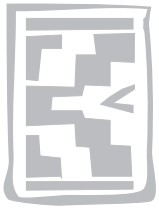

History of bluetongue research at Onderstepoort

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ABSTRACT

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Research on this economically important disease of ruminants, especially sheep, which had been named bluetongue by farmers in the 19th century, has been part and parcel of the activities at Onderstepoort ever since its establishment in 1908 and therefore covers a full century of the OVI's existence.

In view of Onderstepoort's centenary celebration a brief overview of this research is given in terms of the historic milestones which influenced and guided global research on this and other viral diseases of animals.

INTRODUCTION

Arnold Theiler had already proved the filterability of the etiological agent, and therefore its viral nature, in 1905. He had serially passaged the virus in sheep, believed that it had become attenuated and started using the blood of infected sheep as a primitive vaccine in 1906. As a result bluetongue vaccine became the third viral vaccine produced at Onderstepoort, following Theiler's famous rinderpest and human smallpox vaccines.

Later it was realized that Theiler was in fact using a relatively avirulent strain of the virus. Although the vaccine gave variable results it must have been reasonably effective as more than one million doses were already sold in 1916 and it was used with only minor improvements until the 1940s.

A full description of the disease bluetongue can be found elsewhere (Coetzer & Tustin 2004) as well as brief historical reviews of research on bluetongue at Onderstepoort (Bigalke & Verwoerd 2008; Verwoerd & Bigalke 2008).

KEY DISCOVERIES AND MILESTONES

Adaptation to cultivation in embryonated eggs

Following the successful cultivation and attenuation of African horsesickness virus (AHSV) in mouse brains, several attempts during the 1930s to repeat it with bluetongue virus (BTV) failed. Eventually, in 1940, Mason, Coles & Alexander managed to adapt the virus to growth in embryonated hen's eggs and also demonstrated attenuation in this system. However, serial passage was difficult and growth erratic. Nevertheless, they managed to obtain evidence for the existence of different strains, as was the case for AHSV, and by 1942 six strains have been identified, three of which had been adapted to cultivation in eggs.

Discovery of the insect vector of bluetongue

In 1944, the seminal discovery that members of the orbivirus genus, including both BTV and AHSV, is transmitted by *Culicoides* midges was made by R.M. du Toit, a veterinary entomologist, at Onderste-

poort. Farmers had known for many years that stock losses caused by these viruses could be limited by keeping animals indoors after sundown and by avoiding wetland pastures, which suggested the involvement of an insect vector. The development of a light trap which facilitated the catching of large numbers of these tiny insects finally enabled Du Toit to prove the biological transmission of both viruses by *C. variipennis (= imicola).*

Development of an avianized vaccine

The discovery by Alexander in 1947 that the optimal temperature for the cultivation of BTV in embryonated eggs is 33.5 °C and not 37 °C solved the problem of viral propagation. Soon a number of BTV strains were attenuated and in the same year a trivalent (soon followed by a quadrivalent) live attenuated vaccine was launched, replacing the old Theiler vaccine. Initially, however, the vaccine was still produced in sheep. The product was safer and more effective, leading to an increased demand which could not be met using sheep for its production. The vaccine was therefore produced in embryonated eggs but still suspended in sheep's blood to extend its shelf life.

Immunity and cross-protection

Immunological studies carried out by Neitz in 1948 on the available BTV strains clearly demonstrated for the first time that immunization with a particular strain resulted in lifelong solid immunity to the specific strain but little or no immunity to other strains. It explained the vaccine failures encountered with the monovalent Theiler vaccine and also formed the basis for later studies on the serotyping of isolates and for the development of multivalent vaccines giving maximum protection under conditions of varying preponderance of different strains.

Lyophilization to the rescue

In 1950, when Alexander took over as Director of the OVI, he was confronted by a crisis in terms of a short-supply of bluetongue vaccine. Following particularly heavy rainfall and a dramatic increase in the price of wool and wool-producing sheep, the demand for vaccine suddenly jumped from 2.5 million doses the previous year to over 6 million. There was a shortage of eggs and low fertility in those available and to make things worse the vaccine produced in embryonated eggs had a short shelf life. To solve the latter problem lyophilisation was introduced, facilitated by the discovery that buffered lactose-peptone is an excellent stabilizer of the virus. Vaccine could subsequently be stockpiled for periods of greater demand and production increased to more than 24 million doses by 1958.

Internationalization of the problem

The outbreak of bluetongue in Cyprus in 1943 was the first report of the disease outside Africa. In 1952 Onderstepoort also confirmed the presence of BT in Israel. In the same year, a condition named 'sore muzzle' was described in California and its similarity to bluetongue noticed. In 1953 Alexander was invited to the USA for expert advice and he was able to confirm the diagnosis based on preserved material. The virus was subsequently isolated at Onderstepoort and later identified as serotype 10. In 1956 BT outbreaks also occurred in Portugal and Spain and the virus identified as BTV serotype 10.

Cultivation in cell cultures

The next major achievement was the cultivation of the egg-adapted BTV in primary lamb kidney cells by Haig and co-workers in 1956. It first led to the development of a group-specific complement-fixation test and later to a practical and relatively inexpensive neutralization test used by Howell for serotyping isolates. By 1960 he had described 12 and by 1970, 16 distinct serotypes. Onderstepoort was in a unique position to assist other countries in identifying and typing the virus responsible for their outbreaks and was appointed in 1963 by the OIE as a World Reference Centre for bluetongue, a responsibility it still carries today.

Vaccine production in cell cultures

Passaging the various serotypes in cell cultures and testing for attenuation and immunogenicity was the logical next step in order to convert vaccine production from eggs to cell cultures. The number of serotypes in the polyvalent vaccine, first released in the early 1960s, increased rapidly and by 1968 numbered 14. In the same year production was switched to BHK21 cells in roller cultures, another major improvement. However, protection obtained with the polyvalent vaccine was unsatisfactory due to interference between strains and variation in immunogenicity. For the next decade or so Erasmus and Weiss concentrated on solving the problem by plaque-purifying each serotype and selecting suitable vaccine strains. By 1982, 15 such strains had been isolated and was incorporated into three pentavalent vaccines which gave a good immunity when correctly administered.

Molecular biology of bluetongue virus

BTV was selected for molecular studies by Verwoerd in 1964 because so little was known about the structure and replication of this economically important virus. Mass production and purification of the virus was the first step before morphological studies and biochemical analysis could start. Surprisingly electron microscopy revealed two differently sized particles first thought to be two viruses. At the time all known viruses consisted of a protein capsid containing a genome which was either double-stranded or single-stranded DNA or single-stranded RNA. Our chemical analysis clearly indicated RNA but physico-chemical characteristics were those of DNA.

The conclusion that the virus has a double-stranded RNA genome was rather revolutionary and not easily accepted at first. In addition, it was found that the genome consists of ten segments and that the virus possesses a double-layered protein capsid. It was, therefore, clear that we were dealing with a uniquely structured virus, which later gave rise to its classification in a new genus orbivirus (Verwoerd, Els, De Villiers & Huismans 1972).

Molecular genetics

During the 1970s, molecular research concentrated on genetic aspects such as the relationship between the ten genome segments and the viral proteins. It was shown that seven of the segments act as genes coding for viral proteins, the other three for non-viral proteins found in the infected cell. Much effort was spent on elucidating the role of the various components in the replication of the virus in an attempt to better understand the pathogenesis of the disease. It was also the decade during which many new techniques such as cloning and sequencing of nucleic acids were developed which collectively became known as biotechnology. These techniques were also applied to BTV and led *inter alia* to the development of new diagnostic tests using cloned genome segments as diagnostic probes.

First recombinant subunit vaccines

The 1980s were characterized by various attempts to develop recombinant subunit vaccines and the further development of improved diagnostic techniques. Considerable excitement was created when Huismans, Van der Walt, Cloete & Erasmus (1987) demonstrated the immunogenicity of a single outer capsid protein (VP2), supporting the feasibility of developing recombinant vaccines. Genome libraries were constructed and the first recombinant vaccines developed in collaboration with researchers at Oxford, using the baculovirus of insects as vector. Various combinations were shown to be effective as monovalent vaccines but unfortunately problems encountered in upscaling production has so far precluded its commercial application.

Advanced diagnostics

During the 1990s, the cloning and sequencing of the ten genome segments of all 21 South African serotypes continued. Cross-hybridization of these DNA clones identified segment five as the most suitable group-specific probe as it is highly conserved in all serotypes, whereas segment two, coding for an outer protein, is best for serotype-specific probes. In 1991 a series of sequencing studies on BTV isolates from various countries led to the discovery of 'topotypes', or geographical variants within serotypes which can be used to determine the origin of an outbreak. Improving diagnostic tests started to dominate the scene during this period, following the disappointing progress with the development of new generation vaccines.

Globalization of the disease

Between 1956 and 1999 limited outbreaks of BT occurred in a number of countries, mostly in the middle East, South America and southern Europe. The new millennium with its climate change and increased mobility of man and animals brought about an explosion of new outbreaks and the involvement of northern Europe for the first time. Since 1999 there have been outbreaks in Greece, Italy, Corsica, the Balearic Islands and various Balkan countries, involving serotypes 2, 4, 9 and 16. In 2002 BT 4 was also reported in Spain and Portugal. The live attenuated BT vaccine from OBP was used in different countries: BT 2, 4, 9 and 16 in Italy, BT 2 and 4 in Corsica, and BT 2 and 4 in Spain and Portugal. Since 2006 BT 8 has emerged in Northern Europe, presumably due to global warming, also infecting cattle in the Netherlands, Belgium, Germany, Luxembourg and France. In the summer of 2007, BT 8 also spread to the UK, Switzerland, Denmark, Spain, Poland and Italy. It is clear that in spite of all our accumulated knowledge further research is needed to finally conquer the disease.

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