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The History of Avian Reovirus

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In 1957, while studying the pathogenesis of *Mycoplasma synoviae,* Dr. Norman Olson *et al.* (22), at West Virginia University, reported the isolation of an agent producing synovitis from lesions in broilers that exhibited a lack of sensitivity to chlortetracycline and furazolidone. Olson reported in 1959 (23) that this particular agent was not susceptible to streptomycin either, and Kerr and Olson (13) also observed that the newly found synovitis agent was pathogenic only in young chicks, whereas such age resistance was uncommon for *M. synoviae* infections. Subsequently, Olson and his coworkers (25) determined that the synovitis agent was a virus, which they called "viral arthritis agent." It was first misdiagnosed as a poxvirus (25) because of its double-stranded nucleic acid. However, the virus was subsequently identified by Walker *et al.* (39) as a reovirus by electron microscopy.

There was no doubt that this reovirus was capable of causing arthritis and tenosynovitis after experimental infection of chickens. Soon, other isolations of reovirus were reported from various places in the United States and beyond from chickens with tenosynovitis lesions.

In 1972, typical tenosynovitis histologic lesions were induced in the metatarsal flexor tendons of chickens by experimental inoculation of a reovirus isolate by Olson and Weiss (26). This virus, "Fahey–Crawley virus," was first isolated by Fahey and Crawley in 1954 (6) from chickens with chronic respiratory disease.

It was determined that the lesions induced by Fahey–Crawley virus were virtually the same as those described by Kerr and Olson in 1969 (14) when they inoculated their "viral arthritis agent" into chickens. Kerr and Olson also described, in addition to the lesions in the tendon sheaths, another lesion that developed in the synovial membrane of the joint, resulting in a pannus formation, very similar to the lesion of rheumatoid arthritis in humans. Olson had already mentioned such a similarity in 1959 (23).

Avian reovirus was not exclusively associated with viral arthritis/tenosynovitis. It had also been found several times in association with enteric diseases of chickens as early as 1966 by Krauss and Ueberschär (18) and with blue comb disease in turkeys in 1969 by Deshmukh *et al.* (3), Wooley and Gratzek (41), and by Fujisaki *et al.* (7).

Several researchers found that reovirus also caused myocarditis and hepatitis in chickens upon experimental infection (19,28,32). Reovirus isolations from chickens with arthritis/tenosynovitis and/or enteric lesions were reported from several countries (2,17,30). In addition to established reovirus isolates, such as WVU 2937 from Olson *et al.* (24,26), Reo 25 from Deshmukh and Pomeroy (3), and UMI 203 from Johnson (11), a reovirus was isolated from chickens with tenosynovitis by van der Heide *et al.* (33,35) that was named after its diagnostic accession number, S1133, at the University of Connecticut.

Kawamura and Tsubahara (12) reported five different serotypes, Sahu and Olson (31) reported four, and Deshmukh *et al.* (5) reported two. This author's investigations indicated that all isolates from the United States were serologically related, although not all were fully neutralized by S1133 antiserum. However, in Europe and Israel, some true variant serotypes of reovirus were found that were not neutralized at all by S1133 antiserum.

EGG TRANSMISSION AND MATERNAL ANTIBODY PROTECTION

Evidence of egg transmission was first demonstrated under controlled conditions by Deshmukh and Pomeroy (4). A very good serologic comparison of the various isolates from the United States, Japan, and Europe was made by Wood *et al.* (40). Egg transmission was reported as a distinct possibility by Glass *et al.* (8). Egg transmission of reovirus was also demon-

strated by Menendez *et al.* (21) and by van der Heide and Kalbac (35) in 1975.

The role of maternal antibodies to protect progeny of broiler breeders was studied as early as 1975. An inactivated vaccine made from the Connecticut isolate, S1133, in Italy by Cessi and Lombardini (1) was used in breeders to try to protect them during the growout period and to induce protective antibodies for their progeny. Unfortunately, inactivated reovirus vaccines did not appear to result in a high antibody response. Consequently, it was felt that a live reovirus vaccine needed to be developed.

Toward that end, early experiments were performed in broiler breeders with the 73rd chicken embryo passage of the Connecticut S1133 strain (37). In 1976, an initial batch of this vaccine was given by this author to a broiler integrator company with high incidence of tenosynovitis in their broilers. One broiler breeder parent flock was vaccinated at 12 wk of age in the drinking water (with approximately 10^5 mean embryo infectious dose per chicken.) The integrator was advised to vaccinate after 10 wk of age but before 18 wk because the vaccine virus was known to be pathogenic for young chicks and also to be potentially transmitted vertically. Broilers were hatched from this vaccinated flock as well as from nonvaccinated parent flocks, and the incidence of tenosynovitis in the broilers was compared. After 6 mo of eerie silence, the company veterinarian reported that the broilers from the vaccinated breeders were doing well, evidencing no signs of tenosynovitis, whereas the broilers from unvaccinated breeders did have tenosynovitis.

Most test vaccinations were performed in broiler breeders to determine if their progeny could be protected by maternal antibodies. In 1977, Caswell Edison of the University of Georgia conducted field trials involving over 400,000 breeders between the ages of 10 and 17 wk. He found higher tenosynovitis vaccine Ab titers in vaccinates than in nonvaccinated control chickens. The lower condemnation rates for the progeny of the vaccinates further demonstrated the efficacy of the vaccine. In addition, laboratory challenge data generated by Hiram Lasher of Sterwin Laboratories after obtaining the attenuated vaccine from the author also attested to its efficacy. Sterwin proceeded to produce and federally license the first avian tenosynovitis vaccine in 1978. The vaccination of broiler breeders during their growout also had the advantage of preventing such breeders from getting infected with reovirus during production with resulting egg transmission of the virus. Such egg transmission had been shown to result in reduced hatchability due to embryonic death and a high incidence of early tenosynovitis in progeny broilers, as early as 10 days of age. One field experience with experimental broiler breeder vaccination was conducted with Dr. Ken Page in 1980 (38), and it clearly gave evidence of the protective effect of the reovirus vaccine.

However, one challenge that still remained was to be able to protect very young breeders against tenosynovitis. The existing 73rd embryo passage S1133 vaccine was too pathogenic for young chickens. Furthermore, the potential broilers could not be protected against malabsorption syndrome by vaccination with this passage level at day of age for the same reason (see malabsorption syndrome). Therefore, the need for a highly attenuated S1133 vaccine strain became apparent.

To that end, the 73rd embryo passage S1133 reovirus was passed in embryonated specific-pathogen-free chicken eggs (SPAFAS, Inc., Norwich, CT) by chorioallantoic membrane inoculation for a total of 235 passages and then subsequently passed 65 times in chicken embryo fibroblast cell cultures maintained at 32 C in order to promote the development of a temperature-sensitive mutant.

A temperature-sensitive mutant emerged from this treatment (8,31). After an additional 35 chicken embryo fibroblast passages at 37 C, an apathogenic tenosynovitis vaccine was finally obtained for use in day-old chickens (30). The vaccine seed was distributed to several vaccine companies, and the resultant vaccines were used successfully for 17 years since.

After the observation that the reovirus vaccine interfered with Marek's disease vaccination in day-old chicks (26), resulting in increased incidence of Marek's disease condemnation in broilers in areas with high Marek's disease challenge, it was generally advised to vaccinate broiler breeders at 7 days instead of 1 day with the attenuated reovirus vaccine. The initial vaccination was then followed by a second live vaccine administered at 5–6 wk. Then, at 10 or 20 wk of age, or both, inactivated virus vaccinations were given. It should be noted that, de-

spite the safety of the highly attenuated vaccine, reovirus vaccination was carried out only when absolutely necessary, such as in the case where reovirus antibody levels in the breeders were insufficient to passively protect the broiler progeny.

MALABSORPTION SYNDROME

The so-called "malabsorption syndrome" in broilers, first observed in the late 1970s, was originally thought to be caused by reovirus on the basis of reovirus isolations from broilers with clinical disease (34). However, attempts to reproduce the complete syndrome with reovirus did not always succeed. Several investigators also isolated other viruses, such as parvovirus (15), enterovirus (20), and calicivirus (42), as well as certain bacteria (16), from cases of malabsorption syndrome. The cautious conclusion drawn was that malabsorption syndrome possibly had a multi-factorial etiology. On the other hand, two reovirus isolates, 1733 and 2408, isolated by Rosenberger at the University of Delaware, were shown to individually induce malabsorption syndrome. These strains are now contained in some commercial inactivated vaccines. Field trials with broiler reovirus vaccination resulted in improved broiler performance in about 50% of the cases, whereas in the remainder, no difference was observed in feed conversion rates between vaccinated and unvaccinated flocks. In addition, the clinical signs of malabsorption syndrome did not disappear. In those cases, it was concluded that another etiologic factor or factors must be involved.

Hieronymus, Villegas, and Kleven (10) isolated a reovirus from chickens with malabsorption and it was designated CO_8. An inactivated CO_8 vaccine was produced and licensed by Merieux Laboratories under the guidance of Dr. Daniel Gaudry. Extensive field trials were carried out with the CO_8 vaccine by Dr. Caswell Eidson, University of Georgia. However, although the reovirus isolates from chickens with malabsorption syndrome had a different biotype than the standard tenosynovitis reovirus isolates, they all appeared to belong to the same serotype. In an inactivated form, they were therefore no different from the standard reovirus strain. As mentioned earlier, in the United States all reovirus isolates are serologically closely related or identical to the standard S1133 serotype. That was not the case in Europe and Israel, where variant reovirus isolates were found that were not neutralized by S1133 antiserum. In those cases, autogenous reovirus vaccines were produced and used successfully to prevent infections.

CONCLUSION

Avian reovirus has definitely been determined to be the etiologic agent of viral arthritis/tenosynovitis. Reovirus vaccination of breeders offers the benefits of protecting the vaccinates and their progeny against viral arthritis/tenosynovitis. The etiology of malabsorption syndrome is more complex, with reovirus vaccination efforts yielding variable results.

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