Viral Challenges in Turkeys

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Introduction

Viruses are submicroscopic, obligate intracellular parasites, distinct from "living" organisms. Comprised only of an outer shell made of protein, or protein and lipid, surrounding its genome made of RNA or DNA, viruses are not capable of producing energy, synthesizing protein, or performing any biochemical reactions normally associated with life (4). Viral particles are quite simply gene delivery vehicles (9). They exist to shield the viral genome from the elements as it makes its way from one animal or cell to another. Once this task is complete the outer shell is disassembled as the viral genome enters its new host cell. Inside the new host the virus hijacks the cellular machinery to produce more of itself. It is this key aspect of virus biology that makes them difficult to understand, and even more difficult to control.

In agriculture and specifically in poultry the main tool used to control viral infections is that of vaccines. This is a highly successful strategy whereby the immune system of the animal is exposed to the virus in such a manor that no disease (or very limited disease) is produced. Subsequently when that animal is exposed to that same virus in the field its immune system recognizes the threat and responds quickly to prevent infection. The problem with this strategy is viruses mutate and eventually change and are no longer recognized by the host immune system (6). This ever changing game of cat and mouse means it is necessary to keep producing new vaccines to the same pathogen. In addition to changing viruses, the poultry industry has seen the emergence of several new viral pathogens. Development of vaccines to these new pathogens is often a very slow and tedious process, and one that does little good to the industry facing an acute problem from this new threat. The alternative to vaccines as a control for viruses is the use of pharmacological anti-viral agents. These compounds are the viral equivalent of antibiotics for bacterial pathogens. Currently few specific and effective antiviral compounds exist, and their use in an agricultural setting would be prohibitively expensive. This leaves the poultry industry one additional alternative; the use of management and biosecurity. The power of this strategy is in its simplicity. The use of effective biosecurity protocols dramatically reduces the likelihood poultry will come in contact with a particular pathogen. These protocols are then reinforced by use of sound basic hygiene and management practices which ensure the environment is as free of other outside stresses such as crowding, temperature, or deficient diets which can exacerbate disease signs (5).

Much attention and emphasis has been placed on biosecurity practices in the turkey industry in North Carolina primarily related to the emergence of poult enteritis and mortality syndrome (PEMS) (2). During the worst period of these outbreaks, extreme but highly effective security plans were implemented which are largely responsible for the reduction of PEMS cases reported. As effective as those practices were, they were not sustainable, and as the threat diminished, so to has the extent of biosecurity regiments used on a daily basis. The use of these practices is still a very important aspect in the fight against viral diseases in any poultry operation, but the use of biosecurity measures do not have to be as extreme. Ultimately, any security plan needs to be a business decision which weighs the likelihood of disease, the type of pathogen of greatest consequence, the potential cost of an unrestricted outbreak, and the expense of establishing preventative measures. Only after careful consideration of these factors can a protocol which maximizes the health and quality of the birds with the least cost be achieved. There are countless reviews of different specific biosecurity plans recommended for different poultry production systems; however what works for some operations might not work for others. Therefore, this review will only discuss the topic of biosecurity in general terms. Instead this review will focus more on general properties of different viruses and how aspects of a virus's biology should be included in the consideration of a successful plan.

General Biosecurity Concepts

In general, any protocols followed on farms designed to promote the animals health is part of the biosecurity plan. Each aspect of the poultry operation must be evaluated to ensure from the grower to upper management all are working towards promoting biosecurity. On the farm; the location and layout of the houses, what other animals are on the farm, the order in which personnel perform their daily tasks, the cleanliness of the house, the nutritional status of the birds, the controlled movement of vehicles and other personnel on and off the property, the use of vaccines and antibiotics, the cleaning protocol used between flocks, and the length of downtime between flocks are all important factors to consider when establishing a sound biosecurity plan (5, 28). Aside from plans implemented on each farm there are also other company wide and industry wide considerations. The routes delivery trucks and service personnel use in traveling through their respective territories and the order in which company veterinarians and consultants visit farms are particularly important during periods of active outbreaks. Each of these factors can contribute to the ultimate success or failure of any biosecurity plan to reduce the impact of disease.

Applied Poultry Virology

Viral infections in a veterinary setting are generally described and discussed in terms of their clinical signs (11, 31). That is to say viruses that cause respiratory disease are grouped together; viruses that cause enteric diseases are grouped together, etc. From a clinical standpoint this makes sense. We are interested in the animal and fixing the system that is broken; however this implies that all viruses which affect a certain system behave similarly. This misconception can have significant consequences in context of biosecurity regimens. The field of virology, the subfield of biology which studies viruses, has developed a classification system that looks more specifically at the properties of each virus and groups them based on their genetic make up and their architecture (4, 9). These properties can be good predictors of the relative stability a virus may exhibit in the environment. As was discussed before, viruses are not biochemically or enzymatically active outside of a host cell. This means that unlike bacteria and parasites which continue to live and divide outside the bird, viruses can not and this is a weakness which can be exploited.

Viruses come in two main varieties with respect to architecture; enveloped and non-enveloped. These terms refer to whether the viral genome is enclosed in a particle made of a host-cell derived lipid bilayer which will have virus derived attachment proteins protruding through (enveloped) or if the genome is enclosed in a particle made up of numerous repeats of viral proteins which fit together to form a very organized three-dimensional structure held together by hydrogen-bonds and van der Waals interactions (non-enveloped) (9). The main structural element of the enveloped viral particle is the host-derived lipid bilayer. This means these viruses are typically as sensitive to detergents, disinfectants and environmental factors as eukaryotic cells (4). Put simply enveloped viruses are generally fragile in the environment. Conversely, non-enveloped viruses are much more stable in the environment. Compounds designed to disrupt cell membranes, such as non-ionic detergents, have little to no affect on these viruses. Inactivation of non-enveloped viruses requires treatment with compounds which will disrupt the hydrogen-bonds and van der Waals interactions holding the particle together, or conversely compounds which induce massive protein-protein crosslinking which prevent the virus from delivering its genome to a new cell. By considering the structural properties of viruses when developing and implementing a security plan its effectiveness can be maximized.

Representative Turkey Viruses

The following is a brief description of viral pathogens which represent various disease threats to turkeys. This is not by any means an exhaustive list, or meant to suggest that these viruses are of the greatest importance, but rather is a list of known turkey pathogens from representative viral families with different structural properties. There is no standardized test to measure virion stability against different disinfectants. Therefore thermostability for these viral families are listed (Table 1) to demonstrate relative differences.

Avian Influenza

Avian influenza (AI) viruses belong to the viral family *Orthomyxoviridae* and are a well recognized pathogen of poultry. These viruses are classified as either highly pathogenic (HP) or low pathogenic (LP). HP viruses cause severe respiratory disease and are highly fatal in turkeys. LP viruses typically cause mild respiratory disease in turkeys and a can dramatically affect egg production (11, 31). The recent outbreak of LP H7N2 AI in turkeys in Virginia during 2002 costs the industry and the USDA an estimated $200 million (1). Control of AI in the US has typically been based primarily on eradication and biosecurity (22). The virus can replicate in the lungs and in the gut and is spread through inhaled droplets or fecal oral (11). Transport of infected carcasses through non-infected areas and proximity to swine operations are two factors involved in the spread of AI (11, 19, 31). AI is an enveloped virus and therefore is very unstable in the environment. Heating to 56º C (138º F) for 30 min, treating with 70% ethanol, or other routine disinfectants will inactivate AI. Leaving infected chicken manure at ambient temperatures for a few days has also been shown to inactivate AI (18).

Avian Paramyxoviruses

Avian paramyxoviruses belong to the viral family *Paramyxoviridae*. This viral family is also made up of enveloped viruses. One of the viral pathogens of note from this group is Newcastle disease virus (NDV), which can cause severe respiratory disease in most species of birds. Avian paramyxovirus type-2 and type-3 have been reported to cause mild respiratory disease associated with reduced egg production (31). In addition, a new pathogen of turkeys which belongs to a subfamily of paramyxoviruses has recently been described. Avian metapneumovirus (AMPV), also known as avian pnuemovirus (APV) or turkey rhinotracheitis (TRTV) causes a mild respiratory disease in turkeys, however so far this pathogen has been isolated to turkey farms in Minnesota (MN) (24, 31). NDV infections of chickens and turkeys is routinely controlled through a combination of vaccination programs and good general farm hygiene, however sporadic outbreaks still occur (11). Control of AMPV in MN currently combines eradiation attempts and the use of modified live vaccine (25). As with AI, these enveloped viruses are also very unstable in the environment. Most routine disinfectants are effective at inactivating the viruses. Heating of paramyxoviruses to 56º C (138º F) for 6 min is enough to inactivate (17).

Avian Reticuloendotheliosis virus (REV)

REV is a lymphoma causing virus which affects turkeys. This virus is similar to and belongs to the same family as avian leukosis virus (ALV), the family *Retroviridae* (31). These are enveloped viruses which only cause sporadic disease so no specific control procedures have been developed, but given its similarity to ALV a similar eradication program should work for REV if needed (11). The disease can be spread through contact, fecal material, and has been associated with contaminated vaccines. Retroviruses as a family are very unstable in the environment. While there are no specific reports of the specific resistances of REV to different treatments studies of retroviruses from other species suggest that heating to 50º C (122º F) for 30 min is sufficient to inactivate the virion (21).

Turkey Coronavirus

The viral family C*oronaviridae* are responsible for the acute respiratory infection, infectious bronchitis virus in chickens. However in turkeys coronaviruses are reported to cause enteric disease. The first description of turkey enteric coronavirus was known as bluecomb disease (11, 31). More recently a similar turkey coronavirus has been associated with PEMS (8). These viruses cause acute severe diarrhea and growth suppression in young poults. Their transmission is through the fecal oral route involving direct ingestion of feces or the mechanical transfer via personnel, equipment, and even insects (3). Coronaviruses are enveloped viruses and therefore environmentally unstable. Heating to 56º C (138º F) for 20 min has been reported to be sufficient to inactivate coronaviruses (32).

Turkey Hemorrhagic Enteritis (THE)

THE belongs to the viral family *Adenoviridae*. Adenoviruses are non-enveloped viruses associated with several different diseases in different species. THE is reported to cause a severe bloody diarrhea in turkeys four weeks of age or older (11, 31). This disease is often associated with severe mortality ranging from 1-60%, most of which occurs in the first 24hrs following the onset of clinical signs (29). The severity of disease, mortality, and numbers of birds affected are influenced by environmental factors such as crowding, chilling, and nutrition. Like most enteric diseases the primary route of transmission is believed to be fecal oral. There is a vaccine used to control this disease (30). Since THE is non-enveloped it is more stable in the environment and can more readily resist detergents and drying than enveloped viruses. Avian adenoviruses are described as being inactivated following heating to 60º C (140º F) for 30 min (31).

Avian Reovirus

Avian reoviruses, members of the *Reoviridae* family, are non-enveloped viruses capable of infecting and causing several diseases in birds. In turkeys, reoviruses have been associated with respiratory infections, enteritis and tendonitis (11, 31). Transmission of reovirus has been demonstrated through several factors. Oral transmission is likely a major component, however birds with abrasions on their leg joints maybe able to get direct infection of the joints leading to tendon problems. Additionally reovirus is known to be transmitted vertically (11, 31). There are also numerous non-pathogenic strains of reovirus in birds making it difficult to determine their role in larger disease syndromes. One example of this is PEMS. Several laboratories have identified and characterized avian reovirus strains from PEMS affected birds (10, 12, 33). The specific role reovirus plays in the larger PEMS complex is still under investigation. There are a few attenuated vaccine strains of avian reovirus however they are not widely used. As a non-enveloped virus, avian reoviruses are more environmentally stable. Studies with reoviruses suggest that treatment of these particles with some simple detergents may enhance their infectivity (23). Heating to 60º C (140º F) for 30 min is generally sufficient to inactivate these viruses (20).

Turkey Astrovirus (TAstV)

Turkey astroviruses belong to the family *Astroviridae*. Two main types have been described which cause disease; type-1 and type-2 (14). Both types predominantly affect poults and cause severe diarrhea with growth suppression. Type-1 one was first reported in the late 1980s and shown to be highly correlated with enteric disease in the field (26). Type-2 was first identified in 1999 and was shown to be associated with PEMS (16). Type-2 turkey astrovirus was shown to also be highly correlated with enteric disease in the field, and experimentally produces severe diarrhea, lymphoid atrophy, and growth suppression (13, 15). Transmission of TAstV is similar to that coronavirus; direct fecal oral or through mechanical transmission with personnel, equipment, and contaminated litter as the most likely vehicles. Currently there are no specific treatment or containment programs for TAstVs. These viruses are extremely stable. Studies specifically to determine its resistance to disinfectants and environmental stress found most commercially used cleaning products were ineffective, and the virus was still infectious following heating to 65º C (149º F) for 10 min (27). Subsequent studies suggest the virus can withstand higher temperatures for longer periods of time. Similarly, we have demonstrated isolation of infectious virus from litter from thoroughly cleaned houses left empty for weeks (unpublished observations). These findings suggest that on certain farms which have had a history of TAstV infection, this virus may serve as an excellent indicator organism for completeness of cleaning and integrity of the biosecurity plan since treatments and conditions which destroy TAstV will also destroy virtually all other pathogens.

Conclusions

Viruses vary greatly in their biology, host range, and the disease they cause making the development and implementation of specific treatment and protection program for each virus impractical and costly. Selecting elements of the different control programs, matched with specific viral concerns, will greatly maximize the affect of the biosecurity program.

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Table 1: Relative stability of turkey viruses

Virus	Family	Envelope	Heat Inactivation	Ref
Hemorrhagic enteritis	Adenoviridae	No	60C for 30min	(31)
Turkey astrovirus	Astroviridae	No	>65C for 10min	(27)
Avian reovirus	Reoviridae	No	60C for 30min	(20)[a]
Avian influenza	Orthomyxoviridae	Yes	56C for 30min	(18)
Turkey coronavirus	Coronaviridae	Yes	56C for 20min	(32)[a]
Avian metapneumovirus	Paramyxoviridae	Yes	56C for 6min	(7)[a]
Reticuloendotheliosis virus	Retroviridae	Yes	50C for 30min	(21)[a]

[a] Specific information for the inactivation of this avian virus is not available. Temperature listed is for study of a closely related virus.

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