



An appreciation of the seminal contributions of John Brooksby and Fred Brown on foot and mouth disease

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Summary

John Brooksby was an outstanding Scottish veterinary virologist who worked at the Pirbright Institute (Pirbright) for 40 years, including 16 as the institute's director. He devised quantitative methods for measuring neutralising antibodies and perfected a complement fixation test for the diagnosis, typing and strain differentiation of foot and mouth disease (FMD), especially when combined with neutralisation. He identified four of the seven types of FMD virus (FMDV) and many subtypes. Consequently, the institute was designated the World Reference Laboratory for FMD. As director, Brooksby also oversaw advances in the pathogenesis, epidemiology and aerobiology of FMD and other diseases. His advice on the prevention and control of FMD was widely sought by international organisations and individual countries.

Fred Brown was an eminent English biochemist and molecular virologist. He joined the Biochemistry Department at Pirbright in 1955, became head of the department in 1964, and in 1980 became deputy director of the institute. Advances under his leadership included the use of aziridines as inactivating agents for vaccine production, purification of FMDV suitable for biochemical analyses, demonstration of the infectivity of isolated RNA, analysis of the genomic and antigenic structure of FMDV, solving of the atomic structure of FMDV and demonstration of the potential for synthetic peptide vaccines to protect animals against virus challenge.

Keywords

Antigenic structure – Complement fixation – Diagnosis – Foot and mouth disease – Genome structure – Neutralisation – Serotypes – Vaccination – Vaccines – Virus.

Introduction

In 1987, Loeffler and Frosch [1] in Germany demonstrated that vesicular fluid from the tongues of cattle with foot and mouth disease (FMD) that had passed through a bacterial filter would infect further cattle. This was the first demonstration that a disease of animals could be caused by a filter-passing agent, later termed a virus. Following this pioneering work, great advances in knowledge have been made, leading to improved methods to control the disease. Nevertheless, FMD remains a serious problem in livestock production and a major constraint on trade in animals and animal products.

Many scientists working on FMD in various institutions throughout the world have contributed greatly to advancing

knowledge of FMD. This article discusses the work of only two of many exceptional contributors, namely John Brooksby and Fred Brown, who worked at the Animal Virus Research Institute, now The Pirbright Institute (Pirbright), in the United Kingdom from 1939 to 1979 and from 1955 to 1983, respectively. Their investigative work on the control of the disease and the structure and function of the virus was of major significance and was seminal in influencing the evolution of the science of virology and the control of other viral diseases.

Three of the authors of this article were privileged to work with Brooksby and Brown at Pirbright and so knew them personally and had first-hand knowledge of their scientific contributions.

Part one: the contributions of Dr John Brooksby (1914–1998)

John Brooksby (Fig. 1) joined Pirbright in 1939. His early career focused on the diagnosis of FMD virus (FMDV), as well as its serology and quantitation [2]. He contributed to the method of virus titration by cattle tongue inoculation [3] and adapted this method to develop a neutralisation test for assessing vaccine potency. Together with colleagues, he elucidated the antibody response to FMD infection and vaccination [4]. Following the work of Heidelberger, he developed and perfected a complement fixation test (CFT) [5] that was adopted globally and that he used subsequently to identify the Southern African Territories (SAT 1, SAT 2 and SAT 3 serotypes) in 1948 and the Asia 1 serotype in 1954 [2]. The sensitivity and precision of the CFT also led to the identification of subtypes and variants such as A₂₂ in 1964, providing essential information for the selection of vaccine viruses. In addition, the CFT contributed to differential diagnosis between FMD and vesicular stomatitis [2] and later swine vesicular disease, the latter first identified by the institute in 1972 [6].

Brooksby, together with Henderson, investigated the survival of FMDV *post mortem* in meat and found that the virus could persist in the bone marrow, lymph nodes and offal of infected cattle [7]. This led to the application of international control measures including boning out and maturation of meat and heat treatment of offal before importation from infected countries, and greatly facilitated international trade.

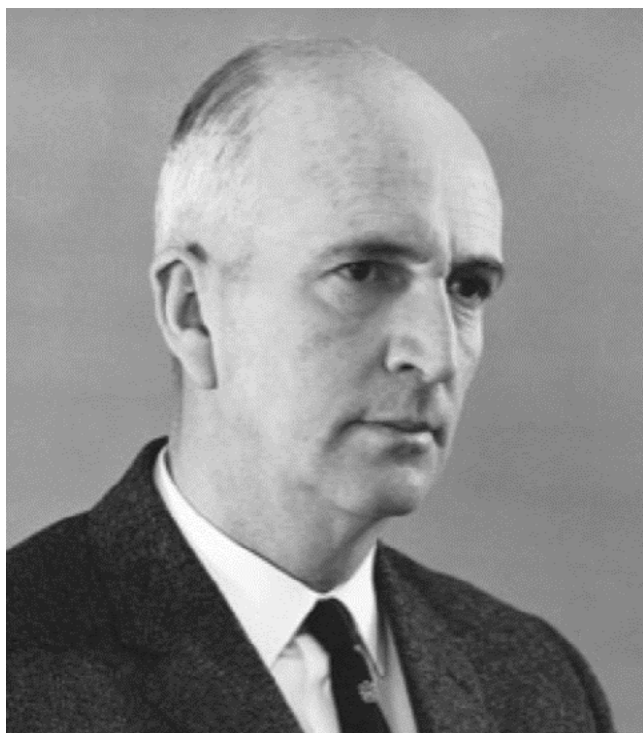


Figure 1
Dr John Brooksby

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Pirbright was extensively involved in research during the 1946 Mexico epidemic of FMD, and Brooksby was the lead scientist, showing the effectiveness of the CFT in diagnosing FMD and of the CFT and serum neutralisation in cattle in distinguishing between strains of FMD and vesicular stomatitis [8-14].

Beginning in 1948, when Brooksby was head of the Pirbright Serology Department, a systematic examination and classification of samples from overseas established a global picture of the distribution of the types of FMD. The task was aided by the good relationships developed between Pirbright and other laboratories, including the Pan American Center for Foot-and-Mouth Disease and Veterinary Public Health in Brazil, the Plum Island Animal Disease Center in the United States and others throughout Europe and Africa. The exchange of scientific information and, in some cases, personnel among these institutions was mutually beneficial.

Proposals made in 1955 for a central reference laboratory [2] were realised by the appointment of Pirbright as the World Reference Laboratory for FMD by the Food and Agriculture Organization of the United Nations (FAO) in 1958 and by the World Organisation for Animal Health (WOAH, then the OIE) in 1960 [15].

In 1957, Brooksby was appointed as deputy director and the institute pursued the improvement of the existing FMD vaccines. A major achievement in the 1960s was the use of a continuous clone of characterised, baby hamster kidney (BHK) monolayer cells for the growth of FMDV and the adaptation of the cells to suspension culture to produce the FMD antigen for vaccine manufacture [16,17]. This was allied to the introduction of aziridine, rather than formalin, for first-order virus inactivation [18], enabling the worldwide production of a safe, potent vaccine on an industrial scale.

In 1963, Dr I.A. Galloway retired and Brooksby became director. In October 1967, type O₁ FMD was reported in the United Kingdom (UK) [2]. Pirbright staff assisted with field investigations, and Brooksby initiated studies on the possible source of the virus and the behaviour of the causal strain in cattle, sheep and pigs. These studies identified the role of the bulk transport of infected milk as an additional important mode of dissemination of FMD, and this mode of excretion was further investigated in pathogenesis studies [19]. The effect of disinfectants and the survival of virus in heat-treated milk were also studied [2]. Brooksby was heavily involved with the subsequent committee of inquiry and in making recommendations for future prevention and control of the disease [20,21].

One of the committee's recommendations was that Pirbright should manufacture and stockpile SAT vaccines for possible use in the event of future outbreaks in the UK. The Frenkel vaccines were produced but never employed in the UK [2]. However, when Pirbright identified the first Middle East

incursions of type SAT 2 in 1962 and of the variant subtype A₂₂ in 1964, such vaccines were supplied for the control of the FMD outbreaks in Israel, Turkey and Greece [2].

Evidence at the start of the 1967 epidemic pointed to airborne spread, and Brooksby asked Dr R.F. Sellers to investigate. Sellers and Parker [22] demonstrated that pigs could be a major source of airborne FMDV. Studies by Burrows *et al.* [19] showed that the respiratory route was the main method by which FMDV infected cattle and sheep, with initial multiplication in the pharyngeal area. Donaldson and Alexandersen carried out extensive studies on the aerobiology of FMDV, laying the foundations for the development of predictive models for airborne dissemination [23].

Brooksby, who had a special interest in the control of animal disease in Africa, seconded staff to assist in the control of FMD, African swine fever and rinderpest in East Africa. In addition, he instigated studies on the potential role of wildlife in the epidemiology of FMD. Cape buffalo (*Syncerus caffer caffer*) were shown to be long-term carriers of the virus, and transmission was demonstrated in both directions between infected buffalo and cattle [24]. Impala (*Aepycerus melampus*) were the most affected of the antelope species investigated and were found to be capable of transmitting FMDV to cattle.

Pirbright also investigated the potential role that Australian marsupials might play in the epidemiology of FMD. Of the 11 species studied, the red kangaroo (*Macropus rufus*) showed only moderate susceptibility to experimental infection, and it was concluded that these animals would not play any significant role in any future antipodean outbreak [2]. During these studies the Australian veterinarian overseeing the project established that primary calf thyroid cultures are the most sensitive cells for detecting live FMDV [25].

The increased volume of samples received from different parts of the world caused problems with subtyping, and in 1967 Brooksby redefined type and subtype [26]. In 1974, Dr H.G. Pereira was appointed head of epidemiology. He proposed, and Brooksby agreed, that field isolates should be related to current strains in vaccines and to reference strains from past outbreaks [27].

As director of the World Reference Laboratory, Brooksby was frequently called on to advise international animal health organisations and individual countries on FMD, including its diagnosis, distribution and spread, the viruses to be included in vaccines and overall measures for control and prevention.

In 1964, countries free of FMD wished to import European breeds of cattle. Brooksby played a leading role in the development of test protocols to enable the safe international export of European cattle to many FMD-free countries, including the UK, the Republic of Ireland, Canada, the United States of America, Australia and New Zealand, enabling the

beef-producing qualities of European breeds to be introduced into those countries [2].

Through the close contact he had with Kenya, he promoted the establishment of an FMD-free zone in that country. Although the zone was not sustained, it was a legacy to Brooksby and served in many aspects as a model for free zones developed elsewhere in Africa, Europe and South America. Brooksby saw the advantages of FMD-free zones in providing greater opportunity for the export of animals and animal products. While the carrier state in African buffalo (*Syncerus caffer*) precludes the global eradication of FMD, greater acceptance of commodity-based trade, as promoted by Thomson *et al.* [28], would increase the export opportunity for animal products from endemic regions worldwide.

Brooksby was chairman of the WOAHC Commission on FMD and of the Research Group of the FAO-led European Commission for the Control of Foot-and-Mouth Disease. He retired in 1979. Among many honours, he was elected as a fellow of the Royal Society in 1980 [2].

Brooksby made a major and lasting contribution to the worldwide understanding, control and prevention of animal disease, in particular FMD.

Part two: the contributions of Dr Fred Brown (1925–2004)

The work of Fred Brown (Fig. 2) at Pirbright included both vaccine development and the molecular biology of FMDV and other viruses. Brown was elected as a fellow of the Royal Society in 1981 in recognition of his contributions to science [29]. As mentioned, the development of the BHK cell system was a massive advance for vaccine production, and it was followed in 1963 by Brown and colleagues showing that aziridines (such as acetyleneimine and binary ethyleneimine) inactivated FMDV with first-order kinetics, removing the uncertainties associated with the use of formaldehyde [18].

The 1950s saw the emergence of molecular virology, and Brown made the seminal observation that purified RNA isolated from FMDV was infectious in susceptible cells [30]. Detailed analysis of the viral proteins had to await further developments in experimental techniques, including devising methods for producing highly purified virus particles, which he achieved in 1963 [31].

Brown's laboratory made extensive use of evolving techniques to determine the protein composition of virus particles and the non-structural proteins involved in virus replication [32–34]. A non-structural protein was recognised by sera from infected animals and was termed VIA, for virus infection associated antigen. Because of the high levels of VIA antibodies in post-infection sera, it was the first antigen used to



Figure 2
Dr Fred Brown

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distinguish vaccinated from infected animals. Subsequent *in vitro* biochemical studies identified VIA as the viral RNA-dependent RNA polymerase [35]. Eventually, *in vitro* biochemical methods, together with molecular cloning and sequencing of the viral genome [36], enabled mapping of the distribution of all the protein coding regions on the FMDV genome [37,38].

Through the application of an elegant combination of biochemical, electron microscope and X-ray crystallographic methods, an immune-dominant feature that coincided with the cell receptor-binding domain of the virus was identified [39]. This initiated a collaborative pioneering study with Richard Lerner's laboratory at the Scripps Research Institute in California, resulting in the demonstration that a synthetic peptide vaccine representing this antigenic site could protect laboratory animals [40]. Subsequent studies identified the importance of helper T cell epitopes in the immune response [41] and became the driving force for determining the atomic structure of FMDV, which was accomplished in collaboration with David Stuart's laboratory at Oxford University [42]. This groundbreaking work was the forerunner of current endeavours to develop recombinant virus-like-particle (VLP) FMD vaccines.

Many unusual features of the FMDV genome were identified, including a long homopolymeric stretch of cytidine residues (the poly C tract) [43] and the triplication of the peptide VPg, which initiates RNA replication [44,45]. Brown's laboratory

also developed T1 mapping for exquisite discrimination of virus strains before nucleotide sequencing was readily available. This method was used to analyse virus samples from 1982 European outbreaks of FMDV, which spread from Brittany in France to Jersey and the Isle of Wight [46]. The study demonstrated that the virus responsible for the outbreaks originated from an incompletely formalin-inactivated vaccine, thus vindicating Brown's earlier demonstration of the superiority of aziridines as inactivating agents for vaccines and provided supporting evidence for wind-borne transmission of FMDV [23].

Brown left Pirbright in 1983 and joined Wellcome Biotechnology, a major manufacturer of FMD and other vaccines. Beginning in 1990, he conducted his final research on FMD at the Plum Island Animal Disease Center in New York. He moved back to the UK in 2003, just five weeks before he died.

Brown's fundamental, collaborative and pioneering research employed and extended the techniques of molecular biology to elucidate the replication, structure and function of FMDV and many other viruses. His work had great practical application and was also strongly influential in the overall development of virus research.

Part three: the Brooksby–Brown legacy

Current research activities build on the achievements of the Brooksby/Brown era. For example, the earlier work by Brown and his team on FMDV-structure function, which led to exploring i) the potential for peptide vaccine and ii) the current endeavours to develop recombinant VLP FMD vaccines, have created prospects for future FMD vaccines that do not require the growth of virus for their production. Furthermore, the future application of artificial intelligence may further enhance the early work on vaccine development by improving antigenicity and product stability.

Such technological developments are likely to stimulate policy strategies towards international FMD control and thereby facilitate global trade, realising Brooksby's vision for the increasing inclusion of developing countries. There are already encouraging signs in at least two areas: i) the establishment by WOA and FAO of Regional Reference Laboratories for FMD in Central, Eastern and Southern Asia [47] and ii) the system of commodity trade involving de-boning and removal of lymph nodes [7] now being championed, especially in Southern Africa [28], which should expand FMD-safe meat export.

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Un hommage aux contributions essentielles de John Brooksby et de Fred Brown sur la fièvre aphteuse

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Résumé

John Brooksby était un virologue vétérinaire écossais de tout premier plan, qui a travaillé pendant 40 ans à l'Institut Pirbright (Pirbright), dont seize années en tant que directeur de cette institution. Il est le concepteur de méthodes quantitatives de titrage des anticorps neutralisants et a également perfectionné une épreuve de fixation du complément pour le diagnostic de la fièvre aphteuse et la caractérisation et différenciation des souches du virus aphteux, en particulier pour son utilisation en association avec la neutralisation virale. Il a identifié quatre des sept types de virus de la fièvre aphteuse ainsi que de nombreux sous-types. Son département a été désigné Laboratoire de référence mondial pour la fièvre aphteuse. En tant que directeur, il a également surveillé l'évolution pathogénique, épidémiologique et aérobiologique de la fièvre aphteuse et d'autres maladies. Plusieurs organisations internationales et pays ont sollicité son avis sur des questions de prévention et de lutte contre la fièvre aphteuse.

Fred Brown était un éminent biochimiste et spécialiste de virologie moléculaire anglais. Il a intégré le département de biochimie de Pirbright en 1955 et a pris la direction de ce département en 1964 ; il a été nommé directeur adjoint de l'Institut en 1980. Nombre d'avancées ont été réalisées sous sa conduite, notamment le recours à des aziridines pour l'inactivation de souches vaccinales, la purification du virus de la fièvre aphteuse à des fins d'analyse biochimique, la démonstration de l'infectiosité d'isolats d'ARN, l'analyse de la structure génomique et antigénique du virus de la fièvre aphteuse, l'élucidation de la structure atomique du virus de la fièvre aphteuse et la démonstration que les peptides de synthèses peuvent conférer une protection aux animaux soumis à une inoculation d'épreuve.

Mots-clés

Diagnostic – Fièvre aphteuse – Fixation du complément – Neutralisation – Sérotypes – Structure antigénique – Structure génomique – Vaccination – Vaccins – Virus.

Reconocimiento de las contribuciones clave de John Brooksby y Fred Brown sobre la fiebre aftosa

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Resumen

John Brooksby fue un destacado virólogo veterinario escocés que trabajó en el Instituto Pirbright (Pirbright) durante 40 años, 16 de ellos como director de la institución. Desarrolló métodos cuantitativos para medir los anticuerpos neutralizantes y perfeccionó una prueba de fijación del complemento para el diagnóstico, la tipificación y la diferenciación de cepas de la fiebre aftosa, especialmente en combinación con la neutralización. Además, identificó cuatro de los siete tipos de virus de la fiebre aftosa (VFA), así como numerosos subtipos. Durante su gestión, el departamento que dirigía fue reconocido como Laboratorio mundial de referencia para la fiebre aftosa. Como director, también supervisó avances significativos en la patogénesis, epidemiología y aerobiología de la fiebre aftosa y otras enfermedades. Su asesoramiento en materia de prevención y control de la fiebre aftosa tenía gran demanda entre organizaciones internacionales y distintos países.

Fred Brown, reconocido bioquímico y virólogo molecular inglés, se unió al Departamento de Bioquímica de Pirbright en 1955. En 1964 asumió la dirección de dicho departamento y en 1980 fue nombrado subdirector del instituto. Bajo su dirección se lograron avances clave, entre ellos el uso de aziridinas como agentes inactivadores en la producción de vacunas, la purificación del VFA a fin de hacerlo apto para análisis bioquímicos, la demostración de la infectividad del ARN aislado, el análisis de la estructura genómica y antigénica del VFA, la resolución de la estructura atómica del VFA, y la demostración del potencial de las vacunas basadas en péptidos sintéticos para proteger a los animales frente al desafío del virus.

Palabras clave

Diagnóstico – Estructura antigénica – Estructura del genoma – Fiebre aftosa – Fijación del complemento – Neutralización – Serotipos – Vacunación – Vacunas – Virus.

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