The History of VALO SPF*

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Heinz Lohmann, the founder of our company, always admired Americans with their scientific approach to business. Between 1956 and 1958 he signed license agreements with American poultry breeders Nichols and Heisdorf & Nelson for die multiplication and distribution of broilers and layers, respectively. With these cooperations he established a basis for transferring knowledge from the USA to Germany between geneticists, nutritionists and veterinarians.

In the late 1950s, a close cooperation and personal friendship developed between veterinarians of the Lohmann Veterinary Laboratory (Drs. Landgraf, Vielitz and Kirsch), their colleagues at the H&N Veterinary Laboratory (Dr. Zander and others) and Prof. Roy Luginbuhl, virologist at the University of Connecticut.

While German veterinarians still knew only two or three typical poultry diseases - coccidiosis, pullorum and Marek's - Luginbuhl had recognized as early as 1958 that eggs used for multiplication of virus had to come from flocks free of latent infections (Luginbuhl *et al.*, 1967). Chicken embryos were first used for virus studies by Rous und Murphy (1911). It took twenty more years before Woodruff and Goodpasture (1931) used chicken embryos fort the multiplication of pox virus.

Beveridge and Burnet (1946) still stated: „there is no authentic report demonstrating that chicken embryos are viral vectors, and it is much easier in poultry to prevent the passage of viral diseases than in other free roaming species". In his review of poultry diseases, Cottral (1952) listed 9 infectious agents which are transmitted vertically by chicken embryos: Leukosis, AE, ND, Infektious Sinusitis (Mykoplasmosis), Psittakosis, Tuberkulosis, Chicken Typhus and Parathyphus. In the following years Adeno virus was shown to be transmitted by hatching eggs, and several reports confirmed Cottral's earlier report. Significant developments were the discovery of „Resistence inducing factors" (RIF) by Rubin (1960) to check for the presence of Leukosis virus and the Cofal test by Sarma (Sarma *et al.*, 1964) a complement fixation test to check for group specific Leukosis antigens. Kottaridis *et al.* (1966) developed a serological test to detect Leukosis antibodies.

Luginbuhl started in 1963 to establish SPF chicken flocks at the University of Connecticut. He used Mount Hope White Leghorns, unfortunately not a very productive strain, and called the program „ SPAFAS" (Specific pathogen free avian supply). According to the definition of the International Society for Laboratory Animals, SPF chickens are kept in flocks routinely monitored for specified pathogens, with negative results to date. Without specifying the pathogens, the label „SPF" is not indicative of the quality of the eggs and their suitability for experimental purposes.

Following Luginbuhl's advice, we started in 1963 to reproduce and rear flocks of chickens in Cuxhaven under isolated conditions und to monitor their health status. Table 1 shows routes of infection in chickens, table 2 the plan for laboratory tests. From 1963 to 1965, we used HNL laying hens, then changed to stock imported from SPAFAS. We compared house type A with negative air pressure vs. house B with positive air pressure and filtered air (FAPP), as described by Drury *et al.* (1969). Results are shown in table 3.

The number of hens per house varied between 200 and 2.000, and the total volume tested between 1963 and 1970 was about 4.000 hens in house A, 14.000 hens in house B. In 1966 the cooperation between SPAFAS and Lohmann was formalized, the contract signed 20.09.1966.

In further trials we tested the effectiveness of air filters. The results shown in table 4 convinced us that FAPP conditions with special high efficiency filters are essential to maintain SPF flocks successfully with predictable results (Vielitz *et al.*, 1974). An important part of our learning process was to determine the optimal air pressure to combine zero disease risk with normal bird behaviour as an indicator of bird welfare (if air pressure is too high, birds may respond with extreme nervousness).

* Based on a presentation at the 40th VALO Anniversary, May 28-29, 2008, Atlantic Hotel Sail City - Bremerhaven.

Table 1: Routes of natural infection in poultry

Egg transmission (a) temporary (acute disease phase)	e.g. AE IB Influenza ND Gumboro Adeno viruses
(b) permanent (wthout clinical disease)	e.g. Leukosis viruses S. pullorum Mycoplasms
Horizontal spread via animal to animal contact dust, feed, litter insufficient sanitation caretaker, equipment	e.g. IB AE Marek Newcastle ILT Salmonella

Table 2: Monitoring scheme for SPF chicken flocks

Infection	Strain or antigen in test system	Type of test
Adeno viruses (e.g. Celo)	Celo-Phelps strain	agar gel precipitation
Avian encephalomyelitis (AE)	van Roekel strain (egg adapted)	embryo-susceptibility test
Fowl pox	a strain of virus producing specific AGP reactions	agar gel precipitation
Infectious bronchitis (IB)	a strain of virus producing specific AGP reactions, Beaudette strain, Mass. 41 strain	agar gel precipitation serum neutralization
Infectious laryngotracheitis (ILT)	a strain of virus producing specific AGP reactions	agar gel precipitation
Influenza (Typ A)	Wilson's strain fowl plaque virus	agar gel precipitation haemagglutination-inhibition
Newcastle disease (ND)	B_1 or F strain	haemagglutination-inhibition
Marek's disease	a strain of virus producing specific AGP reactions	agar gel precipitation
Infectious bursitis (Gumboro disease)	egg adapted strain	embryo-susceptibility test
Leukosis	RSV (type A + B)	serum neutralization: cofal test
Mycoplasma gallisepticum and synoviae	commercially available specific antigens	agglutination
Salm. pullorum	commercially available specific antigens	agglutination

Table 3: Infections occurring in SPF chicken flocks using different ventilation (1963 - 1970)

Housing type	Number of flocks	Infected flocks	
(a) Windowless houses security management negative prssure ventilation no air filtration	14	Celo AE IB Marek	2/14 3'/14 3/14 14/14
(b) Kept in isolated location maximum security management windowless houses psitiv pressure ventilation air filtration	7	Marek all other listed agents	4/7 0/7

Table 4: Prevention of Virusinfection by airfiltration
Transmission trials, exp. house Vet. Labor

Agent (excreted by disease birds)	Number	Filter	Exposure time (days)	Result	
Marek	20 20	without Astrocel*	49	14/20 0/20	seroconversion positive (AGP)
NDV	30 30	without Astrocel	18	30/30 0/30	mortality
IBV	20 60	without Astrocel	24	20/20 0/60	clinically IB-symptoms

* Arocel = Hepafilter, class S (Trial No 62/70, 80/70, 18/71)

In 1968 new FAPP houses were built in Großenhain near Bederkesa, and this reference date was chosen as the basis for the 40th anniversary we are celebrating this year. With the „all in" „all out" system, 2.000 day-old pullets and a corresponding number of cockerels are housed and kept in the same facilities to the end of the laying period.

The SPAFAS hens were soon replaced by more productive HNL White Leghorns. A small house (Farm Elbeck in Altenbruch) was equipped with individual cages, in which HNL hens were kept and subjected to rigorous tests for leukosis virus and other disease agents (Vielitz *et al.*, 1974). The third generation after confirmed 100% negative test results was used as source of hatching eggs to reproduce the first flock from HNL stock for the SPF farm in Großenhain.

In this region Lohmann had leased a large area of woodland in which broiler breeders were reared from 8 weeks of age under free range conditions. We first thought free range management should be beneficial for rearing breeding stock in fresh air with ample exercise, but soon learned that free range also involved high mortality (mainly due to parasitic diseases), resulting in poor development of the chickens – a fact rediscovered in our time, 40 years later, by many poultry people while changing from conventional cages to free range management of layers. Besides poultry diseases, foxes and birds of prey contributed to high losses.

Since 1968 we are using the trade name „VALO" (Vakzine Lohmann).

Several people contributed significantly to the success and increasing sales volume of VALO eggs during the first decades: production manager Widukind Ruttke, sales manager Hartwig Morrise and from the staff of the Veterinary Laboratory especially Dr. Helga Landgraf, Dr. Kirsch, Mrs. Holland-Letz and the author of this review.

The first scientist who used VALO SPF embryos as basis for human vaccines was Prof. Voß at the Robert Koch Institute in Berlin. Our collaboration started in the 1980s in connection with his development of yellow fever vaccines, and we owe many useful suggestions for our laboratory work to his advice.

The sale of VALO eggs was initially organized through TAD, while the Veterinary Laboratory of Lohmann produced and sold vaccines from VALO eggs. I reported on the establishment of SPF chicken populations and their use during an international symposium held at the Veterinary College in Hannover in November 1967. We also reported about our experience with the management of SPF chicken flocks at the 12th International Congress for Biological Standardization in September 1971 in Annecy, France (Vielitz and Landgraf, 1972).

To keep up with the growing demand for SPF eggs, we converted the floor houses to colony cages in the mid 1970s and thereby doubled the capacity.

The SPF status of our VALO flocks has been certified by State Veterinary authorities from the beginning while the Lohmann Veterinary Laboratory continued internal monitoring of all flocks.

In a joint project with the University of Giessen (Prof. Bauer, Dr. Fries), we tried to establish flocks free of endogenous provirus (chf - chicken helper factor), but this turned out to be very difficult. The productivity of chf-negative hens was very poor and no commercial demand for the product developed.

Another research project, in the late 1980s, was conducted in cooperation with the Free University of Berlin (Prof. Monreal, Prof. von Bülow) to establish a flock free of chicken anemia virus (CAV). This project was very successful and enabled Lohmann Tierzucht to produce CAV-free SPF eggs as the first company in the world. From CAV-free VALO eggs, we could then produce the first live CAV vaccine (Thymovac) for world-wide distribution.

VALO has been at the forefront of innovative research during its 40 year history, and SPF chicken embryos will remain essential for research and the production of poultry vaccines in the foreseeable future.

Zusammenfassung

VALO SPF: Erinnerungen an die Entwicklung der SPF-Eierproduktion in Cuxhaven

In diesem Referat wird an die Entwicklung von VALO SPF seit den ersten Anfängen Mitte der 1960er Jahre erinnert. Aus der anfänglichen Notwendigkeit, die eigenen Zuchttierbestände frei von übertragbaren Krankheiten zu halten, entwickelte sich im Laufe der vergangenen vier Jahrzehnte ein wichtiger, hoch spezialisierter Produktionszweig der Lohmann Tierzucht: die Erzeugung von SPF-Eiern für die Impfstoffproduktion. Der Import von Knowhow aus den USA setzte sich fort in eigener Forschungstätigkeit des LTZ Veterinärlabors und in der Zusammenarbeit mit europäischen Forschungseinrichtungen. Zu den herausragenden Beiträgen zur Gesundheit von Geflügelbeständen weltweit gehört die Entwicklung von CAV (Hühneranämie Virus) freien Beständen zur Produktion von Geflügelimpfstoffen aus CAV-freien SPF Bruteiern.

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