An unexpected career in poultry disease research

Early Years

In September 1944, The Netherlands was occupied by Nazi Germany and The Hague was one of the places used by the Germans to send V-1 bombs and V-2 missiles to England. As a consequence, the city was often the target of the Allied bomber fleet. As detailed in a recently discovered diary written by my parents, I was born on September 12 between air raid alarms and the launching of several V-1 bombs. Fortunately, my parents and I survived the last year of the war, which is known as the hunger winter due to the shortage of food and basic supplies. People were surviving on tulip bulbs! Liberation came on May 5, 1945. When I was 6 years old, we (my parents, two younger sisters and I) moved to Zwolle, a city in the middle north-east of The Netherlands. A few years after arriving in Zwolle I already knew that I wanted to become a veterinarian for reasons which are no longer clear, because I did not have a farm background, nor did we have any pets until much later. When I was 10 years old, we moved to a new house in Zwolle, which was fortunate for several reasons. The house was next to grasslands with cattle, a small creek and many opportunities to wander around in the fields and this increased my interest in birdwatching. The second reason is more important for this story: a few houses down
from us lived a veterinarian working for the Provincial Animal Health Service. At that time, Lo van der Vooren, DVM, was mostly involved with checking suspected cases of tuberculosis and Brucella abortus in the province Overijssel. This took him all over the province and he loved to take his son and friends with him, mostly to play hide and seek at the farms while he did the check-ups on cattle. Once I entered High School in 1957, I joined him on my free Wednesday afternoons to observe him in his work, which cemented my interest in becoming a veterinarian.

It is of some interest to describe the Dutch educational system of the 1950’s and 1960’s, which changed drastically in the 1970’s. In order to obtain the right to enter a university you needed to take a five- or six-year high school curriculum, starting at the age of 12. The six-year curriculum required courses in Greek and Latin. Because Greek and Latin were not, and still are not, essential for a veterinary degree my parents enrolled me in the 5-year curriculum. After 3 years, a choice needed to be made to take the alpha or beta track. The latter, required for matriculation into the College of Veterinary Medicine (CVM), had a heavy emphasis on physics (3 years), chemistry (2 years), mathematics (5 years) and biology (5 years). In addition to 5 years of instruction in the Dutch language, we had to take 5 years of French and English and 4 years of German. Promotion to the next year was not automatic and required passing grades in all subjects. Many students had to repeat one year or dropped out. To graduate we had to take nationally administered final exams in these subjects. Written exams in four disciplines of mathematics, physics, and chemistry required a score of >70% to avoid an oral exam. In addition, the foreign language requirements included translation of an essay into Dutch without a dictionary and the ability to speak in the original language about 6 to 10 literary books written in French, German or English. I studied harder for the final high school exam than for any other exam afterwards, but once passed there was automatic acceptance into the CVM at the University of Utrecht. There was no requirement for veterinary experience! The consequence of this system was the matriculation of a large number of students of which a certain percentage would never graduate. This system of admissions has changed over the years and currently more closely resembles the approach in the U.S.A. Fortunately, I passed the high school curriculum and the national exam in 5 years.

Needless to say, the heavy high school curriculum did not leave too much time for many other endeavors. Bird watching was, and still is, one of the hobbies that I developed during high school, getting my first pair of binoculars when I was 12 years old. Playing chess, fishing, politics and debating were other interests during my high school years.

The student years in Utrecht, The Netherlands

Studying for the DVM degree in the 1960s was much more relaxed in The Netherlands than in the U.S.A. First of all, there was no semester system and whenever a specific course was finished you could take the exam within a 3-month period, when you felt ready. The second difference was that the professors did not care if you were present during the lectures as long as you were preparing yourself to pass the exams. For many of the exams you would meet with the professor or his assistant (comparable with assistant or associate professor here) and you would be
questioned for 40 to 60 minutes. I will never forget two of these exams. The first one was the physiology exam which covered 1½ years of lectures. I had the professor’s assistant and he questioned me about muscle physiology. He indicated that my response to a question was incorrect, but I was stubborn and told him that my answer was actually correct, without being able to convince him. The examination continued with a follow-up question and I immediately pointed out that the follow-up question clearly indicated that my previous answer was correct. He looked at me and said “Yes, you were correct!” The rest of the exam was a breeze! The second oral exam I will never forget was the obstetrics exam, which consisted of knowing all there was to know about extracting a calf from a cow when there were problems with the position of the calf in utero. The professor was feared by the students and the oral exam was a nerve-wracking experience for all students. When I came into his office, he asked how I was doing and I answered that I worried about losing my government scholarship because the Secretary of Education, a member of the Labor Party, wanted to change the rules. We started a discussion about politics and the time was ticking, but after a while he switched to obstetrics and gave me the full half hour exam, which I fortunately passed. When I left the room, he called me back and said: “I was once as left-wing as you are and that is OK as long as you know your obstetrics.” I left the exam room 15 minutes past my official time. It was well known that a student leaving the room late did not do well during the exam which most likely would mean trouble for the next student. Indeed, the next student had been worrying more than was needed.

After passing the preclinical years we were able to participate in the organized vaccination campaigns for foot-and-mouth disease and the collection of blood samples for monitoring of brucellosis. Thus, as long as I participated in the required practical classes, I was free to work with a veterinarian. Each day the veterinarian would give me his wife’s car, the vaccine and a list of farms to visit. I was busy for three winters vaccinating cattle and taking blood samples. This opportunity provided a nice income, practical experience working with cattle and, during evenings and nights, a chance to join the veterinarian on emergency calls.

During the years in Utrecht, for philosophical and practical reasons I became more and more interested in working in a developing country after graduation. The latter because working for two years in a developing country would replace serving in the military (we still had a draft at that time). We had an active group of students interested in international development work which was organized into what we called “The Tropical Circle.” I served for a year as president of this group, inviting veterinarians to give lectures about their experiences working in developing countries.

Close to beginning my final practical year, the College started providing scholarships to students seriously interested in working in a developing country. I was the second student to obtain a fellowship and was to spend the first 5 months of 1969 at the College of Veterinary Medicine of the Ahmadu Bello University in Zaria, Northern Nigeria. This was during the war between Biafra, the Ibo homeland that was trying to break away from Nigeria, and the federal
government of Nigeria. Although Zaria was far removed from the actual fighting there were many military roadblocks. Toward the end of my stay I needed to visit the National Veterinary Research Institute in Vom for some library material and to bring back liquid nitrogen to Zaria, because the liquid nitrogen machine in Zaria was broken. Leaving Vom on my return trip, I was stopped by soldiers at a roadblock and needed to explain to them what was in the big liquid nitrogen tank in the back of the land-rover. Somehow, I managed this without having to open the tank, thus avoiding what could have been a very uncomfortable moment! I also had many interesting discussions with the veterinary students, explaining why the Dutch government was no longer supporting the Nigerian federal forces with weapons.

During the 5 months in Zaria I conducted a study on bacterial causes of infertility in Zebu cows owned mostly by Fulani herdsmen. This study was based on the reported high incidence of *Brucella abortus* in these cows. Infertility would be a reason to sell these cows when money was needed. I collected reproductive tracts and blood samples at the local slaughter place and processed the samples for bacteriology (Fig. 1 and 2). I later interviewed some Fulani herdsmen with the help of a Dutch agricultural expert who spoke the local language of the Hausa people. I learned that cows were sold for slaughter 3 to 4 years after the last calf was born — a time when primary pathogens would be difficult to detect. In addition, I was going to some field stations to look for parasites in blood smears from cattle (Fig. 3). The 5-month experience was important for two reasons: It was my first research experience and it confirmed my desire to look for work in a developing country.

After returning to the Netherlands at the end of May 1969, while finishing my practical year, I started to look for positions in developing countries. One of the possibilities was starting a project on Marek’s disease in Mexico City. The project was funded by the Technical Assistance Program of the Dutch State Department in collaboration with the Mexican Department of
Agricultura y Ganaderia. The project would be located in the Instituto Nacional de Investigaciones Pecuarias (INIP) in Palo Alto near Mexico City. The application procedure was rather complicated with the first interview at the State Department, followed by a full day of psychological testing and a final interview with Dr. Bart Rispens, Dr. Henk Maas (co-worker with Dr. Rispens) and Dr. Jeroen Bool, Director of the Central Veterinary Institute (CVI). It was a lucky coincidence that I had visited CVI during my poultry rotation prior to my time in Nigeria. During that visit Bart presented his work on CVI988, which I found fascinating without knowing at that time that Bart would become my first mentor in Marek’s disease research. After a complicated and difficult interview at the State Department, with questions related to my political background, I did get the position prior to graduation. In September 1969 I married Gerda van der Woude and we started to prepare for the move to Mexico once the contract with the State Department was signed.

**The first 5 years after graduation**

After graduation in early 1970, I spent an interesting 6 weeks working as a locum in a large dairy practice. The practice owner gave me the keys to his house after one week and left with the family for a 5-week vacation in Spain. During this 5-week-period I had two unrelated cases of swine fever, a reportable disease in the Netherlands, which had not been seen for a while in The Netherlands. Fortunately, I recognized the possibility of swine fever during my first visit and notified the veterinary authorities after my second visit to the farm. The Veterinary Inspector did not believe my diagnosis, but a sick pig was submitted to the Central Veterinary Institute. Three days later I received a call from the inspector that my diagnosis was correct, although he claimed that it was a case of non-specific swine fever!

In preparation for Mexico we had a six-week immersion course in Spanish, with grammar in the morning and tape-recorder sessions in the afternoon. This was followed by a 5-month training period at the CVI to learn how to prepare and sterilize cell culture media, to perform basic cell culture techniques and to isolate and propagate Marek’s disease virus (MDV). This was a steep learning curve, since it was not part of the veterinary education. During that time, vaccination experiments were being conducted by the CVI Marek’s disease research team and I remember going to a hatchery with Bart Rispens and Henk Maas to vaccinate one-day-old chicks.
In addition, I met Dr. Peter Biggs when he gave a lecture in the Netherlands, not knowing that this would be the very early beginning of a life-long friendship with Peter.

Finally, on January 15, 1971 we left for Mexico City to start the two-year assignment at INIP, which ultimately became a four-year assignment. When we arrived in Mexico City we were met at the airport by Dr. Gosse Bijlenga, a Dutch DVM/virologist working at the joint FAO-INIP project on vampire bat transmitted paralytic rabies. The first month we stayed in a small house on the grounds of INIP before moving to another small house a bit further out of town, but only 3 miles from the Institute. Setting up the laboratory had its own interesting problems - like getting materials from the storage area at the Institute. The first few months were used to train my counterpart, Dr. Jesus Gonzalez del Angel (Fig. 4), in cell culture techniques and isolation of MDV from blood samples. I also needed to train a young woman to prepare glassware, media, etc. This all happened while I had only had a minimal training myself. Fortunately, I could get some help from Dr. Bijlenga when needed.

One month after arriving I had to give my first lecture at an international conference on poultry diseases in Mexico City. Although there was translation from English into Spanish, I decided to give the lecture in Spanish, which generated a lot of goodwill. When we left Mexico in May 1975 several of my colleagues commented on that lecture. The topic of my lecture was “Recent research on Marek’s disease in The Netherlands,” which contained a lot of new, unpublished information as well as personal communications from researchers in other institutes, provided to me by Bart Rispens. After my lecture Dr. Moreno Chan of the UNAM also presented a lecture on Marek’s disease, where he basically stated that his lecture was out of date compared to the information I had presented. Afterwards, the chair of my department at INIP, Dr. Pablo Correa-Giron, told me that I had dealt a major blow to the UNAM group. Apparently, there were some political controversies between INIP and the UNAM group. I later talked with Dr. Chan and he told me not to worry about it.

The 4 years in Mexico provided me with a solid base in trouble shooting cell culture problems and in basic MDV virology, which was a great advantage years later when I started my PhD project on Marek’s disease at Cornell University. In 1971, I had the great fortune to accompany Bart and Will Rispens for a 14 day visit to East-Lansing, Michigan, taking the opportunity to learn some more techniques at the Regional Poultry Research Laboratory (RPRL), now known as Avian Disease and Oncology Laboratory (ADOL). Meeting Drs. Ben Burmester, Dick Witter, Graham Purchase, Bill Okazaki, Lucy Lee and Kevin Nazerian of ADOL was very exciting for me as a young researcher just starting his career in Marek’s disease. Bart, Will and I stayed for
one week with Nancy and Graham Purchase and the second week we took care of the Burmester house when they left for vacation. Bart and I worked very hard during the second week on revising two manuscripts for publication in Avian Diseases (16:108 and 126. 1972). Afterwards Bart and Will stayed with us in Mexico in connection with the World Veterinary Congress (Fig. 5). In 1973 during my home leave in The Netherlands, I spent 8 weeks working with Bart Rispens in the new veterinary facility in Lelystad. Plans were made to start a PhD project with Bart after my return from Mexico. Things didn’t go as planned, however - Bart passed away from cancer on November 11, 1973.

Circumstances took me in another direction. I attended my first American Association of Avian Pathologists (AAAP) meeting in 1972 in New Orleans. When I arrived at the meeting, I was shocked to witness a heated exchange between the speaker and a scientist in the audience who was telling the speaker that he was lying! Welcome to the AAAP meetings in the “good old days”! The AAAP meeting was important for me because it was the first time that I met Dr. Bruce Calnek, my future mentor. We had a nice talk and I had many questions, which Bruce patiently answered. I met Bruce again in 1974 at the Western Poultry Disease Conference (WPDC). He asked about my future plans after my time in Mexico and if I was interested in coming to the College of Veterinary Medicine at Cornell as his PhD student starting in the fall of 1975. I was able to visit Ithaca, NY in the summer of 1974 after participating in the XV World Poultry Conference in New Orleans and decided that it would indeed be a good move to apply to the graduate program at Cornell University.

In the spring of 1975 I participated once more in the WPDC, this time to give a presentation on a tumor disease in Japanese quail. Dr. Julius Fabricant was in the audience and in his typical style was asking many questions of the mostly young presenters, which made me rather nervous to present my paper. To my happy surprise he did not ask me a single question! Years later when he retired, all of us had some funny Julius stories and I reminded him that he was very nice to me in 1975, not asking a single question after my talk. His explanation was typical of Julius: “I knew that I had at least 3 years to educate you - and I failed miserably.”

The time in Mexico was not only filled with work but also with many other activities. I spent time climbing the snow-capped volcanos near Mexico City with a mixed Mexican-Dutch mountaineering club, spelunking with the same group, traveling around Mexico, birdwatching and being the goalkeeper in the soccer team of the Institute. More importantly, our two daughters, Marianne and Marjolein, were born in Mexico in 1971 (November 20, the day of the Mexican Revolution) and on December 18, 1973, respectively.

At the end of May 1975, my time in Mexico was finished and we left by car for a two-month stay in East-Lansing, Michigan where I was able to finish the study of tumors in the
Japanese quail, with the help of Dick Witter (see Avian Dis. 20:154. 1976). From East-Lansing we went to Guelph, Canada where we left the car and our dog before going to the Netherlands for debriefing with the Dutch Technical Assistance Program. In the middle of August, we returned to Guelph to get the dog and the car and drove to Ithaca to start my PhD program, not expecting that I would remain in Ithaca for the rest of my career.

My career at Cornell: the first 3 years

Soon after arriving in Ithaca we bought a house which, based on the PhD stipend (US$5500/year), would not have been possible if I had not been on the payroll of the Dutch Government for another 6 months and savings made during our time in Mexico. In addition, the bank was very helpful in providing the mortgage. The first few months in the house were without our furniture which was in transit from Mexico.

The start of my PhD project went smoothly, as Bruce Calnek and I quickly agreed on my topic: an investigation of cell-mediated immune responses to low pathogenic strains of MDV in chickens. I wanted to use the MDV strain CVI988 (aka Rispens vaccine) for the project but it was not possible to import this strain because it was kept in the closed division of the Central Veterinary Institute in Lelystad where research was conducted on foot-and-mouth disease. This was actually beneficial because it gave me the opportunity to search for and isolate a virus similar to CVI-988. Dr. Randy Cole (Fig. 6) was able to help me out when he mentioned that his flock of 28-week-old, non-vaccinated, highly MD susceptible S-strain chickens did not show any lesions associated with MD. I was able to isolate from this flock the SB-1 strain of MD within 3 months of the start of my PhD project. SB-1 stands for S strain, housed in pen B and biological clone 1. A detailed history of SB-1 can be found in my article “History of the first generation Marek’s disease vaccines: the science and little known facts” in Avian Dis. 60:715. 2016. Once I found that SB-1 was a serotype 2 MDV strain (Gallid alphaherpesvirus 3) and that it protected chickens against challenge with MDV serotype 1 strains (Gallid alphaherpesvirus 2), my thesis research progressed rapidly. As part of my doctoral research I introduced some new techniques into the MD research program of my mentor, Dr. Calnek. In order to study cell-mediated immune responses for my thesis research I needed to use radioactive materials in mitogen-stimulation assays (³H-thymidine) and cell-mediated cytotoxicity assays (Cr⁵¹). These techniques were fairly new at the time for use in MD research and certainly new for use in the department at Cornell. Fortunately, I was able to use some of the equipment in the laboratory of Dr. Ronald Schultz (a member of my PhD committee) in the James A. Baker Institute at Cornell. To avoid any humoral immune responses in the chickens, I decided to use birds that were burssectomized at 18 days of embryonation (EbX chickens).
properly done EbX chickens are not able to develop a humoral immune response because the bursa of Fabricius (BF) is removed just before B cells start to migrate from the BF. The basic technique was outlined in a book describing the technology, but the technique was rather cumbersome and I improved it. I developed an assembly line process with me starting to mark the window to cut open the egg, a technician cutting the windows and me doing the surgery to remove the bursa. We were able to do 30 embryos/hour and achieved a hatchability rate of 60 to 70%. One of the unexpected findings was that the pathogenesis of MDV was altered in EbX chickens (see Schat et al., Infect. Immun. 31:199. 1981 for details). This discovery was an important part of our studies on the pathogenesis of MDV infection as will be described in the next section.

Toward the end of my second year as a PhD student, Bruce Calnek became the chair of the Department of Avian and Aquatic Animal Medicine (DAAAM) at the Cornell Veterinary College. His decision to accept the chair position is described in more detail in his autobiography. To assist him in his NIH-funded research on MDV he asked me if I was interested in joining the Department as a Senior Research Associate upon graduation with the idea to promote me into a tenure-track position upon retirement of some of the senior faculty members. The weekend after he asked me, I was on a hike in the Catskills with the local chapter of the Adirondack Mountain Club. Looking out over the empty expanses of the mountains made the decision rather easy! I received my PhD degree in May 1978 with 5 publications, the SB-1 patent and a job offer.

Overview of my career at the Cornell College of Veterinary Medicine as a faculty member (1978-2011)

This section is subdivided into several parts: a) research during the early period 1978-1986, b) research from 1986 until retirement in 2011, c) teaching and d) international interests. During my 33 years on the faculty I was fortunate to be able to work closely with Bruce and to have a group of outstanding graduate students, postdoctoral fellows, research associates, and visiting scientists. In addition, Priscilla O’Connell was my excellent lab manager from 1987 until her retirement in 2010, which greatly facilitated the smooth sailing over the years. The objectives of several of my long-term research projects were only completed by the dedication of a succession of young scientists in my group. In the next sections some of these projects will be described in more detail, while others are only briefly mentioned. In some instances, the references to papers are also provided in the text and my CV provides a complete list of my references (Appendix 1). Appendix 2 lists all visiting scientists, research associates, postdoctoral fellows and graduate students in my laboratory.

My research career at Cornell: the early years as a faculty member (1978-1986)

After receiving my PhD, I was hired on a 12-month practical training extension of my student visa and Cornell University was going to apply for a green card for me, which would give me permanent resident status in the U.S.A. In order to get the green card as a Senior Research Associate (SRA) Cornell had to demonstrate that I was uniquely qualified, meaning that there was
no US citizen or permanent resident with the same qualifications. It was a lucky circumstance that I had done a minor in fish diseases for my PhD which included a research project, and that DAAAM had responsibilities in aquatic (finfish and shellfish) disease research and diagnosis. Bruce had to advertise the opening for the SRA position and the job description read something like this: “The successful applicant needs to have experience in avian tumor viruses, preferably Marek’s disease virus (MDV), with an emphasis on immune responses to MDV. In addition, he or she needs to have experience in finfish and/or shellfish research.” There was only one other applicant with experience in MD immunology but had no experience in the aquatic part. Without a problem green cards were given to my family and me in the beginning of 1979. In 2002, I became a citizen of the U.S.A.

Thus, I was now officially allowed to stay in the U.S.A. and at Cornell to work on MD research and fish viral disease diagnosis. It was pure coincidence that shortly afterwards there was an outbreak of infectious hematopoietic necrosis in rainbow trout in New York State, which was an exotic disease for New York State. I did isolate the virus and it resulted in a publication with Dr. Jim Carlisle, our fish pathologist at the time (J. Fish Dis. 2:5117. 1979). Early in my career I had one graduate student, Dr. Jan Spitsbergen, DVM, (Fig. 7) (currently Assistant Professor, Oregon State University), working on fish diseases. Her area of research was investigation of immunosuppression by 2,3,7,8-trachlorodibenzo-p-dioxin (TCDD) and Arochlor 1254 on the resistance of rainbow trout to infectious hematopoietic necrosis virus.

The years working with Bruce Calnek and our graduate students on Marek’s disease were extremely productive. Bruce kept an active research program while being chairman of DAAAM by doing his research in the mornings at the Levine Laboratory (Fig. 8, 9), a building which was located about 1 mile from the main Cornell campus, before transferring to his campus office for an afternoon of chairman duties. We shared an office at the Levine Laboratory and many times we would drop what we were doing and start brainstorming.

As mentioned before, the procedure of EbX in chickens changed the pathogenesis of MDV. Previous work by others had used neonatal bursectomy and showed that the B cells did not play a role in the development of MD. However, in contrast with EbX, neonatal bursectomy does not eliminate all B cells from the peripheral organs. Subsequent work by Bill Shek (a Cornell DVM and PhD student at the time), Bruce Calnek, Julius Fabricant and myself (Fig. 10) showed that many of the MDV-positive cells in the thymus were actually B cells and that tumor cell lines consisted of activated T cells. These discoveries were made possible by the generous gift from Dr. Chen-lo Chen (University of Alabama in Birmingham) of monoclonal antibodies (MAb) against
the \( \mu \) chain of IgM and against the so-called Ia-antigen, which we know now detects MHC class II. This gift was made before these MAb were published by her group and without a material transfer agreement! Later she also supplied MAb against CD4 and CD8 identifying T helper cells and cytotoxic T lymphocytes (CTL), respectively, and MAb against the different T cells receptors. These MAb were used to identify cell lines established from MD tumor cells as CD4\(^+\), Ia\(^+\) cells. Thus the “Cornell model” for the pathogenesis was developed as follows: B cells are the first major population of cells to multiply the virus leading to cell death of the infected B cells. CD4\(^+\) T cells become activated and express Ia antigen. These cells become infected and some may die but latency is established in activated T cells and these cells can later be transformed. The absence of B cells does not prevent the presence of small number of activated CD4\(^+\) T cells, which can become infected and initiate the pathway leading to transformation albeit at a lower incidence and requiring more time. \textit{In vitro} infection experiments by our team using different populations of lymphocytes confirmed the fact that activated T cells can become infected in the absence of B cells. It is remarkable that this model was developed by dual staining of cells and analysis of the results using an immunofluorescence microscope. Almost 40 years later the Cornell model has not fundamentally been changed.

During this period reports on MD vaccine breaks started to appear, even in properly vaccinated birds. We were able to isolate one of the so-called very virulent strains, RB-1B, from a flock of chickens at the Babcock Poultry Farm near Ithaca, NY. The chickens were submitted by Dr. Robert Ball, DVM, which explains the abbreviation RB. In our experimental work we demonstrated that HVT or SB-1 alone provided incomplete protection against this strain of MD but the combination of HVT+SB-1 provided adequate protection. At the same time, Dick Witter and his team also tested the combination with similar results and in 1983 the bivalent vaccine (HVT+SB-1) came into commercial use. The Cornell patent on SB-1
provided DAAAM, Bruce Calnek and me (the two inventors on the patent) with a nice royalty income. The DAAAM part of the royalty funds helped fund new directions of research and provided some money for PhD students.

Although Marek’s disease was the main focus of my research activities, my first graduate student, Carmencita Yason, DVM, (Fig. 11) (PhD student, currently a Diagnostic Virologist at University of Prince Edward Island) studied the pathogenesis of avian rotavirus in chickens and turkeys. Her papers in the American Journal of Veterinary Research are still frequently quoted especially by researchers looking at the use of probiotics for the improvement of gut health. She was paid on a departmental fellowship, assisting Dr. Malcolm Peckham in the Avian Diagnostic Section of DAAAM. After Carmencita graduated, she was replaced by TJ Myers, DVM, (Fig. 12) as my next graduate student. TJ continued the work on avian rotavirus using EbX chickens to test the hypothesis that antibodies (especially IgA) are important for the protection against rotavirus infection. However, the hypothesis had to be rejected because pathogenesis experiments using intact and EbX chickens showed only a slight delay in recovery in the EbX chickens compared to the intact ones. He subsequently showed that intestinal NK-like lymphocytes were activated during infection. TJ later spent his whole career with the USDA after a postdoctoral period with Dr. Hyun Lillehoy. He retired in 2016 as the Associate Deputy Administrator for Veterinary Services at APHIS.

My early years on the faculty were also characterized by important events in my private life. My marriage with Gerda ended in a divorce in 1980. Fortunately for me, I obtained sole custody of our daughters, Marianne and Marjolein. The divorce also had a professional consequence. Because I needed security for my children, Bruce went to Prof. Edward Melby, Dean of the College of Veterinary Medicine, and convinced him to change my appointment to a tenure track Assistant Professor line in anticipation of the retirement of a senior faculty member. In 1982 I married Laura Stenzler and when we came back from our honeymoon, Bruce called me into his office, closed the door and told me that I had been promoted to the rank of tenure-track Associate Professor. In 1986 I was granted indefinite tenure and we could plan our first sabbatical leave. In 1989 I was promoted to Full Professor.
My research career at Cornell: from the first sabbatical leave to retirement (1986-2011)

My first sabbatical leave in 1986-87 was used to become more proficient in the use of molecular techniques. Funding from a Fogarty Senior International Fellowship made it possible to spend 12 months at the Houghton Poultry Research Station (HPRS, now closed), part of the Institute for Animal Disease Research in the U.K. Professor Peter Biggs was the Director of the Institute for Animal Disease Research at the time while Dr. Jim Payne was Director of the HPRS. Together with Dr. Norman Ross, I generated the first transcription map for MDV with rather primitive techniques using a lot of $^{32}$P-dCTP. It was a great opportunity to be in England enjoying the friendship with Peter, Jim, Norman and many others at HPRS. It also provided a nice opportunity to visit family and friends in The Netherlands, including a chance to go ice skating on the canals and lakes in the province of Overijsel with my father who was 72 years of age (Fig. 13).

After returning from England I continued a long-term research project on the characterization of MDV-specific CTL. This project was started in the late 1970’s as part of my PhD project when we developed a number of MDV cell lines with defined MHC class I (MHC-I) showing syngeneic restriction of CTL responses and continued by 4 PhD students and one postdoctoral fellow in my laboratory (Fig. 14). When T cell specific MAb became available, Dan Weinstock, DVM, (PhD student, currently working at Janssen Research & Development), used reticuloendotheliosis virus (REV) to transform MHC-I defined spleen cells by in vitro infection. These cell lines were used as target cells to show that REV infection generated REV-specific CTL which were characterized as CD8$^+$ lymphocytes. This was one of the first publications characterizing virus-specific CTL in chickens. Subsequently, Bill Pratt, DVM (PhD student) used these cell lines to develop techniques to stably transfect the cell lines with fragments of the MDV genome and to demonstrate that spleen lymphocytes from MDV-infected or vaccinated chickens were able to lyse the transfected but not the parent cell lines (details in Vet. Microbiol. 33: 93. 1992). Bill Pratt did his PhD in my lab while on leave from the army. After receiving his PhD he worked in the Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Frederick, MD.

Fig. 13. Lunch with my dad while ice skating in the Netherlands (February 1987).

Fig. 14. The four PhD students and one postdoc identifying virus-specific CTL and MDV antigens recognized by MDV-specific CTL. A. William D. Pratt, B. Dan Weinstock, C. Zehava Uni (post doc), D. Rahman Omar, E. Carrie Markowski. Photos provided by the five researchers.
MD. Zehava Uni (currently Professor at the Hebrew University of Jerusalem) continued these studies while a postdoctoral fellow in my laboratory. This approach was further refined by transfecting MHC-I defined REV-transformed cell lines with individual MDV genes. Using CTL from REV and MDV infected or vaccinated chickens, Rahman Omar, DVM, (PhD student, currently Professor in Immunology and Dean of the College of Veterinary Medicine at the Universiti Putra, Malaysia) and Carrie Markowski (PhD student, currently medical doctor in Norway) were able to identify MD proteins that are recognized by MDV-specific CTL. In addition, Rahman showed that these CTL are CD8⁺ TCRαβ¹ T cells (details in Virology 222:87. 1996, Immunology 90:579. 1997 and Vet. Immunol. Immunopathol. 90:133. 2002).

The second sabbatical leave (September 1993-August 1994) was spent in the Netherlands working in the Central Veterinary Institute in Lelystad (now part of the Agricultural University in Wageningen) with Dr. Guus Koch. A generous stipend provided by the Institute made it possible to spend the full 12 months back in my country of birth. The 12-month period also offered the opportunity to visit my parents frequently and enjoy bike rides with them. The year was used to generate some recombinant MDV strains with deletions in an open reading frame originally identified by Kazu Ohashi, DVM, (PhD student, now Professor at the College of Veterinary Medicine, Hokkaido University, Sapporo, Japan).

After returning from the sabbatical leave, studies on molecular aspects of MDV pathogenesis continued with studies on the importance of nitrous oxide (NO) and cytokine induction in vitro and in vivo. Most of that work was done by Zheng Xing (PhD student, now Associate Professor at the College of Veterinary Medicine, University of Minnesota) with some follow-up studies by Keith Jarosinski (currently Assistant Professor, CVM, University of Illinois) (Fig. 15). Keith was in my laboratory from 1999 until 2008 first as a postdoctoral fellow and then as a research associate. He played a major role in a number of papers on the pathogenesis of MDV using deletion mutants, as well as assisting several of my graduate students and was especially important for helping Myrna Miller (DVM, PhD student) on her studies with chicken anemia virus. One of the interesting results of the research of Xinhui Li (PhD student, currently consulting) was the discovery by Michael Piepenbrink (Fig. 15) (postdoctoral fellow, currently scientist II at the University of Alabama in Birmingham) that MDV pp38 alters transcription rates of mitochondrial electron transport and oxidative phosphorylation genes. This was the first time that a specific MD protein was linked to the involvement of mitochondria in the pathogenesis of MD!
In 1979 Yuasa and co-workers published the first paper on what is now known as chicken anemia virus (CAV). When it became clear that this pathogen could be transmitted vertically, we submitted sera samples from our SPF flocks to Dr. Stewart McNulty in Northern Ireland to be screened. Fortunately, these samples were negative and our SPF flocks were considered to be free of CAV. Dr. Benjamin Lucio (Fig. 16) and I decided to start a project on CAV resulting in the isolation of the CIA-1 strain. We were able to import the Cux-1 isolate of CAV from SPAFAS via Bob Wellenstein just before the USDA placed a moratorium on shipments of Cux-1. Dhammi Chandratilleke (my only MSc student) developed monoclonal antibodies to VP3 using the Cux-1 isolate, while Chris Soiné (postdoctoral fellow) developed a nested PCR assay for CAV. One of the interesting differences between CIA-1 and Cux-1 is that the latter one would replicate in our MSB-1 subline while the CIA-1 strain did not. Randy Renshaw (Fig. 16) (postdoctoral fellow, now research associate at the College of Veterinary Medicine (CVM) at Cornell University) made a number of recombinants between the two strains and located a region of high variability in VP1 (J. Virol. 70:8872, 1996). In the meantime, Liang-biao Hu (MSc student with Dr. Lucio) had solved another mystery about the pathogenesis. Using EbX chickens he showed that there was no inherent age resistance to clinical disease: in the absence of antibody responses chickens developed anemia after infection at 5 weeks of age.

In the late 1990’s our SPF flocks became infected with CAV which led to a series of important discoveries. Carol Cardona, DVM, (Fig. 16) (postdoctoral fellow, currently the Pomeroy Chair in Avian Medicine, CVM, University of Minnesota) discovered that CAV or viral DNA could remain present in the gonads of infected SPF chickens up to 60 weeks of age, independent of the antibody status of the chickens (J. Gen. Virol. 81:2067, 2000). This finding was confirmed in a joint project with Dr. Liana Brentano (see International work) using commercial breeders in Brazil. Myrna Miller, DVM, (Fig. 16) (PhD student, currently Associate Professor, Veterinary Science Department, University of Wyoming) noticed that the promoter/enhancer (P/E) of CAV resembled an estrogen response element, which led to two important papers on the control of transcription of CAV. She identified estrogen as a positive regulator of transcription and COUP-TF1 and ΔEF1 as inhibitors of transcription at the P/E site and the transcription initiation site, respectively (J. Virol. 79:2859, 2005; J. Gen. Virol. 89:2998, 2008). These findings provided for the first time a scientific explanation why SPF flocks could break just prior to the start of egg production or afterwards. In addition, Carrie Markowski demonstrated that active CAV replication significantly reduced CTL responses to viral infections.

In 2003, Laura and I spent a six-month sabbatical leave in Geelong, Australia, where I was working in the Australian Animal Health Laboratory (AAHL) of the Commonwealth Scientific and Industrial Research Organization (CSIRO). This turned out to be the start of a long association...
with John Lowenthal, Tim Doran, John Bingham, Ken McColl and many others. From 2006 to 2010 I worked for 3 months at a time at AAHL while 3 months in between were spent at Cornell so that I could do my teaching. The project focused on a specific amino acid mutation at PB2 (AA627) of the H5N1 avian influenza virus (AIV). The mutation from glutamic acid (E) in the chicken isolate of VN/1203 to lysine (K) in the lethal human isolate raised the question if the human isolate infects chickens and ducks in a similar way as the chicken isolate. Only after mutating the HA cleavage site to generate low pathogenic strains still differing in only the PB2 AA627 did we see a slight difference between the two isolates with the chicken isolate causing some neural clinical signs. This project included a collaboration between Dr. Rubin Donis and his team at the CDC, who provided the two genetically engineered strains differing only in the AA at P2 AA627, and the staff of AAHL. It was a different experience for me working in a BL3 zoonotic facility (Fig. 17).

While working on the influenza project I also got a bit involved with the koi herpesvirus (KHV) project. Dr. Ken McColl, DVM, (Fig. 12), a former PhD student with Bruce Calnek, had switched from chickens and MD research (his PhD studies) to working with fish. He was spearheading a project to determine if KHV could be used as a biological control agent against carp, which are nonnative to Australia and a major pest in the Murray-Darling River basin. Based on our shared interests in herpesviruses Ken asked me to get involved.

My final research project as a faculty member was working on Mycoplasma gallisepticum (MG) infection in house finches. Dr. André Dhondt, Professor of Ornithology, Cornell Lab of Ornithology, invited me to become part of his team during his second 5-year grant (NSF-EF grant # 0622705). Jessica Grodio (Fig. 18) (dual degree DVM/PhD candidate, currently Veterinarian at The Center for Avian and Exotic Medicine, New York) developed quantitative techniques to estimate bacterial load in the eyes and an ELISA test to measure MG-specific IgA in lacrimal fluids. These tests were then successfully used in several pathogenesis studies. This project actually finished after I retired.

**Teaching at Cornell**

When I started my career as a faculty member, the Department of Avian and Aquatic Animal Medicine had very little formal teaching responsibilities. In order to strengthen my tenure package, I started to participate in teaching graduate level courses on virology and immunology, ultimately teaching...
my own course on viral immunology. In 1984, I got involved with the student group Veterinarians Interested in Developing Areas (VIDA) and became their faculty advisor. In the same year I developed the course Veterinary Medicine in Developing Nations which I gave in alternate years until 2010. The course was structured so that students would gain some insights into the many aspects of international development such as economics, sociology, agriculture, etc. in addition to a few selected veterinary topics. Many of the participating lecturers came from the Cornell faculty and were working on international development. They were happy to participate every other year. The course was originally restricted to twenty students to encourage discussion, but when the curriculum at the College changed (see below) I had to open it up and the class size grew to more than 80 students.

In 1990, the College of Veterinary Medicine started to look at the curriculum for the future and 4 committees were formed to plan the veterinary education for the 21st century. I participated in the basic science committee and we did not make much progress in the beginning which was frustrating to me and committee members, Nelly Farnum and Roy Pollack. Finally, the three of us developed a plan that was accepted by our entire subcommittee and led to the development of the ‘problem-based curriculum’ which was implemented in 1993. This opened another avenue for me to teach as a tutor in the small group problem-based learning system, which I really enjoyed.

**International work while at Cornell and after retiring**

My involvement with the VIDA students and the development of my course were only a part of the international aspects of my career. In 1985 the College had initiated a program to send veterinary students to developing countries, which is now known as the Expanding Horizons Program (EHP). Students are invited to submit proposals to spend 8 weeks in a developing country, but they have to make the contacts themselves and cannot go with a classmate. Applications are evaluated by a committee and this is the only college committee I still serve on. Funding for this program comes from a variety of sources, including the Ellsworth Foundation, an endowment which I set up when I retired, using some of my leftover college funds from the SB-1 royalty income. In addition, I initiated a second endowment starting with poultry industry support and gifts from individuals. It became fully endowed by a significant contribution from the College Alumni Association. On average each year between 7 and 10 students receive funding and the experience often changes their career interests.

I also continued my personal interest in short-term assignments in developing countries. In 1984 and 1985, I was a short-term expert for the FAO stationed in Concordia, Santa Catarina, Brazil to evaluate avian research projects at the Centro Nacional de Pesquisa de Suínos e Aves (CNPSA) of EMBRAPA. During these two visits I worked with CNPSA scientists and “bolsistas,” one of which was Liana Brentano who subsequently received her MSc degree from Cornell University and PhD degree from the University of North Carolina. Upon her return to CNPSA she started to work on CAV, cooperating with my team in Cornell. During my third visit to CNPSA in 1990, now as a consultant for IICA (Inter-American Institute for Cooperation on Agriculture), Liana and I reported the first isolation of CAV in South America (Avian Dis. 35:793, 1991).
Another interesting international experience was my visit in 1993 to the College of Veterinary Medicine and Agriculture, Universiti Pertanian in Malaysia where Dr. Aini Ideris was my host. We had previously collaborated on field testing the heat-tolerant Newcastle disease vaccine in The Gambia. This was originally an Expanding Horizons project for Dr. Jarra Jagne, now a faculty member at the College of Veterinary Medicine at Cornell, an AAAP member and member of the Board of Directors (BOD) of the AAAP as Director at Large (2019-2020).

In 2000 I served as the Scientific Team Leader for the external review of the Middle East Research Cooperation project on tumor viruses in birds, which included visits to Egypt, Israel, Jordan and the Palestinian Authority. Other scientific members of the team were Drs. Pat Wakenell and Dr. Ching-Ching Wu.

Since my retirement I have kept my connection with AAHL in Australia, working with Tim Doran and his postdoc Arjun Challagulla on using CRISP/Cas9 to generate transgenic birds expressing CRISPR sequences against ICP4 of MDV. Earlier as a consultant for the EW group I had been involved with Tim and many others including Dr. Jim McKay of the EW group in a project to generate transgenic chickens (Transgenic Res. 22:1257, 2013). We have recently published a paper describing our work with CRISPR/Cas9 chickens (Animal Biotech, In press) and a second paper is in preparation.

The American Association of Avian Pathologists (AAAP)

Since 1979 I have participated in all AAAP national meetings except during my sabbatical leaves in England and The Netherlands. After the rather shocking experience in 1972 (see “The first 5 years after graduating”) I found the AAAP very welcoming and very much enjoyed the annual meetings. My involvement with the AAAP includes being on the Board of Directors, representing the Northeast Region (2017-2021), serving on the editorial board and advisory board of Avian Diseases starting in 1989 and 2015, respectively and still continuing. In addition, I served on the Awards Committee including 3 years as chair from 1997 to 2000. Currently I am still involved with the Tumor Virus Committee (since 1985) and the History of Avian Medicine Committee (since 2013). It has been fun and satisfying to be involved with this great association.

The International Marek’s Disease Symposia

I was very fortunate to participate in the first International Symposium on Marek’s disease in 1978 in West-Berlin, thanks to financial support from Dr. Manfred Krasselt of Gist-Brocades. I knew Manfred from my training period in 1970 with Bart Rispens when Manfred learned how to prepare the CVI-988 MDV vaccine. This meeting was very exciting and stimulating for me shortly after receiving my PhD and it became the first one of a long series which I attended. In 1984 Bruce Calnek and I organized the second meeting in Ithaca, which was followed by Symposia at 4 year intervals in Osaka, Japan (1988), Amsterdam, The Netherlands (1992), East-Lansing, U.S.A. (1996), Montreal, Canada (2000), Oxford, England (2004), Townsville, Australia (2008) and Berlin, Germany (2012). Since 1994, there were also molecular workshops in between the Symposia.
until 2010 when it was decided to have the symposia at 2 year intervals with the addition of other avian herpesviruses to the program. These meetings were held in East-Lansing, U.S.A. (2014), Tours, France (2016) and Yangzhou, China (2018). The 2020 meeting was scheduled to be held in Guelph, Canada, but had to be postponed due to the SARS-COV-2 pandemic. At the time of writing it is not clear if it will be held at a later date or that it will be cancelled. I am the only one to have been participating in all MD symposia and workshops. My involvement included organizing the Montreal meeting, spearheading the fundraising for several meetings as well as working on the program committees. It has been a wonderful experience to be part of for such a long term (42 years!) activity of the MD community, a truly international group of researchers.

Retirement

At the end of 2011 I retired from Cornell University but continued my association with the College as emeritus professor, which provides me with office space, access to the library system and some other benefits. In return I give a few lectures to the DVM students and still advise a few students on career options now and then. Professionally I was kept busy with editing the second edition of Avian Immunology and currently am working as a section editor on the third edition; writing book chapters and review papers. The latter included the history of the first four MD vaccine strains (Avian Dis 60:715, 2016). This was very timely because I personally knew all the important researchers in the USA, England and The Netherlands. It was a great opportunity to contact many of the key people to get additional information before it would have been too late. Since writing the paper, two of the Dutch people have passed away.

Other activities at the professional level include international consultancies in India, Mexico and Europe and lecturing in many parts of the world. The consulting with the Venky’s group in Pune, India was very interesting. I was involved in designing a new research facility for viral research and when it was ready I officially opened the building (Fig. 19). I find the international consultancies very inspiring and hope to continue for a few more years once the COVID-19 pandemic has slowed down enough to permit travel again.

Laura and I have also taken the opportunity to travel to Africa, Ecuador and the Galapagos (Fig. 20) for some birding and wildlife trips and hope to have the opportunity to make several more trips. Local birding and bird photography are other activities as well as cross-country skiing and road biking. In addition, we enjoy time with our granddaughters Antonia and Alida, especially during the summer months when they are with us for 2 to 3 weeks.
Looking back

When I matriculated in the College of Veterinary Medicine at the University of Utrecht in 1962 it was with the goal of becoming a dairy veterinarian in The Netherlands, never thinking about becoming a professor at Cornell University and certainly not in poultry diseases. It is 50 years ago that I started my work in poultry disease research not knowing that it would become my life-long career. It has been a great experience and if I had to do it over I would do it the same way. Poultry disease research is important to provide meat and eggs to a growing population and there is a global need for research. This need has provided me with experiences in many parts of the world not only in the research arena but also in my personal life with many new friendships around the world. In addition to the research I also enjoyed teaching the DVM students and I am still in contact with a number of my former DVM students.

My research has been recognized by my peers resulting in several awards (see appendix 1, my CV for details). Of special importance for me are the Bart Rispens Award for the best paper in Avian Pathology (1987) and the Ezra Technology Innovator Award from Cornell University for the SB-1 vaccine which was presented to Bruce Calnek and me in 2015. Both Dr. Rispens and Dr. Calnek were great mentors and are certainly responsible in large part for my successful career in poultry research. In addition to my two mentors I also want to credit my many graduate students, postdoctoral fellows and visiting scientists (see appendix 2 for the complete list) and my lab manager Priscilla O’Connell for my successful career. Without the dedication to their research projects it would have been very different and less successful.

Finally, I want to mention Dr. Julius Fabricant once more. After his retirement he would frequently come to the College to have a cup of coffee and argue with me about any topic. Julius loved to argue about anything but unfortunately, we had similar ideas about a number of issues, which made it somewhat difficult. One morning he came in clearly ready to start an argument by saying “Ton, you do not care about poultry diseases, all you care about is DNA.” This statement was delivered in the typical Julius fashion with his arms going all over the place. I answered in first instance with “Julius that is not true” which resulted in “What is not true” again in the typical Julius fashion. My answer was “Julius, I care also about RNA” which precipitated a long and loud laughing response making me afraid that I had to call the ambulance. I certainly miss Julius.

Fig. 20. Visiting the Galapagos in 2014.
Addendum
Selected aspects of the career of Ton Schat are also documented in Merial Biographies reprinted from Merial Selections Vol 6, No. 1, pages 11-13 and the Merial Video Clips, both have been deposited in the AAAP Archives.

Biography solicited by the Committee on the History of Avian Medicine, American Association of Avian Pathologists.

Additional biographical materials may be available from the AAAP Historical Archives located at Iowa State University. Contact information is as follows:
  Special Collections Dept. & University Archives
  403 Parks Library
  Iowa State University
  Ames, IA 50011-2140