AVIAN DISEASES 54:1136–1143, 2010

Historical Article—

Early History of Regulatory Requirements for Poultry Biologics in the United States

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Received 1 June 2010; Accepted and published ahead of print 10 August 2010

SUMMARY. Congress passed the Virus-Serum-Toxin Act in 1913, giving the U.S. Department of Agriculture (USDA) authority to prevent the importation or interstate shipment of worthless, contaminated, dangerous, or harmful veterinary biological products. The passage of this act marked the beginning of regulatory requirements for veterinary biological products in the United States. In 1913, only a few biologics establishments produced products for the poultry industry. The first license issued by the USDA for a poultry product was in 1918 to the University of California, Berkeley, for fowlpox vaccine. The list of biological products for poultry grew slowly in the 1920s. However, this began to change with the licensing of laryngotracheitis vaccine in 1933; pigeonpox vaccine in 1939; several Newcastle disease vaccines (inactivated in 1946, Roakin strain in 1948, B1 strain in 1950, and La Sota strain in 1952); and the first bronchitis vaccine in 1953. With the development of these and other new products, the biologics industry began to move its emphasis on hog cholera serum and virus to one based on the production of numerous new vaccines and bacterial products. The USDA's approach to the regulation of biologics in the early 1950s was still geared to the production of hog cholera products; however, as a result of the intervention of a group of dedicated poultry scientists, who were concerned about the poor performance of Newcastle disease vaccines, this soon changed. This presentation describes the initiation and development of modern standards for poultry biologics that occurred as a result of this intervention. The development and improvement of standards and regulatory requirements to address mycoplasma, leukosis, and other extraneous virus contaminations in chicken embryo origin products are reviewed. The licensing of products to meet new and emerging disease problems in the poultry industry and the close interaction among research scientists, poultry industry, biologics manufacturers, and government regulatory officials that has been needed to ensure the availability of products that meet appropriate standards of purity, safety, potency, and efficacy are also addressed.

RESUMEN. *Reseña Histórica*—Historia inicial de los requisitos reglamentarios para los biológicos avícolas en los Estados Unidos.

El Congreso aprobó la ley denominada Virus-Serum-Toxin Act en el año 1913, otorgándole al Departamento de Agricultura de los Estados Unidos. (con las siglas en inglés USDA) la autoridad para impedir la importación o el envío interestatal de productos biológicos veterinarios, sin utilidad, contaminados, peligrosos o nocivos. La aprobación de esta ley marcó el inicio de los requisitos reglamentarios para los productos biológicos veterinarios en los Estados Unidos. En el año 1913, sólo unos pocos establecimientos producían productos biológicos para la industria avícola. La primera licencia expedida por el Departamento de Agricultura para un producto avícola ocurrió en 1918, otorgada a la Universidad de California, Berkeley, para la vacuna de la viruela aviar. La lista de productos biológicos para avicultura creció lentamente en la década de 1920s. Sin embargo, esto comenzó a cambiar con la concesión de la licencia para la vacuna contra la laringotraqueítis en 1933, para la vacuna con el virus de la viruela de palomas en 1939; varias vacunas contra la enfermedad de Newcastle (inactivada en 1946, cepa Roakin en 1948, cepa B1 en 1950, y la cepa La Sota en 1952), y la primera vacuna contra la bronquitis infecciosa en 1953. Con el desarrollo de estos y otros nuevos productos, la industria de productos biológicos comenzó a mover su énfasis del suero y virus de la peste porcina clásica a la producción de numerosas vacunas y productos bacterianos nuevos. El enfoque del Departamento de Agricultura para la regulación de productos biológicos en la década de 1950 todavía estaba destinado a la producción de productos contra la peste porcina clásica, sin embargo, como resultado de la intervención de un grupo de dedicados científicos avícolas, que estaban preocupados por el pobre desempeño de las vacunas contra la enfermedad de Newcastle, esto pronto cambió. Esta presentación describe el inicio y el desarrollo de las normas modernas para los biológicos avícolas que ocurrieron como resultado de esta intervención. El desarrollo y la mejora de las normas y los requisitos reglamentarios para hacer frente a los micoplasmas, a la leucosis y a otros de virus extraños contaminantes de los productos con origen en embrión de pollo son revisados. La concesión de licencias de productos para satisfacer los nuevos problemas emergentes de enfermedades en la industria avícola y la estrecha interacción entre los científicos, la industria avícola, los fabricantes de productos biológicos y los funcionarios gubernamentales de reglamentación que ha sido necesaria para garantizar la disponibilidad de los productos que cumplen las normas pertinentes de pureza, seguridad, potencia y eficacia también se abordan.

Key words: poultry vaccine history, regulatory requirements, poultry standards, history

Abbreviations: AHI = Animal Health Institute; ARS = Agricultural Research Service; AVMA = American Veterinary Medical Association; BAI = Bureau of Animal Industry; BPS = Biological Products Section; CEO = chicken embryo origin; COFAL = Complement Fixation Test for Avian Leukosis; CVB = Center for Veterinary Biologics; MD = Marek's disease; pfu = plaque-forming units; PPLO = pleuropneumonia-like organisms; RIF = resistance inducing factor; SAMS = Standard Assay Methods; SPF = specific-pathogen-free; USDA = U.S. Department of Agriculture; VBD = Veterinary Biologics Division; VBLA = Veterinary Biological Licensees Association; VBLC = Veterinary Biological Licensees Committee; VST = Virus-Serum-Toxin

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INTRODUCTION

The first successful field studies with hyperimmune hog cholera serum and virus for the prevention of hog cholera were conducted in Story County, IA, in 1907. The method for producing hog cholera serum and virus was patented by the government, with all rights dedicated to the public. By 1912, 30 states were distributing serum that they either produced or purchased from the rapidly increasing numbers of commercial serum companies (17). However, it soon became evident from field complaints and tests conducted on these products by the Bureau of Animal Industry (BAI) in 1909 that some products being marketed were worthless and/or contaminated, and some method of proper production control was necessary. It also became evident that there was a need for stronger control on the importation of biological materials when an outbreak of foot-and-mouth disease in the United States in 1908 was traced back to contaminated human smallpox vaccine virus, imported from Japan in 1902. As a result, on March 4, 1913, Congress passed the Virus-Serum-Toxin (VST) Act (21 U.S.C. 151-159), granting authority to the Secretary of Agriculture to prevent the preparation and marketing of any worthless, contaminated, dangerous, or harmful virus, serum, toxin, or analogous product. The passage of this act marked the beginning of regulatory requirements for veterinary biological products in the United States.

EARLY YEARS

The authority granted to the U.S. Department of Agriculture (USDA) under the VST Act of 1913 was delegated to the BAI. Within the BAI, responsibility for regulation of hog cholera serum and virus, mallein, and tuberculin was delegated to the Biochemic Division under Marion Dorset, the chemist who led the team that developed the hog cholera serum and virus method of immunization. The responsibility for regulation of other biological products, however, was assigned to the Pathological Division under John R. Mohler. A section was set up in the Biochemic Division under H. J. Shore, called the Office of Virus Serum Control, to establish an inspection program for establishments producing hog cholera serum and virus. By the end of fiscal year 1915, this office had 42 inspectors to monitor the 80 (of 102) licensed biologics establishments that were producing hog cholera serum and virus at that time. Inspectors were stationed at establishments producing hog cholera serum and virus to monitor the sanitary conditions of plants and equipment, the conduct of purity and potency tests, record keeping, cultures, animals, labels, and competence of production personnel (15).

Under the Pathological Division, samples of immune serum, vaccines, and bacterins were procured from time to time on the open market and subjected to bacteriologic and potency tests. The BAI annual report for 1917 indicates that the BAI purchased and tested 281 samples of biological products and found that 121 of these samples, representing 12 different products from eight establishments, lacked potency or were contaminated (16).

Records indicate that the first license for an avian product was issued to the University of California, Berkeley, for fowlpox vaccine on January 13, 1916, ''for the prevention of chicken pox.'' With this beginning, the following additional poultry products were soon licensed:

- Hemorrhagic septicemia vaccine, avian (later designated as a bacterin) to The Royal Biological Laboratories, Kansas City, MO, on August 9, 1918;
- Mixed bacterin for fowls to The Royal Biological Laboratories, Kansas City, MO, on January 1, 1921;
- Avian cholera typhoid bacterin to Cutter Laboratory, Berkeley, CA, on February 14, 1923;
- Pullorin to Gochenour-Collins Labs, Glenmont, MD, on April 18, 1927; and
- Anti–mixed infection serum–Avian to Jensen-Salsbery Laboratories, Kansas City, MO, on November 14, 1927.

By the end of 1929, seven licenses had been issued for fowlpox vaccine, 19 for hemorrhagic-septicemia bacterin-avian, 17 for mixed bacterin-avian, eight for avian cholera typhoid bacterin, four for pullorin, and one for anti–mixed infection serum-avian.

Hudson and Beaudette's 1932 report, indicating that placing bronchitis virus on the cloacal mucosa could prevent bronchitis in birds (1), led to a royalty agreement with Vineland Poultry Laboratories (Vineland, NJ) and to Vineland Laboratories subsequently receiving the first USDA license for laryngotracheitis vaccine on February 16, 1933. Hinshaw first described this disease in1924 as infectious bronchitis, but in 1931, a special committee on poultry diseases of the American Veterinary Medical Association (AVMA) adopted the name laryngotracheitis (1,5). The product was first licensed for distribution intrastate and during this time was sold as infectious bronchitis vaccine (Goldhaft, T. M., Historical Information on Vineland Laboratories, Inc., August 31, 1994), but when licensed by the USDA it was named infectious laryngotracheitis vaccine.

On November 17, 1939, the BAI issued a license to Vineland Laboratories for the production of pigeonpox vaccine for prevention of fowlpox. Reactions to this vaccine were milder, and it could be used to vaccinate birds during the growing period (Goldhaft, T. M., Historical Information on Vineland Laboratories, Inc., August 31, 1994). One must keep in mind that avian virology was in its infancy at this time, and the state of the art was production of virus in the host. Thus, fowlpox vaccine was made from scabs scraped from the comb of birds, infectious laryngotracheitis vaccine from scraping the interior of tracheas removed from infected birds, and pigeonpox vaccine from scabs that formed on feather follicles on the breast of pigeons. However, in 1939 the licensing of an equine encephalitis vaccine, killed virus, the first veterinary vaccine produced in chicken embryos, began an industry-wide conversion to this new method of production (Goldhaft, T. M., Historical Information on Vineland Laboratories, Inc., August 31, 1994). The issuance of a license to Vineland Laboratories on November 17, 1939, for fowl laryngotracheitis vaccine, chicken embryo origin (CEO), live virus, represented the first live virus vaccine produced in chicken embryos.

MODERN VIRUS PRODUCTS EMERGE

The number of biological products available for diagnosis and prevention of diseases in poultry continued to grow in the 1940s with the licensing of the following:

- Avian tuberculin to Ashe Lockhart, Inc., Kansas City, MO, on February 17, 1940;
- Gallinarum Typhimurium bacterin to Gland-O-Lac Co., Omaha, NE, on July 29, 1940;
- Salmonella Typhimurium bacterin to Gland-O-Lac Co., Omaha, NE, on January 21, 1943; and
- Pullorum antigens, regular, turkey, polyvalent, and stained to The Columbus Vaccine Co., Columbus, OH; Fort Dodge Serum Co., Fort Dodge, IA; and American Scientific Labs, Inc., Polo, IL, on July 29, 1948.

The diagnosis of Newcastle disease in the United States by J. R. Beach on the west coast in 1940 and by F. R. Beaudette and J. J. Black on the east coast in 1945 led to the licensing of the first Newcastle disease vaccine, inactivated, to Lederle Laboratories on December 31, 1946. However, the inactivated Newcastle disease vaccines produced at that time did not prove to be fully effective. Beaudette's efforts to screen virus isolates led to his selection of the Roakin strain of Newcastle disease virus that could be safely administered as a live virus vaccine to birds over 4 wk of age by wing web stab. Vineland Laboratories prepared a vaccine from this strain and was issued a license on June 11, 1948. Lederle Laboratories was also issued a license on June 11, 1948, for a live Newcastle disease vaccine prepared from strain MK107 that had been selected by Dr. Van Roekel. These vaccines caused high mortality in birds less than 4 wk of age; however, in 1947 Drs. Hitchner and Johnson selected a mild, B1 strain of Newcastle disease virus that could be given by the intranasal route at 1 day of age. Salsbury Laboratories and Lederle Laboratories obtained licenses for this Newcastle disease vaccine, B1 strain, in 1950. Vineland Laboratories followed by obtaining a mild Newcastle disease isolate selected by Dr. Beaudette and was issued a license for Newcastle disease vaccine, La Sota strain, in 1952 (10). The La Sota strain vaccine was initially recommended for intramuscular administration at 2 wk of age or older; however, it was later found to be safe and effective by several other routes and at younger ages.

Schalk and Hawn identified the virus causing bronchitis in birds in 1931. This disease, at one time confused with laryngotracheitis, was initially managed by controlled exposure of birds in the middle of the growing period (6). However, it was found that this practice could induce chronic respiratory disease in flocks also infected with mycoplasma. Modified strains of bronchitis virus were prepared by repeated passage in embryonated eggs, and the BAI issued a license to Salsbury Laboratories for a live bronchitis vaccine on August 6, 1953. It was initially assumed that all bronchitis strains were antigenically similar, but research done at the Connecticut Experiment Station in 1956 demonstrated that distinct antigenic differences did exist (10). The identification of the Connecticut strain represents the first in a long list of variants of bronchitis virus that have been identified and licensed as vaccines.

Lederle Laboratories Div. American Cyanamid Co. received a license for Newcastle bronchitis vaccine, live virus, the first combination product licensed by the BAI, on October 7, 1954. This started a trend toward combination products in the industry. Delaware Poultry Laboratories was issued a license in 1961 for the first Newcastle bronchitis vaccine containing both Massachusetts and Connecticut types of bronchitis virus. The broader protection noted after 2 yr of commercial use of this combination prompted the USDA Agricultural Research Service (ARS) to recommend that all licensees add this combination of types to their vaccines (Gottlieb, E., and Associates, Press Release 1963). Lederle Laboratories was issued the first license for an avian pleuropneumonia-like organism (PPLO) diagnostic antigen on October 4, 1956.

On March 1, 1949, the USDA published final regulations in the Title 9, Code of Federal Regulations that authorized the Department to issue special licenses for the preparation of products for experimental use under controlled conditions designed to provide safeguards to protect the public and the livestock industry. This authority was applied to the first rabies vaccine, modified live virus, CEO; the first modified live virus hog cholera vaccines; and the first avian encephalitis vaccines to permit further evaluation under field conditions (Herl, O. E., report, Proc. of the 13th Ann. Mtg. of the Animal Health Institute [AHI], April 9, 1953).

E. Elizabeth Jones first described encephalomyelitis in birds in 1932; however, it was not until the 1950s that significant breakthroughs were made in understanding the epidemiology and immunology of the disease in order to permit the development of control procedures. By the late 1940s and early 1950s, the disease had become a significant problem for large breeding organizations. Kimber Farms in California began a vaccination program for its flocks in 1950 using chick brain propagated virus administered by wing web stab and made the virus available to other breeders. An application for license was submitted in 1956, and the Veterinary Biologics Division (VBD) authorized limited field trials to evaluate the virus that were annually renewed. However, the data generated in these studies were not adequate to support licensure since they lacked proper controls and an evaluation of immunity in the vaccinated flocks. By 1960, the demand for a commercial vaccine had increased to the point at which a licensed product was needed. J. M. Hejl requested a meeting of industry and university personnel to try to resolve the problem. He threatened to revoke the permits for field trials if valid data for licensure were not developed. John Taylor and the DeKalb Organization cooperated with Salsbury Laboratories to conduct appropriate field studies using a field strain of virus No. 1143, grown in chicken embryos that Bruce W. Calnek had developed for oral administration. Finally, on March 7, 1962, Salsbury Laboratories was issued a license for avian encephalomyelitis vaccine, live virus, CEO (3).

INTERVENTION AND REINVENTION

With advancements in science and the development of new and improved veterinary biological products, the biologics industry slowly changed from an industry that primarily produced hog cholera serum and virus toward one focused on the production of numerous vaccines, bacterins, and diagnostics. In 1945, the industry produced 158 million doses of vaccine. By 1954, the production of vaccine had increased to 1299 million doses and by 1955 to 2146 million doses, of which 1937 million doses were poultry vaccines (Hejl, J. M., report, Proc. 16th Ann. Mtg. AHI, March 23, 1956). Although the industry was changing, the BAI's approach to regulation under the VST Act prior to 1950 had remained nearly the same as it was when established in 1913. The primary focus of the program was the use of resident inspectors in production facilities; a licensing staff in Washington, DC; and the occasional testing of products purchased from the open market. However, in the early 1950s, some events took place that resulted in a complete reinvention of how veterinary biological products were regulated in the United States.

The USDA was reorganized in 1953, and all responsibility for the control of veterinary biological products was assigned to the newly created Biological Products Section (BPS) of the Animal Inspection and Quarantine Branch of the ARS. O. E. Herl, who replaced Skidmore in 1950, remained the head of this new branch. J. M. Hejl became head of licensing, and Arthur Tellejohn headed the inspection force. In this reorganization, the BAI Pathologic Division was discontinued, as was the function of sampling and testing of biologics purchased from the market (Baker, L., Presentation at Fort Dodge Sales Meeting, August 23, 1968). In a report presented to the AHI, J. M. Hejl stated that as a result of the changes in the industry, the BPS had initiated a redirection of inspection toward vaccines and bacterins, but additional changes were still needed to keep the biologics program in pace with the industry, and a control laboratory would be a tremendous asset to the program (Hejl, J. M., report, Proc. 16th Ann. Mtg. AHI, March 23, 1956).

In 1953, at a meeting of the Interregional Advisory Committee on Newcastle Disease and Other Respiratory Disease of Poultry in Chicago, several reports were given pointing out the inadequacies in the program of testing and licensing of Newcastle disease vaccines. It is logical that this group of research workers from the experiment stations would address such deficiencies, since it included scientists that had isolated and characterized the strains of Newcastle disease virus used for production of these vaccines. These scientists had maintained a strong interest in how these products were performing in the field. At this meeting, committee members reported finding products contaminated with antibiotic-resistant bacteria, outbreaks of pullorum occurring in vaccinated flocks as a result of *Salmonella pullorum*–contaminated vaccines, vials of live virus vaccine on the market with no detectable virus titer, and killed virus vaccines lacking immunogenicity. Such reports led the committee to establish a subcommittee comprising Edwin Johnson, Robert Hanson, Arnold Rosenwald, and Henry Van Roekel, called the Vaccine Standardization Subcommittee of the Interregional Advisory Committee on Newcastle Disease and Other Respiratory Diseases of Poultry, which was designed to prepare recommendations for improving standards for Newcastle disease vaccines. The subcommittee's preliminary recommendations were presented by Robert Hanson at the U.S. Livestock Sanitary Association Annual Meeting in November 1954 (8) and were published in the journal of the AVMA in December 1954 (11). The Vaccine Standardization Subcommittee recommendations included the need for the following:

1. Better communication between the Regional Technical Committees and the BPS,
2. Strengthening of present standards for virus vaccines,
3. Establishment of a program for revision of standards,
4. Establishment of a program for evaluating new products, and
5. Paying for this program by sharing the cost with the biologics industry.

The subcommittee also recommended that the BPS add virologists, bacteriologists, and statisticians to its staff and obtain laboratory facilities with animal isolation units for the testing of randomly selected serials of products (8).

R. P. Hanson, chairman of the Vaccine Standardization Subcommittee, proposed that the committee meet with members of the biologics industry and members of the BPS during the AVMA meeting in Minneapolis, MN, in August 1955 to review the subcommittee's recommendations. It was hoped that this meeting would lead to definite recommendations that could be presented to the Interregional Advisory Committee for consideration. Since the BPS no longer had the capability to test products, the question of whether independent laboratories or experiment station laboratories should do the recommended testing was also on the agenda (Johnson, E. P., letter to Vaccine Standardization Subcommittee Members, March 22, 1955). Such a meeting was arranged at the Hotel Dyckman in Minneapolis, MN, on August 15, 1955. O. E. Herl of the ARS; John Salsbury, chairman of the Poultry Vaccines Subcommittee of the Veterinary Biological Licensees Association (VBLA); and Phil White and Arthur Goldhaft, also of the VBLA, met with M. S. Hofstad, Henry Van Roakel, E. P. Johnson, and A. S. Rosenwald from the Vaccine Standardization Subcommittee. R. P. Hanson, chairman of the subcommittee, was not able to attend (Rosenwald, A. S., personal report of joint meeting on vaccine standards, August 15, 1955).

Two sets of problems were addressed in this meeting: 1) the need for proper standards of experimental design in the evaluation of new products and 2) the need for more critical methods for the evaluation and testing of products currently being marketed. It was admitted that many of the tests required for these purposes were inadequate or unnecessary. The committee also agreed that there was a need for better standardization of tests. However, since many of the tests conducted were developed by licensees and were considered to represent confidential business information, the VBLA agreed to ask its members to put together a list of the tests being used, to note which tests the industry thought needed improvement, and to provide this information to the Vaccine Standardization Subcommittee for review. The subcommittee also offered to provide assistance in the review of protocols for the evaluation of new products; however, O. E. Herl indicated that to maintain the confidentiality of information, he would only do this with the approval of the manufacturer. The group as a whole agreed that it would be most effective if they worked to improve test procedures for currently licensed products and the correlation of laboratory tests with field results. The need for laboratory support for the spot testing of licensed products and research for the development of improved test methods was noted. Since the BPS' ability to test products was lost in the 1953 reorganization of ARS, this was a significant concern to BPS, one that was not resolved until 1961 when some space became available for this purpose in the newly constructed National Animal Disease Laboratory in Ames, IA (Rosenwald, A. S., personal report of joint meeting on vaccine standards, August 15, 1955).

Although the group agreed they should continue to meet on these issues, there were questions concerning how such work would be financed and who should assume the leadership and initiate the next meeting. It was suggested that biologics licensees might band together to assume this role, financed by grants-in-aid from the industry. Such grants-in-aid were also suggested as a means to provide needed laboratory support (Rosenwald, A. S., personal report of joint meeting on vaccine standards, August 15, 1955). Decisions on leadership and financing were left unresolved; however, this initial meeting marked the beginning of a new direction for the regulation of veterinary biological products and of a cooperative effort between research, regulatory, and industry personnel for the improvement and standardization of test requirements based on science.

R. P. Hanson and the Vaccine Standards Subcommittee continued to work on the development of standards for Newcastle disease vaccines following this first meeting through the exchange of drafts and comments based on their research experiences, with input from the biologics industry and the BPS (Rosenwald, A. S., letter to Dr. J. M. Hejl, May 28, 1956). The subcommittee also began the drafting of a manual on methods for the examination of poultry biologics to document the materials and methods used in the production and testing of poultry biologics (Rosenwald, A. S., letter to Vaccine Standards Subcommittee Members, May 7, 1956). A second meeting of the subcommittee, industry, and BPS representatives (joint subcommittee) was scheduled to be held in Chicago on November 30, 1956, to review a draft of "Tentative Proposals for Minimum Requirements, Newcastle Disease Vaccine, Live Virus," which the Vaccines Standards Subcommittee provided to BPS on November 19, 1956. This draft was a major step forward in improving the standards for Newcastle disease vaccines and proposed several new concepts that served as a template for the development of future standards for all categories of biological products. Procedures for identification and characterization of seed stocks and the testing of seed stocks to demonstrate freedom from contamination with bacteria, extraneous viruses, *Salmonella*, and PPLO were provided. Freedom from extraneous viruses, strain identification, potency tests,

bird safety tests, and a bird protection test were prescribed for finished product.

Review of the draft minimum requirements for Newcastle disease vaccine at the joint subcommittee meeting in Chicago resulted in areas of agreement and disagreement. Three major points of disagreement were identified. Vaccine manufacturers considered the tests for bacterial contamination too stringent for egg-origin vaccines and suggested that a tolerance of 1000 organisms/ml should be provided, similar to that allowed for human smallpox vaccine. The test for PPLO was questioned, since no technique had been developed to detect PPLO contamination in the presence of antibiotics, nor to this date had anyone demonstrated PPLO to be a contaminant in avian vaccines. The proposed chicken embryo inoculation test for detection of extraneous viruses raised questions concerning the source of sufficient mono-specific antiserum for neutralizing vaccine viruses and the lack of uniform guidelines for the determination of significant pathology in embryos from possible virus contaminants. Sources of eggs and procedures for handling them were also noted to be critical factors in reducing contamination (Vaccine Subcommittee Ideas, November 30, 1956). The subcommittee recognized that detailed procedures for conducting each of the prescribed tests would be needed to implement these new requirements and that full implementation would need to be delayed until the ''Manual for the Examination of Poultry Biologics'' that they were developing was published.

Minutes of the second joint subcommittee meeting (Vaccine Subcommittee Ideas, November 30, 1956) recommended that those tests found acceptable to BPS, VBLA, and the subcommittee should be included in a revised standard that would become effective following a 3-mo preparatory period. This revised standard would be tentative and subject to being reappraised and revisited by the joint committee following a 6- to 12-mo trial period. Firms should conduct tests proposed in the draft requirement for which agreement was not reached for further research and evaluation. Data from such testing would be used to reevaluate test procedures until satisfactory standards could be established. A. S. Rosenwald suggested (letter to Vaccine Standards Subcommittee Members on December 6, 1956) that biologics manufacturers should conduct the questioned tests for information and research over a period of time and report their results to BPS until adequate data became available to determine the validity of the test procedures and the standards being set.

The BPS took these suggestions under advisement and developed a plan for implementation of updated vaccine standards that was consistent with them. Tests that were considered acceptable were implemented after manufacturers had a period of time during which to become familiar with the new test requirements. Tests that were still under review were conducted for information. The BPS and VBLA began a project involving updating the standards for all categories of products based on the template established for Newcastle disease vaccine. The VBLA established a poultry subcommittee to work with the BPS to develop standards for additional avian biologics. The BPS, VBLA, and ARS continued to conduct research and collect data on testing procedures in order to resolve the issues relating to the parts of the standard for Newcastle disease vaccine for which agreement had not been reached. The Poultry Disease Subcommittee on Animal Health, in cooperation with The Regional Technical Committee on Respiratory Diseases of Poultry of the ARS, continued to work on their manual, *Methods for the Examination of Poultry Biologics*, which was published by the National Academy of Sciences in 1959. This was the first manual to provide standard procedures and methods for investigating poultry diseases and testing poultry biologics. It became an invaluable reference for personnel involved in the research and diagnosis of avian diseases and established the first standard methods for the uniform application of standard requirements for poultry vaccines in the United States (14).

BIOLOGICS TESTING LABORATORY ESTABLISHED

After several years of planning, the USDA was authorized to construct the National Animal Disease Laboratory in Ames, IA. The BPS was assigned 10% of the space in this new facility for the testing of veterinary biological products. In preparation for this event, promising young veterinarians were selected from the BPS inspection force and sent to various colleges for graduate training in the fields of bacteriology and virology to reorient them to the laboratory testing of biological products (Baker, L., Presentation at Fort Dodge Sales Meeting, August 23, 1968). When the new facilities were dedicated in 1961, these veterinarians formed the core of the leadership that organized and initiated the reestablishment of testing functions in the biologics program. The availability of a laboratory dedicated to the testing of veterinary biological products permitted BPS to focus its efforts on the resolution of the testing issues not agreed to in the first draft of the standard requirement for Newcastle disease vaccines and to develop new standard test procedures for biological products.

With the establishment of the laboratory in Ames, BPS also reorganized its approach to inspection. Resident inspectors were removed from vaccine production facilities and assigned to a central office in Ames, IA, and traveled to conduct inspections of facilities and investigations of violations of the VST Act. In 1965, the USDA reorganized BPS to create the VBD of the ARS and appointed J. M. Hejl as the director of all program operations.

STANDARD REQUIREMENTS ISSUED

With the publication of *Methods for the Examination of Poultry Biologics*, Hiram Lasher and Jim Bivins, representing the VBLA Poultry Subcommittee, began working with Luke Sinclair, BPS, Veterinarian in Charge (Pearl River, NY), and later with the BPS Laboratory, Poultry Products (Ames, IA), to draft standard requirements for poultry vaccines for issuance to the industry (Sinclair, L. P., Letter to Dr. G. V. Peacock, April 30, 1962). Their drafts were reviewed by BPS, VBLA, and poultry disease experts, and when they were agreed to be satisfactory, they were published as the following standard requirements:

- V-29, SR for Newcastle disease vaccine (killed virus), July 2, 1962;
- V-21, SR for Newcastle disease vaccine (live virus), January 2, 1964;
- V-27, SR for fowl laryngotracheitis vaccine, September 1, 1966;
- V-39, SR for avian pox vaccines, September 1, 1966;
- O-50, SR for pullorum antigen, August 20, 1969;
- V-68, SR for avian encephalomyelitis vaccine, live virus, July 1, 1970;
- V-69, SR for avian encephalomyelitis vaccine, killed virus, July 1, 1970; and
- V-19, SR for bronchitis vaccine, September 1, 1970.

These first standard requirements were issued as ''Biological Product Memorandums;'' however, in 1968, J. M. Hejl informed the industry that VBD was making arrangements to publish standard test requirements in the Federal Register so that the entire scientific

community could comment on their adequacy (9). Publication in the Federal Register would also provide legal authority for the USDA to enforce these requirements and ensure uniform application by all licensees. In 1969, Thomas Hawkins, from the VBD licensing staff, was assigned the task of getting these documents published for comment, reviewing and responding to the comments received, and preparing final rules for publication in the Federal Register in accordance with the Administrative Procedures Act. Review of these standard requirements by VBD personnel and Veterinary Biological Licensees Committee (VBLC) prior to publication in 9 CFR resulted in several updates, including the application of the master seed concept. James Tanner, VBD Biometrician, also reviewed each standard requirement to ensure the statistical validity of each test procedure. Beginning in 1969, and until he retired in the mid-1970s, Thomas Hawkins was responsible for promulgating the majority of the regulations and standard requirements currently published in Title 9 CFR, Parts 101–124.

In 1964, the AHI offered a free, 1-yr trial membership to members of the VBLA. In 1965, members of the VBLA agreed by tally vote to continue their membership in AHI and began paying dues. Biologics issues were addressed in the VBLC of the AHI.

As BPS began the testing of serials of product in the early 1960s, their test results often did not correlate with those of the licensees. This lack of correlation became a serious issue for licensees. The VBLA, and later the VBLC of the AHI, requested that VBD develop Standard Assay Methods (SAMS) to describe in detail how each standard requirement test was conducted in the VBD laboratory. They also requested that standard references and reagents that were needed to conduct these tests be prepared and made available to the industry (Report of VBLC Poultry Subcommittee Meeting, November 15, 1967). Although meeting this request represented a major project, the VBD agreed to the need for such information and materials, and today the Center for Veterinary Biologics provides references, reagents, and SAMS for use by the industry for conducting standard requirement tests.

The need to recall products tested as unsatisfactory by the VBD Laboratory after being released for marketing by the licensee presented an additional problem for the industry. To avoid the need for such recalls, the VBD drafted Biological Products Memorandum No. 39, which provided procedures for USDA sampling and concurrent testing of serials prior to their release for marketing. There was much discussion concerning Biological Products Memo No. 39 at the November 1967 meeting of the VBLC. Licensees were concerned about the lack of correlation in testing and what procedures would be applied when a licensee found a serial of product satisfactory and the VBD Laboratory found it unsatisfactory. The licensees wanted procedures for retesting and determining the disposition of such serials defined. To aid in avoiding conflicts involving test results, the licensees again requested additional references, reagents, and SAMS for conducting standard requirement tests. In spite of some unresolved concerns by the licensees, Biological Products Memorandum No. 39 was issued, and concurrent testing became effective on January 1, 1968.

CEO ISSUES

Early workers thought the use of chicken embryos for the production of vaccines was a clean system, and they did not consider the possibility that they might be contaminated with unwanted microorganisms. However, by 1952, G. E. Cottral, in a review of endogenous viruses in eggs, described nine diseases of birds in which occasional egg transmission could take place (4). Egg transmission of diseases such as avian lymphomatosis, infectious sinusitis, and avian encephalomyelitis raised concerns with BPS and the biologics industry, since the embryo inoculation test for extraneous viruses in product standard requirements was not adequate to detect these agents. These concerns became a reality in 1966, when Payne *et al.* (13) reported contamination of egg-adapted canine distemper vaccine with avian leukosis virus, and in 1967, when Grass *et al.* (7) reported several outbreaks of chronic respiratory disease in turkeys due to fowlpox vaccine contaminated with *Mycoplasma gallisepticum*.

In response to the mycoplasma findings, Dale Oshel, VBD Poultry Products Laboratory, developed a standard test procedure for the detection of mycoplasma contamination in poultry vaccines that was issued to the industry in 1967. Intense VBD testing revealed that 156 million of the 7 billion doses of poultry vaccine produced that year were contaminated with mycoplasma. Implementation of this new test procedure resolved this industry problem in a very short time, and VBD Laboratory testing of 100% of all serials of poultry vaccines produced in 1969 did not reveal a single mycoplasma-contaminated product (Hejl, J. M., talk presented to New Jersey Poultry Health Seminar, November 13, 1969).

With the report of contamination of vaccine with leukosis virus in 1966, Oshel's laboratory began a project to adapt the Health, Education, and Welfare–developed Complement Fixation Test for Avian Leukosis (COFAL) for use in the detection of lymphoid leukosis subgroups A and B in poultry vaccines. Initial tests conducted on outdated serials of vaccine using this new test procedure revealed that five serials of vaccine produced by two licensed manufacturers showed evidence of lymphoid leukosis contamination (Hejl, J. M., talk presented to New Jersey Poultry Health Seminar, November 13, 1969). The USDA Leukosis Study Committee also voiced its concerns related to leukosis virus contamination in live virus, CEO vaccines and recommended that these vaccines be established as free of leukosis viruses. The VBD presented these concerns to the VBLC Poultry Products Subcommittee at their meeting in May 1967 and requested that the subcommittee recommend an approach to address the leukosis problem. The subcommittee responded (Cooper, R. H., Letter to Dr. J. M. Hejl, June 5, 1967) by recommending that the VBD set up a meeting immediately between veterinary biologics producers, VBD officials, and other experts in the leukosis field to possibly develop a plan of action. The VBD responded (Jones, R. P., letter to VBLC, August 31, 1967) by organizing an avian leukosis working conference that was held October 5, 1967, at the Hotel Burlington in Washington, DC, with B. R. Burmester, H. N. Lasher, B. W. Calnek, R. E. Luginbuhl, R. H. Cooper, E. W. Marty, Jr., D. L. Croghan, D. D. Oshel, W. F. Hughes, R. J. Price, J. R. Ipson, J. W. Walker, B. LaSalle, and L. H. Zollar in attendance. D. D. Oshel and R. H. Cooper were elected co-chairmen of the working committee. D. D. Oshel stated that the committee needed to address the availability of 1) an adequate supply of leukosis-/sarcoma-free fertile eggs for vaccine production, 2) freedom of vaccine seed viruses from leukosis/sarcoma agents, and 3) the need for a simple, practical test procedure for monitoring finished vaccines for contamination. The committee recommended that the USDA develop specific test procedures and reagents for the detection of the leukosis/sarcoma group of viruses in vaccines and discussed methods that could be used to free vaccine seed virus of leukosis virus (Cooper, R. H., and D. D. Oshel, Minutes of the Working Committee for Leukosis Program Proposal, October 6, 1967).

Considering the discussions and recommendations of the working committee, the VBD drafted a three-phase program for preparation

of poultry vaccines free of the leukosis/sarcoma group of viruses that was presented by D. D. Oshel to the VBLC Poultry Products Subcommittee at their November 1967 meeting. Phase 1 of the proposal included the preparation and distribution of the standard reagents and procedures needed to conduct leukosis testing, followed by a meeting of industry and government representatives to review the proposed procedures and test results prior to the next VBLC meeting in May 1968. Phase 2 proposed the use of the standard reagents and procedures to test vaccine seed stocks for contamination and the development of test capability by licensees. Phase 3 proposed the development of egg-producing flocks that would be resistance-inducing factor (RIF)-A RIF-B, and COFAL negative as a source of eggs for live virus vaccine production and the testing of live virus products for contamination by RIF and COFAL techniques. The proposed plan was approved, and VBD, the licensees, and suppliers of fertile eggs began to attack the problem. The VBD developed standard test procedures, provided test reagents to the industry, and on October 1, 1969, issued the requirement that licensees test each serial of live virus poultry vaccine to demonstrate freedom from lymphoid leukosis virus contamination. Licensees prepared clean seed viruses and in turn required that all flocks used as a source of fertile eggs be free of lymphoid leukosis by the COFAL test or another approved procedure. Tests conducted on final product at that time were all negative, indicating that the elimination of leukosis viruses from live virus CEO poultry vaccines had been successfully achieved (Hejl, J. M., talk presented to New Jersey Poultry Health Seminar, November 13, 1969).

To prevent leukosis virus and mycoplasma contamination in CEO poultry vaccines, it is essential that producers of such vaccines obtain their fertile eggs from specific-pathogen-free (SPF) flocks tested and found free of these diseases. Prior to 1969, some poultry vaccine manufacturers had established their own flocks to ensure a consistent supply of eggs. These flocks were not monitored to the extent SPF flocks are monitored today, but they were tested to be free of pullorum and were observed to be free of clinical signs of disease. Vineland Laboratories established one of the first SPF flocks in 1938, when it began to develop production of its vaccines in chicken embryos. The method for determining the SPF status of this flock was included in the production outlines for their products and filed with the BAI. Delaware Poultry Laboratories and American Scientific Laboratories also established their own SPF flocks in the 1950s. The University of Connecticut Department of Animal Diseases established an SPF flock as a result of studies it was conducting with avian encephalomyelitis in 1955, when they found that parental antibodies in eggs prevented the growth of encephalomyelitis virus in embryo tissues. In 1960, Ray Davis, a Connecticut hatchery man, contacted the University of Connecticut and expressed an interest in establishing a commercial source of SPF eggs. Eggs from the University of Connecticut SPF flock were sold to SPAFAS, Inc., to establish the first commercial SPF flock. The first fertile eggs sold from this flock in 1961 were from birds that tested negative for Newcastle disease, infectious bronchitis, adenovirus, *M. gallisepticum*, and *S. pullorum*. This flock was also free of any clinical signs of fowlpox and laryngotracheitis. In 1965, the flock was established to be RIF negative, and in 1966 it was established to be COFAL negative and served as a commercial source of the leukosis-free eggs needed for phase 3 of the plan to ensure leukosis-/sarcoma-free poultry vaccines. In 1970, fertile eggs from SPF flocks tested free of Marek's disease (MD) also became available to the vaccine industry (12).

In the early 1960s, procedures for establishing and monitoring SPF flocks varied from flock to flock. There were no guidelines on the tests needed to establish the status of a flock or the frequency of testing that should be used to maintain the SPF status of a flock. However, on June 24, 1966, the VBD issued Biological Products Memorandum P-30, ''Standard Procedure for Fertile Eggs Used for Production of Poultry Vaccines,'' describing the measures to be taken by producers to ensure that all eggs used for production of licensed poultry vaccines were from healthy flocks. The guidelines in this memorandum were updated in 1968 to provide for testing of flocks for mycoplasma and leukosis and have gone through several updates over the years. Current guidelines are presented in Veterinary Services Memorandum No. 800.65, which no longer specifies the methods by which a source flock should be established or maintained but states that SPF flocks must be tested and found free of 15 different infectious agents, in accordance with procedures described in ''Outlines of Production or Special Outlines,'' prepared by each licensee and filed as satisfactory by the Center for Veterinary Biologics (CVB).

With the successful resolution of the mycoplasma and leukosis contamination issues, the VBD began to focus on concerns over bacterial contamination in products and the 10 microorganisms per dose permitted in poultry vaccines at that time. Antibiotics are added to vaccines to reduce bacterial counts but are not adequate to eliminate contamination and also interfere with the growth of microorganisms when one is conducting tests for bacterial contamination. J. M. Hejl voiced his concerns related to the level of contamination being permitted (Hejl, J. M., talk presented to New Jersey Poultry Health Seminar, November 13, 1969), and on July 1, 1970, he issued a Biological Products Notice setting a target date of July 1, 1972, for all products to be free of bacterial contamination. The VBD bacteriology laboratory evaluated various test media and culture procedures and recommended that for cell culture products, no growth should be detected in nine of 10 tubes of medium inoculated with .2 ml of product restored for use as recommended on the label when tested in liquid soybean casein digest medium. An alternate test procedure using brain heart infusion agar medium was established for CEO poultry products, recommended for administration by other than parental injection, which allowed 1.0 bacterial colony per dose. The VBD also established maximum levels of antibiotics and limits on the number and combinations of antibiotics that could be added to products. Licensees were also required to validate their test procedures to ensure that the antibiotics added as a preservative in their products did not inhibit the growth of test microorganisms in positive controls.

MD VACCINE

In the early 1950s, an acute form of MD began to appear on the eastern seaboard of the United States. This, combined with the beginning of compulsory inspection of poultry at slaughter in 1961, resulted in enormous losses to the poultry industry. The breakthrough on the cause of MD came in 1967 with the simultaneous reports from workers at the Houghton Poultry Research Station in England and the U.S. Regional Poultry Research laboratory in East Lansing, MI, of the isolation of a cell-associated herpesvirus from birds exhibiting signs of MD. With the Churchill *et al*. report of the attenuation of MD virus in 1969 and Witter's isolation of a turkey herpesvirus, both of which demonstrated the ability to immunize birds against laboratory challenge, the poultry industry began to clamor for a licensed vaccine. Some poultry producers began to use controlled exposure in an attempt to vaccinate their flocks against the disease. Poultry producers were reported to be placing used turkey litter and scattering turkey feathers in houses where chicks were brooded or placing turkey poults under brooders with chicks in their attempts to immunize their flocks. Turkey blood was also used as a vaccine. Unlicensed turkey herpesvirus vaccines, and state-licensed turkey herpesvirus vaccines that were

illegally moved across state lines, also began to appear (2). The VBD was being strongly pressured by the poultry industry to license a commercial MD vaccine as soon as possible so that the poultry industry would not need to resort to these unsafe and untested procedures. However, some consumer groups raised concerns about the safety of a vaccine derived from a cancer-causing agent that produced a persistent viremia in vaccinated birds and about the possible public health issues involved in this practice. Some animal health groups cautioned that vaccination might not be the best approach to use to control MD and that eradication should be the goal. They were concerned that the vaccine would contribute to the spread of the virus and severely complicate any future eradication efforts (Hejl, J. M., talk presented to New Jersey Poultry Health Seminar, November 13, 1969).

In an attempt to address these concerns and provide guidance to license applicants, Bernard LaSalle, VB Licensing Staff, drafted "Tentative Licensing Requirements for Marek's Disease Vaccine," which were issued on September 15, 1969. Some felt these guidelines were overzealous and may have actually delayed the licensing of the vaccine. However, applications for product licenses were filed in 1970 and field studies were authorized. On March 1, 1971, Special Licenses were issued for MD vaccine, live turkey herpesvirus, tissue culture origin, to the following laboratories: Merck & Co., Inc; Salsbury Laboratories, Inc.; and Sterwin Laboratories, Inc. These products were frozen cell-associated vaccines that were distributed to customers in liquid or vapor-phase nitrogen containers. A draft standard requirement was prepared for MD vaccine in March 1972 and was reviewed with licensees and applicants in April 1972. At their meeting in November 1973, the VBLC Poultry Product Subcommittee approved the publication of standard requirements for pox, laryngotracheitis, avian encephalomyelitis, and MD vaccines for publication in 9 CFR that included provisions for testing these vaccines in accordance with the master seed concept, in which the titer of product at its highest permitted passage from a master seed is correlated to its immunogenicity in the host (Minutes of the VBLC Poultry Products Subcommittee, November 1, 1973). In the case of MD vaccine, the titer demonstrated to be efficacious was 1500 plaque-forming units (pfu) per dose at release and not less than 1000 pfu/dose throughout the dating period.

The broiler industry found that the price of MD vaccine added significantly to their cost of operations. However, they soon discovered that they could dilute licensed vaccines and only give 1/4 to 1/8 of the recommended dose and except under conditions of maximum dilution and severe exposure still achieve acceptable reduction in MD condemnations. To achieve maximum dilution, broiler producers began to ask licensees for the titer of each serial that they purchased. The CVB objected to licensees providing the titers of their products to customers because this would facilitate the use of the product in contrast to label recommendations (Price, R. J., comments in Minutes of the VBLC Poultry Products Subcommittee, November 1, 1973). Licensees reluctantly complied; however, their customers soon found independent labs to conduct titrations of serials of vaccine they purchased and to provide them with the titers.

ADJUSTING TO CHANGING NEEDS

Although the list of licensed biological products was expanded to address some of the major diseases of poultry in the 1950s, these products were not the final solution. As major diseases were suppressed, different pathogens and different strains or types of known pathogens began to emerge. However, close communications and interaction between the poultry industry and research and diagnostic laboratories at state universities, biologics manufacturers, ARS regional research laboratories, and federal biologics regulatory authorities provided a system for rapid identification of emerging diseases and the development and licensing of new products to control them. As the poultry industry became more integrated with larger flocks, new methods of mass administration as well as changes in the marketing of products were also needed. Many new products and policies were developed to meet these changing needs; however, space does not permit us to address the history of regulatory requirements for these later years at this time. This could possibly be a topic of interest for another historical review in the future.

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ACKNOWLEDGMENTS

We recognize Dr. Rebecca Hyde, APHIS, VS, Center for Veterinary Biologics, for providing licensing dates for products, names and license numbers for early licensed companies, and other dates and information from CVB files in the preparation of this article. Title 9, Code of Federal Regulations, Subchapter E, and Veterinary Biologics Notices and Memorandums were also used for reference.