STRAINS OF AFRICAN SWINE FEVER VIRUS
ISOLATED FROM DOMESTIC PIGS
AND FROM
THE TICK ORNITHODOROS MOUBATA
IN SOUTH AFRICA

BY

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SUMMARY

Between 1973 and 1975, 21 outbreaks of ASF were confirmed in the endemic area of the northern Transvaal after an interval of 10 years in which the disease was silent. The new series of outbreaks coincided with the isolation, for the first time in South Africa, of two HAd\(^{-}\) strains of ASF virus. The first of these virus isolates, Lillie-148, was obtained from swine which, judging from circumstantial evidence, had been infected by a warthog carrier of virus. The pigs on the farm were affected by a form of disease with a lower pathogenicity than that observed in previous epidemics. The second HAd\(^{-}\) strain 24823 was obtained from a case from which neither clinical nor pathological observations were available. From the results of the biological tests carried out at the laboratory, however, it was deduced that the disease in the field may have had a chronic course.

When the carrier status of populations of the argasid tick *Ornithodoros moubata* collected from warthog burrows was investigated, it was found that the situation in South Africa is analogous to that in East Africa. Twenty five per cent of burrows were found to be infected; the mean infective titres of the tick suspensions varied between \(10^{4.5}\) and \(10^{5.2}\) BCHA\(_{50}\) and the mean percentage of infected argasids varied between 1.62 and 3.45. Infected ticks were also found in the Marico district, which is adjacent to the endemic area, but ASF has never been recorded there. From tick suspension TS237, showing both delayed and reduced haemadsorbing effect in buffy coat cell cultures, a HAd\(^{-}\) population of ASF virus was segregated.
From these observations it was inferred that ASF virus may mutate from the HAd\(^+\) to the HAd\(^-\) form in the primary virus reservoir. Furthermore, the virus appears to be evolving towards less pathogenic forms irrespective of prior adaptation of the infectious agent to domestic stock under the epidemiological conditions prevailing in South Africa.

During this investigation it was found that LLC-MK\(_2\) cell cultures were susceptible to ASF virus. Cytopathic effects were observed in primary isolation and peak infectivity coincided with complete destruction of the cell monolayers, attained after three to four serial passages. The sensitivity of LLC-MK\(_2\) cells for estimating the virus content of porcine tissues was in two instances comparable to that of buffy coat cells, but in another three cases it was 100 to 1000 times lower. It was concluded that LLC-MK\(_2\) cells were a suitable complement to buffy coat cultures for the cultivation of ASF virus, particularly for HAd\(^-\) isolates.

After 35 to 45 serial passages in LLC-MK\(_2\) cells the HAd\(^+\) strains of ASF virus lost their haemadsorbing characteristics. A similar mutation, but more gradual, was also observed in buffy coat cell cultures.

The feasibility of plaque production was studied in LLC-MK\(_2\) cell monolayers. Plaques were obtained with all the strains studied, irrespective of their adaptation to LLC-MK\(_2\) or buffy coat cells when 0.4% Agarose was used as a solidifying agent. The diameter of plaques ranged from 0.3 to 3.0 mm and this characteristic was unrelated to the haemadsorbing properties of the strains used. Plaque technique was successfully used to detect the presence of HAd\(^-\) virus particles in HAd\(^+\) populations by subculturing selected virus-plaques into buffy coat cultures.

The results of biological tests suggested that HAd\(^-\) strains have a reduced virulence which can vary within broad limits. The experience with strain Lillie-148 and 24823 showed that either acute or chronic or subclinical disease can follow infection of pigs with these isolates of virus. The results obtained with the two virus populations of strain TS237 emphasized the different degree of pathogenicity between HAd\(^+\) and HAd\(^-\) virus. While the former was responsible
for a peracute or acute form of disease, the latter produced chronic or subclinical infections. In pigs mild forms of ASF also developed following the inoculation of HAd+ strains obtained after serial passages in cell cultures. It was concluded that haemadsorption and pathogenicity are two characteristics that are not linked and can be modified independently.
Gedurende die tydperk 1973-1975 het Afrikaansevarkpes (AVP), na 'n afwesigheid van 10 jaar, weer sy verskyning gemaak in die endemiese gebied van Noord Transvaal en altesaam 21 bevestigde gevalle is aangemeld. Die nuwe reeks uitbrake het saamgeval met die eerste isolasie in Suid-Afrika van twee HAd-v stamme van AVP. Die eerste virusstam wat geïsoleer is, was Lillie-148. Hierdie virusstam is geïsoleer van 'n vark wat volgens omstandighedegetuienis deur 'n vlakvark besmet is. Die virus waarmee die varke op die plaas besmet is, het 'n laer patogenisiteit gehad as virusse van vorige uitbrake. Die tweede HAd-v stam nl. 24823 is verkry van 'n geval waar geen kliniese of patologiese waarnemings beskikbaar was nie. Uit die resultate van laboratoriumtoetse is die gevolgtrekking gemaak dat die siekte wel moontlik 'n kroniese verloop kon gehad het.

Uit ondersoeklike na die vektorstatus van populasies van die sagte bosluis Ornithodoros moubata, wat verkry is uit vlakvarkgate, is gevind dat die situasie in Suid-Afrika soortgelyk is aan dié in Oos-Afrika. Daar is bevind dat 25 persent vlakvarkgate besmet is; dat die gemiddelde virus konsentrasies van bosluismissies van bosluismissies varieer tussen $10^{4.5}$ en $10^{5.2}$ BCHAd $50$, en dat die gemiddelde persentasie van besmette bosluisse wissel tussen 1,62 en 3,45. Besmette bosluisse is ook aangetref in die Marico-distrik wat aangrensend is aan die ensoötiese gebied en waar AVP nog nooit voorgekom het nie. 'n HAd-v populasie van AVP virus is geïsoleer van 'n bosluismissie, TS237, wat in wit selkulture 'n vertraagde en verminderde heem-adsorberende effek getoon het.
Uit hierdie waarnemings is die gevolgtrekking gemaak dat AVP virus in die aanvanklike virus reservoir instaat is om van HAd+ na HAd- te muteer. Hieruit blyk dit dat onder die huidige epidemiese toestande, wat tans in Suid-Afrika heers, die virus skynbaar verander na 'n vorm van laer patogenisiteit. Dit geskied ongeag vroeere aanpassing van die infektiewe agens by die plaaslike varkpopulasie onder heersende epidemiologiese toestande in Suid-Afrika.

Gedurende hierdie ondersoek is dit aangetoon dat LLC-MK₂ selkulture vatbaar is vir AVP virus. Primêre virus isolasies toon sitopatogeniese effekte. Infektiwiteit bereik 'n piek na drie tot vier agtereenvolgende oorspuitings met algehele vernietiging van sellae. Die gevoeligheid van LLC-MK₂ selle vir die bepaling van die virus inhoud van varkweefsel was in twee gevalle vergelykbaar met die van wit selle. In drie ander gevalle was dit 100 tot 1000 keer laer. Die gevolgtrekking is gemaak dat LLC-MK₂ selle 'n geskikte aanvulling is vir wit selkulture vir die kweek van AVP virus, veral vir HAd- isolate.

Die heem-adsorberende eienskappe van die HAd+ stam van AVP virus het verlore gegaan na 35 - 45 agtereenvolgende oorspuitings in LLC-MK₂ selle. In wit selkulture is 'n soortgelyke mutasie waargeneem, hoewel dit meer geleidelik plaasgevind het.

Die moontlikheid van plakete vorming in LLC-MK₂ sellae is ondersoek. Wanneer 0,4% agarose as stollingsagens gebruik is, het alle stamme wat ondersoek is plakette opgelever ongeag of hulle aangepas was vir LLC-MK₂ selle of wit selle. Plakette se deursnee het gelykstelling tussen 0,3 en 3,0 mm. Hierdie eienskap is egter nie gekorreleer met die betrokke stamme se heem-adsorberende eienskappe nie. Die teenwoordigheid van HAd- virus partikels in HAd+ populases is aangetoond deur subkulture van geselekteerde plakette in wit selkulture te maak.

Uit die resultate van biologiese toetses is die gevolgtrekking gemaak dat die HAd- stamme 'n verlaagde virulensie het wat kan wissel tussen wye grense. Die ondervinding met stam Lillie-148 en stam
24823 het aangetoon dat varke wat met hierdie virus stamme besmet raak akute, kroniese of subkliniese siekte toestande ontwikkel. Die graad van verskil tussen die patogenisiteit tussen HAd\(^+\) en HAd\(^-\) virus is beklemtoon deur die resultate wat verkry is met die twee virus populasies van stam TS237. Die HAd\(^+\) stam veroorsaak perakute of akute vorms van die siekte terwyl HAd\(^-\) stam kroniese of subkliniese infeksie tot gevolg het. Matige vorme van AVP is ook verkry nadat varke geïnokuleer is met 'n HAd\(^+\) stam wat 'n aantal oorspuitings in selkulture ondergaan het. Die afleiding is gemaak dat heem- adsorpsie en patogenisiteit twee eienskappe is wat nie verbonde is nie en dus onafhanklik van mekaar gemodifiseer kan word.
INTRODUCTION

CHAPTER I: Epidemiological studies on ASF in domestic pigs and argasid tick Ornithodoros moubata.

CHAPTER II: Susceptibility of cell culture lines to ASF virus.

CHAPTER III: Replication of ASF virus in LLC-MK₂ cell cultures.

CHAPTER IV: Influence of serial passage in cell cultures on the haemadsorbing characteristics of ASF virus.

CHAPTER V: Plaque production by ASF virus in LLC-MK₂ cell cultures.

CHAPTER VI: Biological tests in domestic pigs.

DISCUSSION

REFERENCES

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SUMMARY

OPSOMMING
INTRODUCTION

African swine fever (ASF) is an infectious disease of domestic pigs caused by a DNA virus (Haag & Larenaudie, 1965; Plowright, Brown & Parker, 1966; Enjuanes, Carascosa, Moreno & Vinuela, 1976b) that has been provisionally included in the family Iridoviridae (Fenner, 1976). The electron microscopic studies reported by Breese & De Boer (1966), Almeida, Waterson & Plowright (1967), Moura Nunes, Vigário & Terrinha (1975) all confirm its icosahedral symmetry. The virion appears to have at least two capsid layers surrounded by an outer envelope and an average diameter of 204 nm (Els & Pini, 1977). The number of capsomeres has not been counted, but it has been estimated to be in excess of 812 (Almeida et al., 1967).

Leukocyte cell cultures are the only system where the virus of ASF replicates without requiring previous adaptation (Malmquist & Hay, 1960). The erythrocytes that are added or are present in this system become adsorbed to the infected cells and this phenomenon has been used for some years as a convenient diagnostic test.

Infectivity of the virus is destroyed by treatment with ether, chloroform and heating at 60°C for 20 min but is not affected by variations of pH within the range of 4 to 13.4 (DeTray, 1963; Plowright & Parker, 1967).

The virus of ASF is indigenous to the African continent south of the Sahara. In this area warthogs (Phacochoerus aethiopicus), bushpigs (Potamochoerus porcus), giant forest hogs (Hylochoerus meinertzhageni) and argasid ticks (Ornithodoros moubata) may act as viral reservoirs (Steyn, 1932; De Kock, Robinson & Keppel, 1940; DeTray, 1963; Heuschele & Coggins, 1965; Plowright, Parker & Pierce, 1969a).
The disease made its appearance with devastating effects, at the beginning of the century when the balance between the natural host and the infectious agent was altered by the introduction of domestic pigs into Africa (Montgomery, 1921). The virus has now escaped from this continent and has become established in the Iberian peninsula (Manso Ribeiro, Azevedo, Teixeira, Braco Forte, Ribeiro, Oliveira, Noronha, Pereira & Vigário, 1958; Manso Ribeiro & Azevedo, 1961; Anonymous, 1961). The disease has also occurred in France (Larenaudie, Haag & Lacaze, 1964), in Italy (Mazaracchio, 1968) and in Cuba (Anonymous 1971).

In South Africa where stringent control measures have been systematically implemented, the virus has on the whole been successfully contained in the natural reservoirs inhabiting the endemic area of the northern Transvaal and there is no indication, at present, that domestic pigs are playing a role in the epidemiology of the disease (Pini & Hurter, 1975). The lesions observed under field conditions are those of the acute form consisting of degenerative changes of the lymphoid tissue and vascular system leading to oedema, infarction, necrosis and extensive haemorrhages (Hess, 1971).

In Portugal, Spain and other countries where the virus has become established in the domestic swine population, ASF may be regarded as an evolving disease characterized by slow progression and mild pathogenicity (Hess, 1971; Coggins, 1974). The number of animals which survive the infection is increasing and chronic, subclinical and inapparent forms of disease have been reported (Manso Ribeiro, Nunes Petisca, Frarao & Sobral, 1963; Nunes Petisca, 1965; Sanchez Botija, 1965; Vigário, Terrinha & Moura Nunes, 1974). The lesions seen in swine affected by these types of infection include pericarditis, pneumonia, lymphadenitis, hepatitis and meningoencephalitis (Moulton & Coggins, 1968).

Since pigs which recover from the infection normally withstand challenge with a homologous but not with a heterologous strain, it has been concluded that several immunological types exist (Walker, 1933; Henning, 1956; Malmquist, 1963). Systematic investigations into this aspect have been hampered, however, by the absence of demonstrable neutralizing antibodies in sera of pigs surviving the natural or experimental infection (Hess, 1971). These sera have an inhibitory
effect on the haemadsorption of the erythrocytes to the infected leukocytes but do not inhibit subsequent cell lysis (Malmquist, 1963; Carnero, Larenudie, Ruiz Consalvo & Haag, 1967; Coggins, 1968a). This inhibition of haemadsorption appears to be isolate specific (Hess, 1971). By this technique Vigario et al. (1974) have grouped nine strains of virus into three serological groups while five further isolates could not be classified antigenically as they were devoid of haemadsorbing characteristics.

In South Africa the first recorded outbreaks of ASF occurred in the Potgietersrus district of the northern Transvaal in 1926 (Steyn, 1928). The control measures that were applied consisted of slaughtering both affected and in contact swine, destruction of manure, a prohibition on the restocking of farms within a period of 3 months and control over the movements of live pigs and pork products. In spite of these measures, outbreaks of disease occurred in the Cape Province between 1933 and 1939. The infection, which had apparently entered the region through the movement of pigs from the Transvaal, was eradicated by 1939, because the control measures which were implemented did not allow the virus to become established in the domestic swine population or in any other reservoir (Pini & Hurter, 1975). On the contrary, in the endemic area of the Transvaal, where the eradication of ASF would require the elimination of the natural virus reservoirs, the disease still persists and between 1926 and 1972, it exhibited an apparent cyclic occurrence. Two cycles representing active disease, the first lasting 13 years from 1926 to 1938 and the second 12 years from 1951 to 1962, have been observed. In the intervening periods between 1939 and 1950 and between 1963 and 1972 the virus was apparently inactive.

The opportunity to undertake this investigation was provided by the re-occurrence of the disease in 1973, 1974 and 1975 and by a virological survey conducted to determine whether argasid ticks *Ornithodoros moubata* play a role in the epidemiology of ASF.