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The Commercial Production of Specific-Pathogen-Free Eggs and Chickens: The Evolution of an Industry

R. E. Luginbuhl

Retired President, SPAFAS, Inc.
Professor Emeritus, Department of Pathobiology, University of Connecticut, Storrs, CT 06269

Abbreviations: COFAL = complement fixation test for avian leukosis; FAPP = filtered air, positive pressure; MD = Marek's disease; MG = *Mycoplasma gallisepticum*; REV = reticuloendotheliosis virus; RIF = resistance-inducing factor; SPF = specific-pathogen free

Chicken embryos were first used to study viruses in 1911 by Rous and Murphy (21). However, years elapsed before the use of the embryo was recognized for its great potential in virus research when Woodruff and Goodpasture (34) used chicken embryos to study fowl poxvirus. The early workers did not consider that chicken embryos could carry unwanted organisms, which could compromise the integrity of experiments. In 1946, Beveridge and Burnet (4) stated, "There is no well authenticated report of a chick embryo being a natural carrier of any virus, and the technical problem of preventing entry of unwanted viruses to it is immensely simpler than with any type of free-living animal."

The first infectious organism reported to be naturally transmitted from the hen into the embryo was *Salmonella pullorum*. Data accumulated quickly, and by 1952, Cottral (9), in a review on endogenous viruses in the egg, indicated that there were nine diseases of birds, namely avian lymphomatosis, avian encephalomyelitis, Newcastle disease, infectious sinusitis, psittacosis, pullorum disease, avian tuberculosis, fowl typhoid, and paratyphoid infections, in which egg transmission may occasionally take place. The list continued to expand. For example, in 1966, latent adenolike virus was isolated from chicken kidney cell cultures (6), and avian reovirus was found to be an egg-transmitted virus in 1975 (18,29).

Transmission of various infectious disease organisms from hen to progeny through the embryo was a major concern for commercial poultry producers, who had been attempting to deal with (*S. pullorum*) infections since early in the 20th century. Vertical transmission of infectious agents became a major focus for research workers as well.

Early production of poultry vaccines was also complicated by embryo-transmitted organisms that occasionally contaminated vaccines, often with dire consequences to both producer and manufacturer. Undoubtedly, some cases of vaccine contamination were never recognized, and others were recognized only years later. Yellow fever vaccines administered to many military personnel in the 1940s were produced in chicken embryos. Samples of this vaccine, when tested later, were found to be heavily contaminated with avian leukosis virus.

By 1960, it was apparent that progress in poultry disease research and the production of quality biological products in chicken tissues depended on an adequate supply of specific-pathogen-free (SPF) eggs and chickens. This need was probably recognized by many, a few of whom also recognized the commercial potential of this idea. Before commercialization could become practical, it was also necessary to have enough knowledge about specific organisms, their modes of transmission, and reliable and cheap tests to detect their presence. It was also necessary to develop housing and management procedures sufficient to protect infection-free flocks from adventitious introduction of organisms. Finally, it was necessary to have a transportation industry that could ensure prompt delivery of a perishable and fragile product to customers.

BEFORE COMMERCIALIZATION

In the 30-yr period beginning in 1940, there was an explosion of knowledge about the cause and transmission of poultry diseases. One by one, important diseases were recognized, the causative organism identified, the mechanism of transmission revealed, and convenient labo-

Table 1. Quality control form for SPF eggs.[A]

Adenoviruses Group I—Celo Type 1
Adenoviruses Group 1
Adenovirus Group II (Hev)
Avian Adenovirus Group III (EDS)
Avian Encephalomyelitis
Avian Influenza (Type A)
Avian Nephritis Virus
Avian Paramyxovirus Type 2
Avian Paramyxovirus Type 3
Avian Reovirus
Avian Rhino Tracheitis Virus
Avian Rotavirus
Avian Tuberculosis
Endogenous GS Antigen
Fowl Pox
Hemophilus Paragallinarum
Inf. Bronchitis Ark
Inf. Bronchitis Conn.
Inf. Bronchitis JMK
Inf. Bronchitis Mass.
Infectious Bursal Disease Virus
Infectious Laryngotracheitis Virus
Lymphoid Leukosis A,B
Lymphoid Leukosis Viruses
Marek's Disease (Serotypes 1,2,3)
Mycoplasma Gallisepticum
Mycoplasma Synoviae
Newcastle Disease Virus
Reticuloendotheliosis Virus
S. Pullorum-Gallinarum
Salmonella Species

[A]SPAFAS, Inc., Norwich, CT.

ratory assay systems developed. The embryo-transmitted diseases were especially relevant to the development of SPF flocks because one first needed to break the cycle of transmission or identify uninfected parent flocks in order to derive pathogen-free chicks. However, even those infectious diseases not known to be transmitted vertically were of interest because SPF flocks needed also to be free of antibodies to as many diseases as possible.

The list of diseases relevant to the development of SPF flocks is too extensive to be discussed in detail. Including the original eight diseases tested in 1965, avian leukosis, Newcastle disease, infectious bronchitis, infectious laryngotracheitis, adenovirus (Phelps), avian encephalomyelitis, *Mycoplasma gallisepticum* (MG), and *S. pullorum,* SPF flocks are currently tested for 31 diseases (Table 1).

A. D. Goldhaft, in his book *The Golden Egg* (12), stated that he used SPF chickens to make laryngotracheitis vaccine in the early 1930s. He also stated that by 1938 his laboratory (later called Vineland) had a SPF flock producing hatching eggs for propagating vaccine viruses. The method utilizing SPF eggs was included in a production protocol submitted to the USDA emphasizing that the eggs for vaccine production would come from their own SPF flock.

In 1939, the USDA, with the help of the poultry industry and agricultural experiment stations, established the Regional Poultry Research Laboratory in East Lansing, MI. The mission of the laboratory was to work on tumor diseases of chickens. Anticipating that these diseases might be transmissible, the laboratory was equipped with what was then considered state-of-the-art biocontainment. Elaborate quarantine procedures were put in place involving showers and clothes changes for the poultry workers, all done in an effort to establish or maintain disease-free chickens. Ultimately, this goal was achieved but not until more knowledge about the specific diseases was acquired.

S. B. Hitchner and R. Winterfield were producing poultry vaccines in American Scientific Laboratories in the 1950s and were concerned about the egg supply for virus propagation. Hitchner convinced his family to buy a farm near Madison, WI, for the production of chicken embryos to be used by American Scientific Laboratories. An isolated leghorn breeder whose birds were free of MG was selected as the source of this special flock. This flock then provided MG- and *S. pullorum*-free embryos to the laboratory until 1960. In 1961, American Scientific Laboratories began buying eggs from the SPF flock at the University of Connecticut to establish its own breeding flock for an egg supply (Hitchner, pers. comm.).

The original motivation for the University of Connecticut's Department of Animal Diseases to establish a SPF flock had come from a study conducted in 1955. While attempting to grow avian encephalomyelitis virus in embryonating eggs, researchers found that parental antibodies contained in the egg yolk prevented the growth of the virus in embryo tissues (27). So researchers in the department procured hatching eggs from Mount Hope leghorns at the University of Massachusetts. These chickens had already tested free of MG.

For 5 yr, eggs were hatched, chicks housed on an isolated farm in conventional poultry houses were periodically bled, and the serum was tested for antibodies to Newcastle disease, infectious bronchitis, avian encephalomyelitis, avian adenovirus (Phelps), MG, and *S. pullorum.* No evidence of fowl pox or infectious laryngotracheitis was observed. Specific interest in the leukosis complex began in 1963. The birds remained free of antibodies to the above-mentioned diseases (17).

Avian leukosis virus posed some special problems because it was not only transmitted through the embryo but was impossible to detect early in life. The resistance-inducing-factor (RIF) test developed by Rubin *et al.* (22) provided a convenient tool. Walter Hughes teamed with Rubin and developed for Kimber farms a flock free of infection with avian leukosis virus—a landmark achievement in the leukosis field and a technology later adapted by commercial SPF flocks producers. During the early 1960s, Bang and Foard (2,3), working with Rous virus, recognized the importance of establishing a flock free of antibodies to this disease in order to continue meaningful research with this virus.

Houghton Poultry Research Station in Houghton, England, was very active in poultry disease research, and in 1969, P. M. Biggs published a paper (5) describing methods for the establishment of flocks of chickens free from leukosis and Marek's disease (MD). These procedures were used by those attempting to produce SPF embryos commercially.

The Regional Poultry Disease Research Laboratory of the USDA in East Lansing, MI, was also very active in poultry disease research and recognized the need for SPF embryos. They established their own flock and cooperated very willingly with those attempting to produce SPF embryos commercially.

THE EARLY COMPANIES

In 1950, William Lasher started to produce SPF eggs for his brother Hiram, who was producing poultry vaccines in the Delaware Poultry Laboratories. The Lasher family farm is isolated in the Catskill Mountains of New York State and continues to operate as Sunrise Farms, Inc. Sunrise Farms, Inc. is a major contributor to the production of SPF eggs and has steadily increased production facilities to meet the needs of the industry (16).

It was in 1960 that Ray Davis, a Connecticut hatcheryman who had been hatching chicks used by farmers to produce diethylstilbestrol-inoculated chickens for the New York kosher market, came to the Department of Animal Diseases looking for an alternative use of his facilities because his business ended when this procedure was no longer allowed. During discussions, it was suggested that there may be a need for the commercial production of SPF embryos for poultry vaccine production and virus research. Davis expressed an interest in the project and was willing to finance it if this author would provide the professional help necessary. Permission to work with Ray Davis was obtained from the university, and eggs from the SPF flock were sold to SPAFAS, Inc. to establish the first SPAFAS, Inc. commercial flock. This flock was housed in a conventional poultry house and maintained under maximum-security management and without vaccination. Periodic tests on serum samples revealed the flock negative for Newcastle disease, infectious bronchitis, adenovirus (Phelps), MG, and *S. pullorum.* The flock did not have any sign of fowl pox or laryngotracheitis; however, it did develop antibodies for avian encephalomyelitis.

No other chicken was introduced to the line during these early years, and flocks were reproduced from the original line. By 1962, some of the chickens were trapnested and tested for the RIF of lymphoid leukosis (15,22).

Once it was demonstrated that poultry could be managed and monitored for the known poultry diseases and kept free of these diseases, the commercial producers of poultry vaccines became very interested in the product, especially after one company had produced a contaminated vaccine when using embryos from a nonmonitored breeder flock. The first SPF eggs from SPAFAS, Inc. were sold in 1961.

It was in 1965 that Lohmann & Co., Cuxhaven, Germany, started their SPF program under a license from SPAFAS, Inc. Groups of 40–800 chickens were housed in small units of conventional design away from commercial poultry. These chickens became serologically positive for infectious bronchitis and avian encephalomyelitis but remained negative for the other pathogens. In 1968, Lohmann moved their operation into filtered-air, positive-pres-

sure (FAPP) facilities. The monitoring of the Lohmann flocks was conducted on the SPAFAS principle with blood samples being drawn at regular intervals. The sera were tested in their own laboratory as well as different university laboratories in Germany (32,33). In 1973, Lohmann decided to convert their SPF houses to cages. Problems developed with nervousness, which was eventually remedied by adjusting the positive pressure to the lowest possible amount (31). Control of the infectious diseases, except for an occasional positive serology for reticuloendotheliosis virus (REV), had been accomplished. During this period of development, another line of white leghorns was introduced into their SPF program. Lohmann produced SPF eggs under license to SPAFAS until 1976, when SPAFAS was sold to Hubbard Farms, Walpole, NH, a subsidiary of Merck Inc., Rahway, NJ. Lohmann continues to produce SPF eggs in Germany under the name of VALO (Vielitz, pers. comm.).

It was in the late 1960s that Larson Lab Eggs was started in Iowa with bloodlines from Hy-Line International for their SPF flock. Their operation continued to grow, and they supplied SPF eggs until their merger with Hy-Vac (Chase, pers. comm.).

Intervet International in Boxmeer, The Netherlands, established their own SPF flocks in 1968 with their own breeding nucleus. These flocks are free of the known pathogens including lymphoid leukosis (Hein, pers. comm.).

In 1973, Wickham Laboratories in the United Kingdom set up a SPF flock under the management of Pat Gibbings. Some of the breeding stock for this operation came from the Intervet flock in Boxmeer.

In 1986, Hy-Vac (Lohmann, USA) started a SPF project in Iowa under the direction of Bill Chase with Hy-Line breeding stock. In 1988, Hy-Vac and Larson Lab Eggs joined forces and Russ Larson became the manager (Chase, pers. comm.).

In the 1980s, Solvay Laboratories, Charles City, IA, and Select Laboratories, Gainesville, GA, two primary producers of poultry biologics, decided to establish their own SPF flocks as insurance for the supply of SPF embryos.

By 1977, SPF flocks were also monitored for adenovirus group 1, types 1–11. By 1980, the antigens for hemorrhagic enteritis virus (adenovirus group II) and EDS 76 (adenovirus group III) were routinely included in the SPF monitoring program as well as a specific test for *Hemophilus gallinarum* and REV. By 1981, SPF flocks were being monitored for 20 antigens or antibodies.

In 1983, a spurt in international production of SPF embryos occurred, with units being established by Rezende in Brazil, Alpes in Mexico, and Western Hatcheries in India. All of these units were developed on the principles used in the United States and under the guidance of SPAFAS, Inc.

In 1988, a SPF poultry production facility was opened in the College of Veterinary Medicine at Cornell University, which was substantially funded by a gift from Dr. Hiram Lasher.

EVOLUTION OF TESTING PROCEDURES

By 1965, SPAFAS, Inc. had established its own laboratory for monitoring all its flocks for Newcastle disease, infectious bronchitis, infectious laryngotracheitis, adeno (Phelps), avian encephalomyelitis virus, MG, and *S. pullorum* at 3-mo intervals. A quality control sheet for the flock supplying the eggs was furnished to the customer. This was the first year embryos from avian leukosis virus–negative stock, as determined by the RIF test (22), was offered to the industry. In 1966, SPAFAS, Inc. started using the complement fixation test for avian leukosis (COFAL) (24) on birds that had been leukosis free by the RIF test for four generations.

The COFAL provided another major step toward the production of flocks free of the infectious RNA virus, which causes the clinical disease, because it detected the group-specific antigen for the virus. Once a flock was established as free of the lymphoid leukosis virus, the enzyme-linked immunosorbent assay test (7,26) was used to test for antibodies during the flock-monitoring program.

In 1968, SPAFAS, Inc. had SPF birds in production on a new farm in Roanoke, IL. MD control and testing in SPAFAS, Inc. birds was started in 1968. In 1970, embryos from MD-negative stock were offered to the industry. Since 1968, the chickens have been housed in FAPP (11,19) houses and tested by the agar-gel precipitin procedure for antibodies to MD virus. For the initiation of FAPP housing, credit

should be given to Dr. Charles Beard and others at the USDA-ARS laboratory (Southeast Regional Poultry Laboratory) in Athens, GA, for their creativity in developing this system for poultry houses (11,19).

By 1970, SPF flocks were being monitored for lymphoid leukosis viruses (types A and B), MD, Newcastle disease, adenoviruses, fowl pox, avian encephalomyelitis, infectious laryngotracheitis, MG, *Mycoplasma synoviae,* and *Salmonella pullorum-gallinarum.*

In the early years of SPF production, the birds were tested for antibodies just before the start of egg production and then at 3-mo intervals. By 1972, 5% of the flock was bled at monthly intervals in addition to the testing of 100% at the beginning of production. In 1984, some SPF flocks were also monitored weekly for avian encephalomyelitis, infectious bursal disease, reovirus, MD, adenovirus, and lymphoid leukosis virus in an effort to detect a change in disease status as soon as possible.

The USDA, after consulting with the biologics producers, sent a memo to all producers of live virus chicken embryo–produced vaccines suggesting that they use embryos from SPF flocks and included the procedure in their production protocol. It is now recognized in the United States as USDA Veterinary Biologics Memorandum 800.65 (30), and it is also included in the European Pharmacopoeia regulations (20). These regulations have contributed to the common practice today of using SPF eggs for the production of live poultry and human vaccines and other pharmaceutical products.

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