are commonly associated with human illness. Direct transmission of pathogens to poultry workers is uncommon; however, it should not be discounted. Human infections with certain lineages of H5N1 and H7N9 avian influenza viruses are recent high profile examples of bird-to-human transmission. Foodborne pathogens such as *Salmonella* and *Campylobacter* are primarily a concern for consumers, but also have the potential to infect individuals working in production and processing environments. Educating poultry workers with respect to zoonotic pathogens and their modes of transmission is an important step toward disease prevention.

Introduction

Poultry and humans have dramatically different physiologies, and many pathogens are incapable of crossing the species barrier between birds and man. Nonetheless, there are a number of diseases that humans and poultry share (1). These can be zoonoses (poultry to humans), reverse zoonoses (humans to poultry), or diseases acquired from a common environmental reservoir. Most zoonotic diseases can be prevented through an understanding of basic disease transmission principles and the adoption of preventive practices.

Infectious organisms may be 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 (95). 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 alcohol-based 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 coveralls that can be sanitized between uses, head covers to help keep the hair and scalp free of 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 about zoonotic disease prevention. 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 humans. 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.

Viral Diseases

Arboviral Encephalitis

Arbovirus is a generic term referring to viruses transmitted to vertebrates by the bite of arthropod vectors including mosquitos, ticks, and flies. More than two dozen arboviruses are capable of causing neurological disease in humans (40). However, the discussion here will be limited to three 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.

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 1–17%, respectively (3, 66).

Occurrence

The distribution of arboviruses is dependent 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, Australia, and North and South America. Between 1999 and 2007, the number of human WNV neuroinvasive cases reported in the United States was 11,125, compared to 80 cases for EEEV and one case for WEEV (70). In North America, approximately 90% of human cases are identified between July and September. Neuroinvasive disease is more likely in the elderly and in persons infected through organ transplantation (66).

Reservoirs and Sources of Infection

The principal mode of transmission to humans is from the bite of an infected arthropod vector. For all three of the viruses discussed here, the primary enzootic transmission cycles occur between birds and mosquitos. Transmission is not limited to ornithophilic mosquito species, however, as several other genera of mosquitos have the potential to act as bridging vectors between birds and humans. The incubation period is typically 3–14 days (40). Humans do not produce a sufficient viremia to serve as an amplifying host; however, non-vector-borne transmission has been documented via blood transfusion, organ transplantation, breastfeeding, and needlestick injury. Infections have been reported in workers on goose and turkey farms during WNV outbreaks, although it is not clear whether this may have been attributable to contact with infected birds or concurrent transmission from infected mosquitos (9, 21).

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. Human disease caused by direct infection with poultry-adapted viruses is rare, but has been reported sporadically with some specific genetic virus lineages (i.e., A/goose/Guangdong/1/1996 [H5N1], Gs/GD lineage, and A/Anhui/1/2013 [H7N9], Anhui lineage) in some subtypes: H5N1, H5N6, H6N1, H7N2, H7N3, H7N7, H7N9, H9N2, H10N7, and H10N8 (31). 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. H7N7 Eurasian virus has mainly been associated with conjunctivitis and mild influenza-like illness, while H5N1 and H5N6 Gs/GD lineage and H7N9 Anhui lineage viruses have been associated with more severe respiratory disease (13). 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 and H5N6 Gs/GD lineage and H7N9 Anhui lineage virus infections, which have reported case-fatality risks of 60% and 40%, respectively (13, 41).

Occurrence

AI viruses are found worldwide, although the distribution of specific subtypes varies. Most cases of human infection reported in recent years have been caused by the H5N1 Gs/GD lineage and H7N9 Anhui lineage subtypes (31). Between 2003 and March 2017, 858 human illnesses and 453 deaths due to H5N1/H5N6 AI viruses (Guangdong lineage) were reported to the World Health Organization (WHO) from 16 countries across Asia, Africa, and the Middle East (94). Between March 2013 and February 2017, annual epidemics of H7N9 Anhui lineage virus infections in China were associated with 1,258 human cases (41). All other AI virus subtypes combined accounted for approximately 124 documented human infections between 1959 and 2014, with 93 of these cases being attributed to H7N7 Eurasian lineage (31).

Reservoirs and Sources of Infection

Wild aquatic birds belonging to the orders Anseriformes and Charadriiformes are the natural reservoirs for AI viruses. Influenza viruses are shed in the feces and respiratory secretions of infected birds. Direct or indirect exposure to infected poultry or contaminated environments is believed to be the most important route of transmission to humans. 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 cooked poultry or eggs. The incubation period for H5N1 viruses is typically 2–5 days, but may be as long as 7 days (13). Person-to-person transmission of AI viruses is uncommon, but limited transmission has been reported among relatives having close contact with persons infected with the

H5N1 Gs/GD and H7N9 Anhui lineage viruses. Viral RNA of H5N1 viruses has been identified in the respiratory secretions of infected persons as long as 3–4 weeks after onset.

Preventive Measures

The US 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 (20). The 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) (25, 84). 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. 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 (38). Patients typically make a complete spontaneous recovery.

Occurrence

Newcastle disease virus 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 (84). Most reported cases have been in diagnostic and vaccine laboratory workers, veterinarians, and processing plant workers (25).

Reservoirs and Sources of Infection

Birds are the natural reservoir for NDV; over 240 species have been reported to be susceptible to infection (43). Transmission between birds occurs by inhalation of respiratory droplets or the fecal–oral route. 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 (38). Most references flatly state that secondary transmission has never been documented. Transmission from infected humans to susceptible poultry, however, is a potential concern (84).

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*, *C. butyricum*, and *C. argentinense* (68). Although *C. botulinum* is considered a single species, different strains can be distinguished by the type of toxin they produce. There are seven 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 (19). Cattle and sheep are susceptible to type C and D toxins, however, and several outbreaks in these species have been linked to poultry litter exposure (81).

Nature of the Disease in Humans

Four naturally occurring forms of botulism are recognized in humans: foodborne 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 have improved the case-fatality risk.

Occurrence

Clostridium botulinum has a worldwide distribution. Most foodborne 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 (33). 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. Adult intestinal toxemia is rare, and occurs in patients with a history of abdominal surgery, gastrointestinal abnormalities, or recent disruption of the normal flora because 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 foodborne intoxications is typically 12–36 hours, but may range from 6 hours to 10 days (19). Contact transmission from animal-to-person or person-to-person does not occur.

Clostridium perfringens Infection

Clostridium perfringens causes two different types of foodborne disease as well as gas gangrene in humans (14). Foodborne 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

Clostridium 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 (80). *C. perfringens* type C food poisoning is primarily mediated by beta-toxin and is associated with necrotic enteritis (NE) in humans. Symptoms include acute abdominal pain and distension, bloody diarrhea, and sometimes vomiting (14). The case-fatality risk for type A food poisoning is less than 0.1%, while that for type C food poisoning is 15–25% (14, 74).

Occurrence

Clostridium perfringens type A is one of the most common causes of foodborne disease worldwide. In the United States, it causes an estimated one million illnesses annually (74). *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. Young children and the elderly are at increased risk for severe illness due to type A food poisoning, while malnourished individuals and diabetics are at increased risk for developing NE due to type C food poisoning (14, 80).

Reservoirs and Sources of Infection

Clostridium perfringens is a common inhabitant of soil and intestinal tracts of animals and humans, and is commonly isolated from retail meat products. Between 1998 and 2010, poultry was implicated in 30% of foodborne *C. perfringens* outbreaks in the United States that could be attributed to a single food commodity (36). Improper food handling, especially the inadequate cooling and reheating of meat-containing dishes, is a common contributing factor. 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. Direct exposure to infected persons or animals does not constitute a disease risk.

Campylobacteriosis

Campylobacteriosis is an enteric infection caused by members of the genus *Campylobacter* (77). 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 (50). Most patients recover within one week without antimicrobial treatment. Bacteremia and other extraintestinal infections are uncommon complications. Sequelae of enteric *Campylobacter* infections may include reactive arthritis in 1–5% of patients, irritable bowel syndrome in 1–10% of patients, and Guillain-Barré syndrome in approximately 0.1% of patients (45).

Occurrence

Campylobacter is one of the most commonly reported causes of bacterial gastroenteritis worldwide and is a common cause of traveler's diarrhea. In the United States, *Campylobacter* causes an estimated 845,000 cases of foodborne illness and 76 deaths each year (74). Most cases are sporadic; outbreaks are uncommon, but have been linked to unpasteurized milk, contaminated water, and the ingestion of undercooked poultry. In developed countries there is a male predisposition, a seasonal peak in cases during the late spring and summer, and an increased incidence in children under 5 years old (50). 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.

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 the most important source of human infections. Transmission occurs by ingestion of the organism, and approximately 80% of domestically

acquired infections in the United States are considered to be foodborne (74). Transmission to poultry processing plant workers has been well documented (29). 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 (50). 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.

Preventive Measures

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

Chlamydiosis (Psittacosis)

Psittacosis, also known as ornithosis or parrot fever, is a respiratory disease of humans caused by *Chlamydophila* (or *Chlamydia*) *psittaci* (73, 79). The corresponding disease in birds is referred to as avian chlamydiosis. *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 (5, 79). Typical symptoms include fever, chills, headache, and myalgia. A nonproductive cough is common and may be accompanied by respiratory difficulty. Potential complications include endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, and encephalitis. Most cases respond well to antibiotic therapy.

Occurrence

Psittacosis and avian chlamydiosis have a worldwide distribution. Psittacosis is a reportable disease in most countries, but the number of reported cases is likely an underestimate because many cases are mild, the symptoms are nonspecific, and diagnosis can be difficult. 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 (5). Most cases are sporadic, but outbreaks have occurred in people exposed to infected pet birds and poultry. There is no evidence to suggest that immunocompromised individuals are at increased risk for infection.

Reservoirs and Sources of Infection

Birds are the natural reservoir for *C. psittaci*, and *Chlamydophila* spp. have been identified by culture or serology in 467 bird species from 30 different orders, including all of the major domestic poultry species (44). Human infections are most frequently associated with exposure to psittacines, pigeons, turkeys, and ducks. Different serotypes have been identified more frequently in certain bird species, but all serotypes are considered to be potentially infectious to humans (5). 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. Secondary transmission of *C. psittaci* has been reported but is believed to be rare (88).

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 (79). 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 *E. rhusiopathiae* in humans is called erysipeloid, which is distinct from human erysipelas; a condition that is usually caused by *Streptococcus pyogenes* (69).

Nature of the Disease in Humans

Three forms of *E. rhusiopathiae* infection are typically recognized in humans (90). Erysipeloid 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. Diffuse cutaneous and systemic forms of the infection are much less common, although endocarditis is a frequent complication in systemically infected patients (34, 67).

Occurrence

Erysipelothrix 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 (34). Transmission from infected quail and laying chickens to processing plant employees and animal caretakers has previously been reported (59, 60).

Reservoirs and Sources of Infection

Erysipelothrix 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 the most important reservoir, although several poultry species including turkeys, chickens, ducks, and emus are also susceptible (90). *Erysipelothrix rhusiopathiae* can survive weeks to months in farm and marine environments, and is commonly found in the mucoid slime coating of fish. 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 erysipeloid is 2–7 days. Person-to-person transmission has not been documented.

Escherichia coli Infection

Most strains of *E. 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 one of six pathotypes: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enteroaggregative (EAEC), or diffuse-adherence *E. coli* (DAEC) (57). Strains with recognized extraintestinal virulence factors or that demonstrate enhanced virulence in an animal model of extraintestinal infection have been designated as extraintestinal pathogenic *E. coli* (ExPEC). Of the six intestinal pathotypes, only the EHEC strains (e.g., O157:H7) are considered to be zoonotic pathogens. Avian pathogenic *E. coli* (APEC) have not been associated with human intestinal infections, but there may be some overlap between APEC strains and human ExPEC strains (55). 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 (65). 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 (55).

Occurrence

Enterohemorrhagic *E. coli* strains are recognized as an important problem in North and South America, Europe, Japan, and Australia (57). 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 (74). Extraintestinal *E. coli* infections are estimated to account for 70–90% of community-acquired urinary tract infections (55).

Reservoirs and Sources of Infection

EHEC strains of Shiga toxin producing *E. coli* have previously been identified in retail chicken samples, and chickens are also readily colonized with *E. coli* O157:H7 in experimental trials (75). Cattle and other ruminants are the most important source of human EHEC infections, however, and avian species are not considered an important reservoir. Transmission of EHEC strains occurs via contaminated foods, person-to-person contact, or by 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 (52). The incubation period for EHEC strains ranges from 2–10 days, with a median of 3–4 days (57). Secondary transmission of EHEC strains is common, especially among children in daycare centers. The duration of shedding is typically one week or less in adults, but may be three or more weeks in children.

Listeriosis

Listeriosis is caused by *Listeria monocytogenes*. Thirteen serotypes have been described, although three are most frequently associated with human disease: 1/2a, 1/2b, and 4b (62).

Nature of the Disease in Humans

Listeria monocytogenes can result in a variety of clinical syndromes, ranging from febrile gastroenteritis to severe invasive disease (2). Septicemia and meningoencephalitis are most frequently reported in neonates and immunocompromised adults. Focal infections may include brain and hepatic abscesses, cholecystitis, conjunctivitis, endocarditis, joint infections, skin infections, and osteomyelitis. Infection of pregnant women may result in fetal infection, stillbirth, or abortion. The average case-fatality rate for invasive infections is 20–30% (83).

Occurrence

Listeria monocytogenes has a worldwide distribution. In 2010, it was estimated to cause 23,150 illnesses; 5,463 deaths; and 172,823 disability-adjusted-life years (DALYs) globally (49). Populations at increased risk of systemic infection include pregnant women and neonates, immunocompromised adults, and the elderly. Veterinarians and farm workers are at increased risk for cutaneous infections, particularly following large-animal obstetric procedures (54). Most cases of listeriosis are

sporadic, but common source outbreaks are frequently identified.

Reservoirs and Sources of Infection

Listeria monocytogenes is widely distributed in nature and can be identified in soil, water, silage, and animal feces. *Listeria* is commonly found on poultry farms. It is an important source of environmental contamination in processing plants because of its ability to grow at refrigeration temperatures and form biofilms that are resistant to routine sanitation procedures. Most cases of human listeriosis result from foodborne transmission. Several outbreaks have been associated with ready-to-eat delicatessen meats and soft cheeses made from unpasteurized milk. The overall incubation period has been reported to range from 1–67 days, with longer median incubation times for pregnancy-associated cases (27.5 days) compared to non-pregnancy associated bacteremia (2 days) or central nervous system disease (9 days) (35). The median incubation period for non-invasive gastroenteritis is approximately 24 hours. 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). At the time of writing, 169 NTM species have been recognized, but only around 20 have been associated with human illness (78). 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 (85).

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 (87). 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.

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 preexisting lung disease, in elderly women with no history of underlying lung disease, and in adolescents with cystic fibrosis (78). 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. 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 (85). While birds may serve as an important reservoir of some *M. avium* strains, molecular studies suggest that bird-type *M. avium* isolates are genetically distinct from those that are typically isolated from humans and swine (86). Person-to-person transmission of MAC has not been documented, but has been reported for *M. abscessus* in cystic fibrosis patients (47).

Salmonellosis

Nontyphoidal *Salmonella* infections may be caused by any of the non-host-specific *Salmonella* serotypes that commonly affect both animals and man. *Salmonella enterica* serotypes Enteritidis and Typhimurium have a broad host range and are two of the most common nontyphoidal serovars isolated from humans. *Salmonella* Gallinarum and *Salmonella* Pullorum are host-restricted to poultry (76).

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 (4). Most cases are self-limiting, with diarrhea resolving without treatment after 3–7 days. Bacteremia is a potentially serious complication that occurs in 1–5% of cases (15). Possible sequelae of bacteremia include endocarditis and disseminated focal infections.

Occurrence

Salmonella has a worldwide distribution. There were an estimated 153 million human cases of nontyphoidal salmonellosis and 57,000 deaths globally in 2010, with 78.4 million of these cases resulting from foodborne transmission (46). Children, the elderly, and people with compromised immune systems are more likely to develop severe disease. Poultry and eggs are frequently identified as sources of infection in foodborne salmonellosis outbreaks. Of the 224 foodborne salmonellosis outbreaks identified in the United States between 1998 and 2012

that could be attributed to a single food commodity, 64 (29%) were attributed to poultry (22).

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, reptiles, and rodents. Transmission occurs by the fecal-oral route. Forty-five US outbreaks between 1996 and 2012 were linked to contact with live poultry from mail-order hatcheries (6). 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 4–6 weeks after infection, although *Salmonella* can still be identified one year postinfection in 5% of children younger than 5 years of age and in 1% of adults (4, 15).

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.

Nature of the Disease in Humans

Staphylococcus aureus causes a wide variety of clinical manifestations ranging from minor skin pustules to septicemia and death (39, 91). 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 foodborne intoxication is mediated by the production of heat-stable enterotoxins in uncooked or inadequately refrigerated foods (8). Signs include acute onset of nausea, abdominal cramps, vomiting, and often diarrhea. Most cases of foodborne intoxication resolve without treatment in 1–2 days. *Staphylococcus aureus* is also the causative agent of toxic shock syndrome in humans.

Occurrence

Staphylococcus aureus has a worldwide distribution and is one 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 (91), and causes approximately 240,000 foodborne intoxications in the United States each year (74). Newborn infants and the chronically ill are at increased risk for developing *S. aureus* skin infections (39).

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 non-carriers (91). Transmission is by direct or indirect contact. Hands are the most important vehicle for transmission, and at least two-thirds of infections are believed to result from autoinfection (39). Airborne transmission is uncommon but may result from 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 foodborne intoxication. Signs typically appear within 3–4 hours after ingesting staphylococcal enterotoxins (8). Person-to-person transmission of *S. aureus* is common. Live poultry have been implicated as a potential source of livestock-associated MRSA (32).

Fungal Diseases

Cryptococcosis

Cryptococcosis is a fungal infection caused by members of the genus *Cryptococcus*. There are more than 30 species belonging to this genus, although only *C. neoformans* and *C. gattii* are considered major pathogens. Cryptococcosis is not considered to be a zoonosis; rather humans and animals both acquire infection from environmental sources (10).

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. Even with appropriate antifungal treatment, the six-month case-fatality risk for cryptococcal meningitis in HIV-infected patients may exceed 35% (28).

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. 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 (64).

Reservoirs and Sources of Infection

Humans are infected with *Cryptococcus* by inhalation of desiccated encapsulated yeast cells or basidiospores from the environment. 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 (53, 61). 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 (89).

Dermatophytosis (Favus)

Microsporum gallinae is a contagious zoophilic fungus that is responsible for causing dermatophytosis in poultry and in humans (11, 30). 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. 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.

Occurrence

M. gallinae possibly has a worldwide distribution, although it is rarely reported as a cause of disease in either poultry or man. Miyasoto et al. identified 44 human cases that had been reported in the literature as of 2010, with 34 of these cases being reported in Nigeria and Iran (56). 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 the most important reservoir for *M. gallinae*. Transmission occurs by direct contact with infected animals or humans, or indirect contact via contaminated fomites. The incubation period is unknown, but for other dermatophytes has been reported as 4–14 days (12). 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 (26).

Histoplasmosis

Histoplasmosis is caused by the fungus *Histoplasma capsulatum* (17). 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 (17, 92). Acute pulmonary histoplasmosis is characterized by fever, myalgia, non-productive cough, dyspnea, and chest pain. The infection is usually self-limiting, but in immunocompromised patients or in 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 preexisting 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

Histoplasma capsulatum var. *capsulatum* causes histoplasmosis across the Americas, parts of Africa, eastern Asia, Australia, and rarely in Europe. In the United States, *H. capsulatum* is endemic in the Ohio and Mississippi River valleys. 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 near bird roosts (7, 17).

Reservoirs and Sources of Infection

Histoplasma grows in the soil, particularly in areas contaminated with bird and bat droppings. In the United States, the presence of birds, bats, or their droppings was noted in 77% of reported outbreaks between 1938 and 2013 (7). Humans become infected by inhalation of airborne microconidia. The typical incubation period is between 4 and 14 days (17). Transplacental transmission has been reported, but contact transmission from animal-to-person or from person-to-person does not occur (17, 93).

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). *Ornithonyssus bursa* (the tropical fowl mite) is a less frequent cause (18). Synonyms include gamasoidosis, acariasis, and fowl mite dermatitis.

Nature of the Disease in Humans

Typical clinical signs in humans include pruritic erythematous papules marked by a pinpoint red spot (18). Excoriation of lesions is common. Avian mites have also been associated with occupational asthma and otitis externa in poultry workers (48, 71). Symptoms are alleviated when the source of mites is removed. Mites are not typically observed on human skin as they leave quickly after biting.

Occurrence

Avian mites have a worldwide distribution. Avian mite dermatitis is not reportable and the incidence is unknown. Most published case reports result from proximate exposures to abandoned bird nests in an urban setting (16, 63). Mite infestation of layer and breeder flocks is common, however, and is recognized as a frequent cause of discomfort in poultry workers (82).

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 five months and three weeks, respectively, without a host (63). 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.

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 (72).

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 (23, 27). The median duration of illness in immunocompetent persons is 10–14 days, and signs may persist for up to one 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 one 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 (24, 72). Young children, immunocompromised persons, and people with occupational exposure to infected animals are at increased risk of infection.

Reservoirs and Sources of Infection

Cryptosporidium meleagridis has been identified in a wide range of avian and mammalian hosts including turkeys, quail, chickens, partridges, parakeets, deer, mice, dogs, and humans (24). Birds are not considered a major reservoir for *C. parvum*, which is the most common zoonotic species. Infectious oocysts shed in the feces of infected animals and humans can survive for several months in the environment. Transmission to susceptible hosts occurs by the fecal–oral route. The incubation period is 3–12 days (27). Large numbers of oocysts are shed in the feces of infected individuals, and person-to-person transmission is common. Oocyst shedding can continue for several weeks after the resolution of clinical symptoms (23).

Toxoplasmosis

Toxoplasmosis 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 (58). 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 (51).

Occurrence

Toxoplasma gondii has a worldwide distribution and exposure to the organism is common. The overall seroprevalence in the adolescent and adult US population for 2009–2010 was estimated at 12.4%, and the prevalence among women from 15–44 years of age was estimated at 9.1% (51). The incidence of congenital toxoplasmosis in the United States has been estimated to total approximately 365 cases per year. 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. Commercial poultry raised in confinement are rarely infected with *T. gondii*, however, infection of free-range chickens is common (37). The incubation period is 5–23 days (42). *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. Person-to-person transmission does not occur by direct contact, but transmission by organ transplantation or blood transfusion has been documented.

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