Avian reoviruses are members of the orthoreovirus genus in the Reoviridae family (4, 6). Ubiquitous in commercial poultry, they can be differentiated by antigenic configuration, pathotype, relative pathogenicity, growth in cell culture, sensitivity to trypsin, and host specificity (2, 3, 4, 5, 8, 9, 10, 11, 12).

Reoviruses have been isolated from a variety of tissues in chickens affected by assorted disease conditions, including viral arthritis/tenosynovitis, stunting syndrome, respiratory disease, enteric disease, immunosuppression, and malabsorption syndrome (9, 10, 11, 12). They have frequently been found in chickens that were clinically normal. The nature of the disease that occurs following reovirus infection is very much dependent upon host age, immune status, virus pathotype, and route of exposure. Interactions with other infectious agents have been documented (9, 11, 12) and may result in differences in both the nature and severity of reovirus-induced disease expression.

In young meat-type chickens, economic losses related to reovirus infections are frequently associated with increased mortality, viral arthritis/tenosynovitis (9), and a general lack of performance including diminished weight gains, poor feed conversions, uneven growth rates, and reduced marketability of affected birds (1). Breeder flocks that develop viral arthritis just prior to the onset of or during egg production may in addition to lameness be characterized by increased mortality, decreased egg production, suboptimal hatchability/fertility, and vertical transmission of virus to progeny—all of which can contribute to increased costs for poultry producers.

The best defined and most readily diagnosed reovirus-associated disease in chickens is viral arthritis (9). The disease has been recognized in virtually all major poultry-producing areas worldwide in both heavy and light chicken breeds. Other disease conditions associated with reovirus infections can be demonstrated experimentally or are inferred by isolation from clinical accessions. However, these conditions are often difficult to recognize and definitively diagnose in the commercial setting. Because of the differences in disease expression, viral arthritis and other reovirus-associated diseases will be described separately in this chapter.

REFERENCES

Viral Arthritis
John K. Rosenberger

INTRODUCTION

Viral arthritis is an economically important disease of chickens that can be caused by different serotypes and pathotypes of avian reovirus (26, 35, 41, 78, 81). The disease is considered to be most important in meat-type chickens but has been diagnosed in commercial layers (39, 89, 99) and turkeys (1, 2, 18, 61, 69, 91, 106, 115). When inoculated into turkeys, selected reoviruses pathogenic for chickens produced lesions consistent with viral arthritis (106).

The disease in chickens typically is controlled by vaccination with live attenuated and/or inactivated whole virus vaccines. Derivatives of the S1133 strain of reovirus are most commonly used as vaccines and have proven to be efficacious in most parts of the world. Autogenous vaccines can be used to provide protection against different serotypes (26, 85, 100). Turkeys and other avian species are not routinely vaccinated for viral arthritis.

HISTORY

In 1954, Fahey and Crawley (16) made what was later confirmed by Petek et al. (70) to be the initial isolation of avian reovirus from the respiratory tract of chickens with chronic respiratory disease. The Fahey-Crawley virus, when inoculated into susceptible chickens, produced a moderate respiratory disease, liver necrosis, and an inflammation of the tendons and synovial membranes.

Olson et al. in 1957 (68) described a naturally occurring synovitis in chickens from which they were able to isolate an agent insensitive to chlorotetracycline and furazolidone and serologically unrelated to either Mycoplasma gallisepticum or M. synoviae. This agent, later named the "viral arthritis agent" by Olson and Kerr (64), eventually was identified as a reovirus by Walker et al. in 1972 (109). Dalton and Henry (7) used the term tenosynovitis to define the changes in the tendons and tendon sheaths associated with a condition they considered different from that caused by M. synoviae. This difference was substantiated by Olson and Solomon (66) when they reported tenosynovitis in commercially produced chickens that had been derived from M. synoviae-free broiler chickens. An isolate obtained from these birds had characteristics identical to those described for the "viral arthritis agent" and was shown to be antigenically similar to the Fahey-Crawley virus (67). Since the first reports of tenosynovitis in the United States and England, the disease has been described in many other countries. Several reviews document the incidence of reovirus-induced tenosynovitis (47, 75, 99).

Control of viral arthritis was greatly facilitated by recognition of the role of maternal antibodies in conferring protection to progeny (101, 105). Both viable and inactivated vaccines were subsequently developed to induce antibodies in breeder flocks for the protection of progeny from contact and transovarian transmission (101, 107, 108). The first commercially available live vaccines were developed by van der Heide et al. (103, 107) from the S1133 strain of avian reovirus. This same strain has been extensively used as an inactivated vaccine alone or in combination with other reovirus pathotypes.

Reoviruses that cause viral arthritis also have the potential to induce other pathological changes in chickens, particularly if introduced via the transovarian route or shortly after hatch (8, 36, 41, 56, 59, 77, 78). Disease conditions associated with some reoviruses that have arthrotrophic characteristics include ruptured gastrocnemius tendons, pericarditis, myocarditis, hydropericardium, uneven growth, and mortality (27, 46, 77, 78, 95, 99).

INCIDENCE AND DISTRIBUTION

Reovirus infections are prevalent worldwide in chickens, turkeys, and other avian species. Viral arthritis is observed primarily in meat-type chickens but can be found in lighter breeds (39, 89) and turkeys (1, 2, 18, 61, 69, 91, 106, 115). It should be recognized, however, that reoviruses are commonly found in the digestive and respiratory tracts of clinically normal chickens and turkeys (44, 70, 91, 115) and have been identified as a vaccine contaminant. It is estimated that greater than 80% of reoviruses isolated from chickens are apathogenic (100).

ETIOLOGY

Reoviruses, which replicate in the cytoplasm, are nonenveloped with an icosahedral symmetry and a double-shelled capsid. Intact virus particles have a diameter of approximately 75 nm and a density in cesium chloride of 1.36–1.37 g/mL (21, 87, 88, 93). The viral genome consists of 10 ds RNA segments (22, 23, 93), which can be sepa-
Reoviruses can be classified using serologic procedures or grouped according to their relative pathogenicity for chickens. Kawamura and Tsubahara and Kawamura et al. (43, 44) identified five serotypes of reovirus from 77 isolates originally obtained from feces, cloacal swabs, and tracheas. Sahu and Olson (84) found four serotypes from intestines, respiratory tract, and synovial isolates. Wood et al. (113) calculated the relatedness of reoviruses originating from the United States, the United Kingdom, Germany, and Japan and found at least 11 serotypes, although there was considerable cross neutralization among heterologous types. Hieronymus et al. (27) grouped five reovirus isolates into three serotypes, and Robertson and Wilcox (74) assigned 10 Australian isolates into three groups with considerable cross-reactivity. It is apparent that reoviruses may frequently exist as antigenic subtypes, rather than distinct serotypes.

Rosenberger et al. (80) and Sterner et al. (95) inoculated specific-pathogen-free chickens by various routes with plaque-purified, antigenically similar viruses and demonstrated clear strain differences based on relative pathogenicity and virus persistence.

### Laboratory Host Systems
Reoviruses grow readily in the embryonating chicken egg following inoculation via yolk sac or chorioallantoic membrane (CAM). The yolk sac is preferred for original isolation and generally results in embryo mortality 3–5 days after inoculation, with affected embryos exhibiting a purplish discoloration due to massive subcutaneous hemorrhage. Mortality in CAM-inoculated embryos usually occurs on day 7–8 PI; embryos are slightly dwarfed with occasional enlargement of the liver and spleen. Necrotic foci may occur in both the liver and spleen, particularly in embryos that survive longer than 7 days PI. Small, discrete, slightly raised white lesions may be found on the CAM. Histologically, areas of necrosis of the ectoderm with only moderate stimulation of the epithelial cells are seen. Mesoderm adjacent to the lesion is edematous and contains numerous inflammatory cells. Edema alone may be found. Embryo mortality is less consistent following inoculation via the chorioallantoic sac.

The virus grows in primary chicken cell cultures of embryo, lung, kidney, liver, macrophages, and testicle. Primary chicken kidney cells from 2–6-week-old chickens are satisfactory, but for plaques and isolation, primary embryo liver cells are preferred (2, 22). Chicken embryo fibroblasts are suitable for reovirus growth, but the virus often requires adaptation (3, 25, 40). Chicken-origin cell cultures infected with reoviruses are characterized by the formation of syncytia, which may occur as early as 24–48 hours, followed by degeneration, leaving holes in the monolayer and giant cells floating in the medium. Infected cells exhibit intracytoplasmic inclusions that may appear either eosinophilic or basophilic (75). Of many established cell lines tested, virus has been grown on Vero (84), BHK 21/13, ITT, feline kidney (CRFK), Georgia bovine kidney (GBK), rabbit kidney (RK), porcine kidney (PK) (3), a Japanese quail cell line (QT35) derived from an induced fibrosarcoma (6), chicken lymphoblastoid cells (90), and subpopulations of chicken lymphocytes (58).

### Pathogenicity
Although normally associated with arthritis, reoviruses have been identified as the etiology of other disease conditions as well, including growth retardation, pericarditis, myocarditis, hydrop ericardium, enteritis, hepatitis, bursal and thymic atrophy, osteoporosis, and acute and chronic respiratory syndromes (16, 18, 24, 35, 46, 61, 62, 63, 77, 95, 100). The pathogenicity of selected reovirus isolates was enhanced by coinfection with *Eimeria tenella* or *E. maxima* (82, 83). Exposure to infectious bursal disease virus or particular dietary regimes increased the severity of...
tenosynovitis resulting from infections with the WVU-2937 isolate (4, 5, 94). Reoviruses may also exacerbate disease conditions caused by other pathogens including chicken anemia virus (15, 54), Escherichia coli, and common respiratory viruses (73, 81). The increased susceptibility to other infectious agents following or concomitant with reovirus exposure may result from immune system compromise (73, 77, 81, 82, 83).

PATHOGENESIS AND EPIZOOTIOLOGY

Natural and Experimental Hosts

Although reoviruses have been found in many avian species, chickens and turkeys are the only recognized natural or experimental hosts for reovirus-induced arthritis. Reoviruses have been isolated from turkeys with arthritis (69), and Van der Heide et al. (106) found a turkey isolate to be pathogenic for chickens. The turkey isolate was neutralized by chicken reovirus S1133 antiserum. High mortality in turkey pouls has also been associated with reovirus (91), although turkeys were shown to be more resistant than chickens to reovirus-induced tenosynovitis (1). McFerran et al. (53) identified a reovirus in turkey feces that shared the group-specific antigen with chicken isolates but was not neutralized by available reference antiserum.

Reoviruses were found in clinically affected ducks, pigeons, geese, American woodcock, and Psittacine species, but a firm etiologic relationship was not always established (9, 75). A disease in Muscovy ducks characterized by a general malaise, diarrhea, and stunted growth has been reported in several countries (17, 42, 50) and reproduced experimentally with isolated reoviruses (17, 50). Attempts to establish active infection in the canary, pigeon, guinea pig, rat, mouse, hamster, and rabbit failed; however, Phillips et al. (71) reported liver lesions in neonatal mice after oral and nasal infection, and Nersessian et al. (61) produced stunted growth and incoordination in suckling mice inoculated intracerebrally with several turkey isolates.

Age-Associated Resistance

Kerr and Olson (45) were the first to report an age-related resistance to reovirus-induced arthritis. The disease can be readily reproduced in 1-day-old chickens free of maternal antibody (36, 105), whereas older chickens are infected, but the disease is generally less severe, and the incubation period is longer. Similar results were reported by Rosenberg (80) with reoviruses isolated from birds with an apparent stunting syndrome and arthritis. Jones and Georgiou (36) suggested the age-associated susceptibility may be related to the inability of young birds to develop an effective immune response.

Transmission

Horizontal transmission of reovirus has been extensively documented (75, 99). There is considerable variation, however, among strains of virus in their ability to spread laterally. Although reovirus may be excreted from both the intestinal and respiratory tracts for at least 10 days postinoculation, virus generally appears to be shed from the intestine for longer periods, suggesting fecal contamination as a primary source of contact infection (39, 49). Roessler et al. (77) demonstrated that 1-day-old chickens are more susceptible to reovirus introduced via the respiratory route than orally. Virus may persist for long periods in the cecal tonsils and hock joints, particularly in birds infected at a young age (37, 51), implicating carrier birds as potential sources of infection for contacts.

Menendez et al. (56) and Van der Heide and Kalbac (102) have clearly demonstrated that avian reoviruses can be vertically transmitted. Menendez et al. (56) showed that following oral, tracheal, and nasal inoculation of 15-month-old breeders, virus was present in chicks from eggs laid 17, 18, and 19 days postinfection. Egg transmission rate was low (1.7%). Reoviruses were also isolated from chicken embryo fibroblast cell cultures prepared from embryonated eggs derived from experimentally infected hens (102).

Incubation Period

The incubation period differs depending upon the virus pathotype, age of host, and route of exposure (75, 99). For inoculated 2-week-old chickens, the incubation period varied from 1 day (foot pad inoculation) to 11 days (intramuscular, intravenous, intrasinus inoculation). The incubation period following intratracheal inoculation and contact exposure was 9 and 13 days, respectively (66).

Often, infections are inapparent and demonstrable only by serology or virus isolations. Mature birds inoculated by oral and respiratory routes with the FDO isolate had virus in all organs tested at 4 days postinfection. The number of virus isolations was greatly reduced by 2 weeks, and no virus was present 20 days postinfection. There was frequent localization of virus in the flexor and extensor tendons of the pelvic limb, although gross lesions were not evident (57). Foot pad inoculation of 1-day-old chickens with an arthrotropic reovirus (R2) produced a more rapid progression of disease than either the oral, subcutaneous, or articular routes (32). When infected by the oral route, which appears to be a likely mode of naturally transmitted virus, the initial site of viral replication, which occurred within 2 to 12 hours postexposure, was the epithelium of the intestine and the bursa of Fabricius. This was followed by virus distribution in a wide range of tissues, including the hock joint, within 24–48 hours (41). Many reoviruses cause microscopic inflammatory changes in the digital flexor and metatarsal extensor tendons without development of gross lesions (65).

When viral arthritis does result from naturally occurring infection, it is usually seen in young birds 4–7 weeks old but may be seen in much older chickens as well (99). Morbidity can be as high as 100%, and mortality is generally less than 6%. The virus can persist in the tendons for at least 22 weeks (78).
Chapter 3  Infectious Bronchitis

Signs
In acute infections, lameness is present, and some chickens are stunted. With chronic infection, lameness is more pronounced, and in a small percentage of infected chickens, the hock joint is immobilized. In a flock of 36,000 broilers, the infection, first diagnosed as infectious synovitis, appeared in 8 of 16 pens when the chicks were 3–4 weeks old. Approximately 550 birds died or were removed because of lameness by 7–8 weeks. Another 4,500 birds were stunted.

In another flock of approximately 15,000 broilers, no clinical signs of viral arthritis/tenosynovitis were observed, but approximately 5% of the birds had enlargement in the area of the gastrocnemius or digital flexor tendons when observed at slaughter. At 9 weeks, birds from this flock had an average weight of only 3.66 lb; feed conversion was 2.45; mortality totaled 5%; and the condemnation rate was 2.6%. Virus was isolated from two birds condemned for toxemia; of 80 serum samples obtained from this flock, 89% had reovirus antibodies detected in a precipitin test. This unapparent infection probably caused the poor performance of these broilers.

Similar observations have been made by other workers (21, 34). Rupture of the gastrocnemius tendon, especially in male roaster birds 12–16 weeks old, is often associated with reovirus infection (34, 40). A similar lesion has been seen in 5–8-week-old turkeys (69). The typical uneven gait in bilateral rupture of the tendon results from the inability of the bird to immobilize the metatarsus. The latter is often accompanied by ruptured blood vessels.

Gross Lesions
Gross lesions in naturally infected chickens are observed as swellings of the digital flexor and metatarsal extensor tendons. The latter lesion is evident by palpation just above the hock and may be readily observed when feathers are removed (Fig. 11.1).

Swellings of the foot pad and hock joint are less frequent. The hock usually contains a small amount of straw-colored or blood-tinted exudate; in a few cases, there is a considerable amount of purulent exudate resembling that seen with infectious synovitis. Early in the infection, there is marked edema of the tarsal and metatarsal tendon sheaths (Fig. 11.2). Petechial hemorrhages are frequent in the synovial membranes above the hock (Fig. 11.3B).

Inflammation of tendon areas progresses to a chronic-type lesion characterized by hardening and fusion of tendon sheaths. Small pitted erosions develop in the articular cartilage of the distal tibiotarsus. These erosions enlarge, coalesce, and extend into underlying bone (Fig. 11.3B,C). An overgrowth of fibrocartilaginous pannus develops on the articular surface. Condyles and epicondyles are frequently involved (46). In inoculated chickens, the diaphysis of the proximal metatarsal of the affected limb is enlarged.

Histopathology
Histologic changes have been described by Kerr and Olson (45). In general, they are the same for naturally occurring and experimental infections. During the acute phase (7–15 days following foot pad inoculation), edema, coagulation necrosis, heterophil accumulation, and perivascular infiltration are seen. There also are hypertrophy and hyperplasia of synovial cells, infiltration of lymphocytes and macrophages, and a proliferation of reticular cells. These latter lesions cause parietal and visceral layers of the

11.1. An 8-week-old broiler showing marked swelling of digital flexor and metatarsal extensor tendons. Diagnosis frequently can be made on the basis of the bilateral swelling of these tendons.

11.2. Marked edema of digital flexor tendon sheaths (left); normal (right).
11.3. Viral arthritis lesions in distal posterior tibia of inoculated chickens. A. Normal. B. Cartilage erosions and hemorrhages of synovial membrane 35 days postinoculation. C. Erosions of cartilage and marked thickening of synovial membrane 212 days postinoculation.
tendon sheaths to become markedly thickened. The synovial cavity is filled with heterophils, macrophages, and sloughed synovial cells. Periostitis characterized by increased osteoclasts develops. During the chronic phase (starting by 15 days postinfection), the synovial membrane develops villous processes, and lymphoid nodules are seen. After 30 days, inflammatory changes become more chronic. An increase in the amount of fibrous connective tissue occurs, and a pronounced infiltration or proliferation of reticular cells, lymphocytes, macrophages, and plasma cells also can be seen.

The same general inflammatory changes develop in the tarsometatarsal and hock joint areas. Development of sesamoid bones in the tendon of the affected limb is inhibited. Some tendons are replaced completely by irregular granulation tissue, and large villi form on the synovial membrane.

At 54 days postinfection, orally infected birds showed chronic fibrosis of tendon sheaths, with fibrous tissues invading tendons and resulting in ankylosis and immobility (104).

Linear growth of cartilage cells in the proximal tar-sometatarsal bone becomes narrow and irregular. Erosions on the hock joint cartilage are accompanied by a granulation pannus. Osteoblasts become active and lay down a thickened layer of bone beneath the erosion. Osteoblastic activity is present on the condyles, epicondyles, and accessory tibia, producing osteoneogenesis and subsequent exostosis (46). Ultrastructurally, the gastrocnemius tendon and sheath in broilers infected with reovirus at 1 day of age by the oral route were characterized by degenerative changes in fibroblasts including cytoplasmic vacuolization, membrane disruption, loss of ribosomes from the endoplasmic reticulum, and generalized mitochondrial and cellular disruption (29).

Lesions found in the heart have been described in detail (46, 66). An infiltration of heterophils between myocardial fibers is a constant finding. In some cases, it is accompanied by proliferating mononuclear cells, probably reticular cells. The pathogenicity of avian reoviruses for day-old chicks revealed the arthrogenic potential for many strains and marked hepatic necrosis (24).

Erythrocyte, hematocrit, and total leukocyte determinations are generally within the normal range, although there may be a rise in the heterophil percentage and a decrease in the lymphocyte percentage.

**Immunity**

Avian reoviruses possess a group-specific antigen discernable with gel diffusion techniques (112) and a serotype-specific antigen demonstrable with neutralizing antibody in plaque-reduction or chicken embryo assays (75, 99). Neutralizing antibodies can be detected 7–10 days following infection, and precipitating antibodies at approximately 2 weeks. Neutralizing antibody appears to persist longer than precipitating antibody, but this may be a reflection of assay sensitivity. The importance of antibody in establishing protection is not well understood, because birds may become persistently infected in the presence of high levels of circulating antibody (38). It is apparent, however, that maternal antibody can afford a degree of protection to 1-day-old chickens against naturally occurring and experimental challenges (103, 105). Relative protection afforded by antibody appears to be related to serotype homogeneity, virus virulence, host age, and antibody titer (72, 80, 96, 105, 110).

Induction of intestinal IgA, which may be important in limiting the pathogenic potential and dissemination of reovirus, is affected by route of exposure, age, and sensitivity to trypsin (59). Chickens infected at one day of age or with trypsin-sensitive reovirus by the oral route do not have a detectable intestinal IgA response.

Interferon production by avian reoviruses has been demonstrated in vitro and in vivo. The S1133 attenuated strain induced interferon in chick embryo cell cultures, and in vivo interferon was detected in the lungs but not in other tissues (13, 14, 111). A more pathogenic reovirus elicited the production of interferon detectable in serum samples (13, 14). Hill et al. (28) reported that the suppression of T-cell-mediated immunity by cyclosporin A resulted in increased mortality in reovirus-infected birds, but the relative severity of tendon lesions was unaffected.

**DIAGNOSIS**

A presumptive diagnosis of viral arthritis may be made on the basis of signs and lesions. Involvement of primarily the metatarsal extensor and digital flexor tendons (see Fig. 11.2), and heterophil infiltration in the heart, assist in differentiating the infection from bacterial and mycoplasmal synovitis. Demonstration of reoviruses in the tendon sheaths by fluorescent antibody techniques (75) or virus isolation in chickens embryos or chicken embryo liver cells provides further evidence (75, 99). The relative pathogenicity of a reovirus obtained from an affected joint can be confirmed by inoculation into the foot pad of susceptible 1-day-old chickens. If pathogenic, the virus will induce a pronounced inflammation of the foot pad within 72 hours postinoculation.

Reoviruses can be readily differentiated from other viruses by their typical physicochemical characteristics and the presence of a group-specific antigen demonstrable with the agar gel precipitin test. For preparation of the antigen, 9–11-day-old embryonating chicken eggs are inoculated by the CAM route, and CAMs are harvested from dead or affected embryos within 7 days postinoculation. The CAMs then are homogenized and used as antigen (67). The precipitin test can be used to identify isolates as reovirus if known positive antisera is available, or it can be used as an indication of antibody status in affected flocks.

Lesion-associated reovirus proteins or nucleic acid can be detected in formalin-fixed tissue using immunoperoxidase procedures (97) or nucleic acid probes (30, 116). This approach may assist in assigning etiological relationships to specific reovirus isolates.
Serology
Reovirus group-specific antibody can be detected readily with the agar gel precipitin test (43, 67) or indirect fluorescent antibody (IFA) assay (31). The IFA test is more sensitive and, therefore, better suited for quantitative evaluations. Virus neutralization, based on plaque reduction in chicken kidney or chicken embryo liver cell cultures and several cell lines, has been routinely used for determining serotype differences with rabbit or chicken antiserum and monoclonal antibodies (44, 48, 96, 110, 113). Although several serotypes have been described, considerable homogeneity exists among reovirus isolates, with many being classified as antigenic subtypes rather than distinct serotypes. In vitro measurements of reovirus antibody specificity may not always correlate with protection against homologous and heterologous challenge of birds with maternally derived antibody (114), and the type specificity of neutralizing antibody is less for chickens immunized with inactivated reovirus than for chickens immune following infection (55).

Slaght et al. (92) were the first to describe an enzyme-linked immunosorbent assay (ELISA) for detecting avian reovirus antibody. The S1133 strain was used as antigen and found to react with antibodies to the Reo-25 and WVU-2937 isolates; homologous antibody gave the highest titer. The ELISA systems now available from commercial sources are apparently suitable for assessing reovirus antibody levels on a flock basis (98).

PREVENTION AND CONTROL
The ubiquitous nature of the avian reoviruses and their inherent stability, coupled with modern, high-density confinement rearing practices, suggests that elimination of virus exposure may be difficult. The virus can be transmitted both vertically and horizontally and, because of its resistance to inactivation, may be frequently carried by mechanical means. Thorough cleaning of a poultry house appears to prevent infection with pathogenic virus in subsequent groups following the removal of an infected flock from the premises. Because of the relative stability of the avian reovirus group, commercially available disinfectants should be validated for efficacy before use. Lye and 0.5% organic iodine solutions are considered to be effective inactivating agents.

Chickens are most susceptible to pathogenic reoviruses at 1 day of age and then develop an age-associated resistance beginning as early as 2 weeks. Because of this enhanced period of susceptibility, vaccines and vaccination programs have evolved that are directed at providing protection at 1 day of age. Active immunization can be achieved by vaccination with viable attenuated reovirus that is usually applied by the subcutaneous route (107), although immunization by coarse-spray application of vaccine has also been used (20). Protection from subsequent challenge can be demonstrated, but the S1133-derived reovirus vaccines may interfere with Marek's disease vaccination if administered simultaneously (79, 86). The interference is most pronounced with herpes virus of turkeys (HVT) derived Marek's disease vaccines (73, 79). A reovirus vaccine derived from a naturally apathogenic strain of reovirus (2177) isolated in the United States (77, 78, 95) may be more suitable for simultaneous day-of-age administration with Marek's disease vaccine than are several S1133 derivatives (108). The vaccine should be used with caution if Marek's disease vaccine titers are low and/or Marek's disease virus challenge is significant. Reovirus vaccination of breeding stock can be done with viable or inactivated vaccines or combinations of both. The inactivated vaccines are more efficacious if preceded by vaccination with live vaccine (100, 114).

If a live vaccine is used, it should be administered prior to the onset of egg production to prevent transovarian transmission of the vaccine virus (19). The advantages of this type of immunization program include immediate protection of 1-day-old progeny provided by maternal antibody and a limitation of the potential for vertical transmission that has been shown to be economically significant (8). Vaccination of breeders is an efficacious method of controlling viral arthritis and other pathogenic reoviruses, but it should be recognized that protection is assured against homologous serotypes only (26, 72, 85). When the field virus is clearly different from that included in commercially available vaccines, an autogenous vaccine may prove effective (26, 85).

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Other Reovirus Infections
Richard C. Jones

INTRODUCTION

Reoviruses are recognized as a cause of tenosynovitis/viral arthritis in chickens (see preceding section, “Viral Arthritis”), but they have also been isolated from several other disease conditions in chickens and turkeys. From time to time, they have been recovered from a variety of other healthy or diseased avian species. In many cases, attempts to demonstrate that the reoviruses were the cause of the condition have been unsuccessful, or experimental work has not been done. Thus, their association with disease has not always been established. On other occasions, serological surveys have been conducted, which have shown the presence of antibodies to at least the avian group antigens in other avian species. In these cases, the role of reovirus infection is completely unknown.

Some strains isolated from birds other than chickens and turkeys, however, have been shown capable of causing pathological changes (mainly in the hock joints) in chickens, suggesting the possibility of cross-species transmission (26). The role of other avian species as carriers and reservoirs of infection for domestic poultry has never been established.

THE VIRUSES

In almost all cases, in which reoviruses have been isolated, cultivation has been achieved using methods described for the viral arthritis strains—namely, chick eggs inoculated via the yolk sac, or chick embryo fibroblasts, liver or kidney cells, or chicken kidney cells. Isolates usually have been identified by cultural characteristics and

typical reovirus morphology under the electron microscope. Few comparisons have been made of reoviruses from viral arthritis and those from other conditions of poultry or other avian species.

Rekik et al. (45) examined reoviruses isolated from 9 flocks of broiler chickens in Quebec. Serum neutralization tests showed the presence of types antigenically different from the vaccine (S1133) strain. They asserted that some reoviruses isolated from conditions other than viral arthritis could be antigenically different. Heffels-Redmann et al. (23) examined two reoviruses isolated from Muscovy ducks. Although the basic electrophoretic Mobility Patterns of immunoprecipitated polypeptides closely resembled those of chicken strains, considerable strain-specific variation was seen at the protein level. Based on cross-neutralization tests, the two duck strains were grouped in one serotype, with no cross-reactivity with the chicken serotype S1133.

Lozano et al. (31), using polyacrylamide gel electrophoresis, compared the genomic profiles of a total of 70 avian reovirus isolates, comprising 60 from turkeys, 8 from chickens (including strain S1133) and one each from a canary and a cockatiel. Greater heterogeneity of the migration pattern was seen among the turkey reoviruses as compared with the 8 chicken viruses, particularly in the S (small) genome fragments. A characteristic migration pattern according to species could not be determined because of the high polymorphism existing in the Mobility Patterns from chickens and turkeys. However, the canary and cockatiel viruses had strikingly similar migration patterns, which were different from the chicken and turkey viruses. The authors indicate the difficulty of assessing the significance of these differences, because these two viruses were processed separately, and virus isolation time was different.

Further detailed comparisons are needed between reovirus strains from different avian species.

Diseases in chickens

Reoviruses have been isolated from a wide range of disease conditions in commercial chickens other than tenosynovitis. They include respiratory disease, enteric disease, inclusion body hepatitis, hydropericardium, hepatitis in young chicks, generalized disease, blue wing disease, and the runting/malabsorption syndrome. At the same time, they can be isolated easily from the intestines of apparently healthy chickens. In addition to differences in tissue tropism between strains, a range of virulence exists, from high to virtually harmless. Several reports describe varying degrees of reduced weight gain due to reovirus infection, presumably indicating varying effects on function of the gastrointestinal tract.

A study by Robertson et al. (47) investigated the presence of reoviruses in healthy commercial chickens and in other flocks affected with the runting syndrome or tenosynovitis. The viruses could be isolated from almost all fecal samples from healthy flocks of 3 weeks of age or older, from several tissues of chicks aged 2 weeks or more with the runting syndrome, and from older birds with tenosynovitis. In addition, all broiler breeder flocks examined had antibodies to avian reovirus. The finding of widespread reovirus infection, apparently in the absence of disease, strongly suggests that isolation of reovirus from tissue specimens does not necessarily imply that they are causing disease.

Reports of reoviruses being associated with conditions in chickens other than joint disease include the following.

Respiratory Disease

The so-called Fahey-Crawley virus (15), whose identity was later confirmed as the first avian reovirus (44), caused a mild respiratory disease of baby chicks (54), but older chicks were resistant. Another respiratory isolate (UGA) was unable to cause respiratory disease alone, but in combination with a strain of Mycoplasma gallisepticum of low pathogenicity, respiratory signs and lesions were observed (53). However, reoviruses generally are not regarded as primary agents of respiratory disease in poultry.

Enteric Disease and Systemic Infections

Several descriptions exist of reovirus-associated enteric disease. An agent characterized as a reovirus was isolated from young chicks suffering from ulcerative enteritis by Krauss and Ueberschar (28), but it was not confirmed that this virus was the cause of the disease. Further early reports described enteric disease (11) and cloacal pasting and mortality (13) in young chicks.

A commercial farm with a history of poor feed conversion and chronic feed-passage problems was investigated (3). Abnormal tissue pathology was seen in broilers from 9 days of age. Avian adenoviruses and reoviruses were isolated, and although SPF chicks were inoculated with isolated reoviruses, their relationship to the initial problem was inconclusive.

In a recent study, adenoviruses and reoviruses isolated from commercial broiler chickens were tested for gastrointestinal pathogenicity in day-old chicks (29). Chicks in inoculated groups developed wet unformed fecal droppings, but although adenoviruses caused marked gizzard erosions, necrotizing pancreatitis, and proventriculitis, reoviruses effects were mild by comparison, including hyperplasia of lymphoid aggregates and mild gizzard erosions.

Some reports highlight the synergistic effect of reovirus in dual infection with other pathogens. For example, reovirus and Cryptosporidium baileyi produced a systemic infection (22), and Ruff and Rosenberger (51) showed that reoviruses can potentiate coccidial infection, although the outcome depends on the reovirus strain used.

Other reports record more generalized infections, with several organs affected. Four outbreaks of disease in broiler chickens in Victoria, Australia, were examined by Bagust and Westbury (4). Affected flocks range from 4–38 days. Sudden deaths and starvation were variously associated with hepatitis, ascites, hydropericardium, pale kid-
Reoviruses were isolated consistently from the tissues of the affected birds. Inoculation of these viruses into day-old SPF chicks intraperitoneally or orally induced sporadic deaths but no clinical syndromes. It was speculated that other factors may interact with the reoviruses to induce these problems.

Blue wing disease is a condition affecting broilers characterized by mortalities of 10%, subcutaneous and intramuscular hemorrhages, and atrophy of the thymus, spleen, and bursa. Engstrom et al. (14) showed that it was caused by a synergistic effect between chicken anemia virus and a reovirus. McNeilly et al. (34) also showed a synergistic effect between these two agents, so that dually infected animals had significantly lower weight gain and more severe damage in several tissues than chicks inoculated with either alone. However, the severity of effects depended on the strain of reovirus.

Avian reoviruses have also been shown to enhance the pathogenicity of other infectious agents of chickens such as Escherichia coli (49) and infectious bursal disease virus (37).

**Inclusion Body Hepatitis; Hepatitis in Young Chicks**

The liver of the chicken is considered to be one of the target organs for reovirus infection. McFerran et al. (33) isolated reoviruses and adenoviruses from outbreaks of inclusion body hepatitis. However, it is now recognized that adenoviruses rather than reoviruses play an important role in the pathogenesis of this disease. More recently, mortality in broiler chicks up to 10 days of age has been reported in Poland and attributed to reoviruses (Z. Minta, personal communication). In affected chicks, the prominent feature is acute hepatitis. The reovirus, which has a distinctive monoclonal antibody pattern, is able to reproduce the condition after inoculation of SPF chicks. A killed vaccine has been prepared for vaccination of parent flocks, to protect the progeny by maternal antibodies.

**Hydropericardium**

Bains et al. (5) described serious mortalities (10–18%) in broiler chicks in Queensland, which occurred in birds less than 14 days old. At necropsy, hydropericardium, with in some cases up to 3 ml fluid, was a consistent feature together with small spleens. Reoviruses were isolated from the hearts of these birds in fertile eggs or cell cultures, but there was no attempt to show that reoviruses were the cause of the condition. However, Jones (25) described a similar investigation but showed that intravenous inoculation of the isolated reovirus induced hydropericardium in experimentally infected chicks. The pathogenesis of this condition in relation to reoviruses has never been studied.

**The Runting-Stunting/Brittle Bone Disease Syndrome in Broilers**

A disease syndrome that first appeared in broilers in the late 1970s and chiefly is characterized by lowered body weights and variously described as the runting-stunting, pale bird, malabsorption, brittle bone, helicopter wing syndrome has been linked with several possible causative agents, including reoviruses (9, 17, 41, 43, 48, 56). However, several studies have suggested that reoviruses probably play a secondary role in these conditions rather than a primary one. In a recent report, Montgomery et al. (36) attempted to reproduce the syndrome using various infectious agents isolated from affected Mississippi broilers. These included an infectious bronchitis virus (IBV) isolate and a reovirus. Although IBV with the reovirus caused weight depression, it was concluded that none of the isolated agents was the ultimate cause.

So, although several reports indicate that isolated reoviruses are sometimes capable of causing varying degrees of enteritis (50), or simply reduced weight gain (50, 55), the consensus view appears to be that the most important pathogen is a small virus that can be seen in the enterocytes but which has escaped cultivation (36). Nonetheless, some commercial reovirus vaccines are produced, which are claimed to have beneficial effects against the stunting or malabsorption syndrome. The claims may indeed have some justification, but the vaccines are unlikely to protect against the primary causes.

**DISEASES IN TURKEYS**

**Tenosynovitis**

Reoviruses have been isolated from tenosynovitis in turkeys (30, 42), but the relationship of the viruses with this disease is unclear. Al-Afaleq and Jones (1) examined three chicken and three turkey reoviruses each isolated from hock joints. All viruses induced microscopic tenosynovitis lesions in chicks, but none produced them in turkey pouls. Even when reovirus was given together with Mycoplasma synoviae, only minimal joint lesions were induced in experimentally infected turkeys (2).

**Enteric Disease**

Reoviruses have been isolated from the intestines of normal turkeys and turkeys with enteric disorders (10, 16, 39, 40, 52). When such strains have been tested in vivo, effects have been variable: Some have been found to be pathogenic, and others nonpathogenic or of low pathogenicity. Dees et al. (10) compared different isolates and found that although strain BC-7 was nonpathogenic, BC-3 induced enteritis, involving destruction of the intestinal villi. Goodwin et al. (18), using a brilliant red powder in the diet, found that gastrointestinal transit time in reovirus-infected turkeys was significantly longer than in normal turkeys.

**REOVIRUSES IN DUCKS AND GEESE**

Several early reports describe the isolation of reoviruses from different species of ducks, including mallards (32), healthy Pekin ducks (26), and diseased ornamental ducks (19). All shared a common group antigen with chicken
Reoviruses, but their relationship with disease in ducks was not determined. However, the strains from Pekin ducks (26) were able to cause microscopic lesions of tenosynovitis in specific-pathogen-free chicks.

Subsequent evidence of pathogenicity of reoviruses for ducks has been forthcoming. Reoviruses were isolated from a disease of Muscovy ducks with 30% morbidity and 20% mortality by Malkinson et al. (35). At autopsy, necrotic foci were in the liver, spleen, and kidneys. Intramuscular inoculation of the reovirus caused mortality without clinical signs within 2 days and necrotic foci in the liver and spleen.

Heffels-Redmann et al. (23) considered that the two duck strains they examined were antigenically distinct from the standard chicken strains.

The isolation of reoviruses from geese with Derzsy’s disease has been reported (7), but their role is unknown as the condition is now known to be caused by infection with a parovirus.

Serological evidence of reovirus infection in geese has been recorded in two reports. Kaleta et al. (27) detected neutralizing antibodies to a virus originating from Muscovy ducks and the standard chicken strain S1133 in sera of domestic geese (Anser anser domesticus). Hlinak et al. (24) examined sera from bean geese (Anser fabalis) and white-fronted geese (Anser albifrons) in Germany. Avian reovirus antibodies were detected in 29% of blood samples, and there was no difference in seroprevalence between the two species. The authors indicate that although the role and significance of wild geese in the epidemiology of avian diseases remains to be determined, it is possible that they could be of some importance as reservoirs and carriers of some diseases of domestic poultry.

**REOVIRUSES IN OTHER AVIAN SPECIES**

McFerran et al. (32) isolated reoviruses from pigeons and Gough et al. (19) from diseased pigeons, pheasants, parrots, and other exotic avian species. Jones and Guneratne (26) isolated a reovirus from the feces of a zoo wedge-tailed eagle (Aquila andax). This virus caused microscopic lesions of tenosynovitis in SPF chicks. All these reoviruses shared a common group antigen with chicken reoviruses, but their importance as pathogens in the host species was not determined.

Graham (20) isolated a reovirus from the liver of an African grey parrot submitted for necropsy with subcutaneous hemorrhages, multiple foci, and necrosis in the liver, spleen, bone marrow, intestinal lamina propria, airsacculitis, and epicarditis. Experimental inoculation of two African grey parrots with the isolate was fatal and reproduced the hemorrhages and necrotic lesions of the original condition.

A virus associated with mortalities in American woodcock (Scolopax minor) was identified as a reovirus (12). A consistent necropsy finding was emaciation of the carcass. The authors considered that the reovirus infection was systemic and to be responsible for the deterioration in bodily condition of the birds. Transmission was thought to be by the fecal-oral route, but again, the true association with the disease was not confirmed.

An enteric disease in bobwhite quail (Colinus virginianus), which resulted in increased mortality in birds from 5 days to 5 weeks was described by Ritter et al. (46). A reovirus was isolated from the feces, and intestinal cryptosporidia were also present. In attempts to reproduce the condition experimentally, the reovirus induced subclinical infection, but the cryptosporidium caused changes resembling the natural disease. Infection of quail with both agents produced systemic infection (21).

An outbreak of disease in pheasants in Turkey attributed to reovirus infection was described by Mutlu et al. (38). Twenty-seven of a flock of 100 were affected between 3–5 months of age. In addition to being in poor condition, affected birds were short of breath, had greenish diarrhea, and died within a week. Pathological findings comprised fibrinous tracheitis, catarhal inflammation of the gut, severe hepatic necrosis, and fibrinous pericarditis. A reovirus was isolated from several organs, but whether this was the only agent involved was not investigated.

Curtis et al. (8) reported tenosynovitis in 6–7-week-old pheasants. Staphylococcus aureus and an avian reovirus related antigenically to strain S1133 were isolated from the swollen hock joints of lame birds. The association between the reoviruses as a cause of the disease was presumed but not confirmed.

Antibodies to avian reoviruses (and to other poultry pathogens) were detected by ELISAs in ostriches (Struthio camelus) from Zimbabwe (6). Again, the significance of this finding is unknown.

**CONCLUSIONS**

Reoviruses are very common among domestic poultry and other avian species. They are viruses that are relatively easy to cultivate, and when they are looked for, serum antibodies are often found, so there is a temptation to implicate them as a cause of several conditions from which they have been isolated. Apart from tenosynovitis in chickens, where a clear relationship occurs between reovirus infection and the clinical disease, the role of reoviruses in avian disease is frequently unclear. In exotic birds, reports of reoviruses have been sporadic. There appears to be a wide range of pathogenicity among isolates, but most are probably harmless.

There may be differences in tissue tropism, although all appear to replicate in the gut, and pathogenic strains affect the liver. In most cases, the serologic or molecular relationship of reoviruses from exotic species to the tenosynovitis strains is unknown. Where exotic strains have been tested in chickens, a predilection exists for the hock joints or tendons, suggesting the potential for cross-species infection. However, exotic species have never been proven to be reservoirs of infection for domestic poultry.

Because of the inconsistency of disease associated with reoviruses in species other than the chicken, vaccines have not been developed.
REFERENCES


