Present results of serological and immunological studies of foot-and-mouth disease virus at the Botswana Vaccine Institute

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Summary: The Botswana Vaccine Institute, located in Gaborone, Botswana, in the middle of Southern Africa, has a double vocation: to produce FMD vaccines, and also to study the various types and subtypes of FMD viruses present in Southern Africa. This work is carried out jointly with the technical assistance of IFFA-Mérieux.

Since the end of 1978, more than 35 different strains belonging to SAT 1, SAT 2 and SAT 3 types, have been studied from the serological as well as the immunological aspect. All the results of studies of serological relations, unilateral or bilateral, and of immunological studies, are listed in detail.

Therefore it is possible to justify the choice of vaccine strains and to recommend vaccination programmes which — when applied rigorously — enable a country to get rid of foot-and-mouth disease plague. Botswana is a very good example.

INTRODUCTION

The Botswana Vaccine Institute (BVI) is located in Gaborone, the capital city of Botswana, a country in Southern Africa. Foot-and-mouth disease (FMD) is a disease which has been present in this part of Africa for a great many years. It was described for the first time in 1795 in South Africa by Le Vaillant and subsequently by others, amongst whom General Kruger in 1858. It was not before 1931, however, in Rhodesia, that it was actually identified as such, and the different types of viruses involved (three) were discovered in 1948 by Galloway; due to their origin, these types were called SAT 1, SAT 2 and SAT 3 (Southern African Territories). Although these three types of FMD virus are those most frequently encountered in Southern Africa, other types have also been known to be at the origin of the disease: type O in Mozambique and types O and A in Angola and Tanzania.

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In Botswana, further to a reappearance of the disease in 1977 and the impossibility of containing the epidemic with vaccines available at the time, the Government of Botswana decided to build its own FMD Vaccine Production Institute in cooperation with the French firm: IFFA-Mérieux.

The vocation of the BVI is two-fold:

a) To study the different strains of FMD virus present in Southern Africa or even in other African countries; on a practical level, this has resulted in an increasing demand from neighbouring countries for the rapid diagnosis of suspected disease.

b) To produce efficient FMD vaccines specifically suited to the epidemiological context of the different countries concerned. In October 1981, the production capacity of the new industrial unit will be 21 million monovalent doses per year.

In September 1978, studies could first be started on strains of Botswana origin using a certain number of hyperimmune sera kindly supplied by the Animal Virus Research Institute (AVRI), at Pirbright, U.K. But rapidly, studies were extended to strains originating from neighbouring countries. The diverse origins of the different strains of the FMD virus studied to date explain to what extent the different African countries with FMD problems (especially Southern African countries) have understood the importance of having, in the centre of Southern Africa, a laboratory of regional vocation to help them in the control of FMD.

This was the reason behind a meeting held in April 1980 in Lusaka between the Heads of State or Government of numerous countries (Angola, Botswana, Lesotho, Malawi, Mozambique, Tanzania, Zambia, Zimbabwe and Swaziland) where it was decided to entrust the Government of Botswana with the responsibility of ensuring the coordination of FMD control in the countries concerned.

This paper describes the results obtained:
— by serological studies and the influence they had on the selection of the vaccine strains;
— by immunological studies (direct or indirect) which led to the selection of the vaccine strains which could be used for the industrial production of FMD vaccine.

MATERIALS AND METHODS

A. MATERIALS

1. Virus.

All the viruses (both collection viruses and field viruses) were multiplied on living lingual expliants using Frenkel's technique with the aim of preparing
the antigens required in serology, and of allowing the study of the various parameters of the virus cultures with a view to the preparation of industrial vaccines.

For immunological studies, collections of titrated viruses were constituted:
- viruses titrated on susceptible cattle, using Henderson's conventional technique, which are required by the cross challenge in vaccinated animals;
- viruses titrated for cross seroneutralization in cells.

In the different types, the following viruses were concerned:

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### SAT 1

- SAT 1 Bot. 1/68 Botswana 1968, AVRI Collection
- SAT 1 Moz. 3/77 Mozambique 1977, AVRI Collection
- SAT 1 SAR 4/74 S.A.R., AVRI Collection
- SAT 1 Rho. 12/78 Rhodesia Dec. 1978, identified by BVI
- SAT 1 Bot. 1/77 Botswana 1977, AVRI Collection
- SAT 1 Iran 1962 Iran 1962, Razi Institute Collection
- SAT 1 Bot. 8/79 Botswana Aug. 1979, identified by BVI
- SAT 1 SAR 11/79 S.A.R. Nov. 1979, identified by BVI
- SAT 1 Rho. 5/80 Rhodesia (Zimbabwe) May 1980, identified by BVI
- SAT 1 SAR 7/80 S.A.R., July 1980, identified by BVI
- SAT 1 Nam. 10/80 Namibia Oct. 1980, identified by BVI
- SAT 1 Ken. 7/79 Kenya 1979, Kenya Collection
- SAT 1 Zam. 11/80 Zambia Nov. 1980, identified by BVI
- SAT 1 Zam. 1/81 Zambia Jan. 1981, identified by BVI

### SAT 2

- SAT 2 Bot. 3/77 Botswana 1977, AVRI Collection
- SAT 2 Ken. 183/74 Kenya 1974, AVRI Collection
- SAT 2 Moz. 2/77 Mozambique 1977, AVRI Collection
- SAT 2 Rho. 2/72 Rhodesia 1972, AVRI Collection
- SAT 2 SAR 2/78 S.A.R. 1978, AVRI Collection
- SAT 2 Bot. 8/78 Botswana Aug. 1978, identified by BVI
- SAT 2 Rho. 2/79 Rhodesia Feb. 1979, identified by BVI
- SAT 2 SAR 4/79 S.A.R. April 1979, identified by BVI
- SAT 2 Bot. 7/79 Botswana July 1979, identified by BVI
- SAT 2 Bot. 11/79 Botswana Nov. 1979, identified by BVI
- SAT 2 Bot. 2/80 Botswana Feb. 1980, identified by BVI
- SAT 2 Bot. 4/80 Botswana April 1980, identified by BVI
- SAT 2 Rho. 5/80 Rhodesia (Zimbabwe) May 1980, identified by BVI
- SAT 2 Moz. 6/80 Mozambique June 1980, identified by BVI
- SAT 2 Bot. 9/80 Botswana Sept. 1980, identified by BVI
- SAT 2 Ken. 49/80 Kenya 1980, Kenya Collection
- SAT 2 Ken. 113/80 Kenya 1980, Kenya Collection
- SAT 2 SAR 4/81 S.A.R. April 1981, identified by BVI

### SAT 3

- SAT 3 Bec. 1/65 Bechuanaland (Botswana) 1965, AVRI Collection
- SAT 3 Rho. 1/74 Rhodesia 1974, AVRI Collection
- SAT 3 SAR 1/80 S.A.R. Jan. 1980, identified by BVI

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2. Sera.

The sera used for the complement fixation tests are sera of guinea-pigs which are hyperimmunised by repeated injections of virus previously adapted to guinea-pigs.
The sera used for seroneutralization tests are collected from cattle undergoing immunity tests. Blood samples are taken just before the virulent challenge.

3. Animals.

All cattle used for immunological studies are animals from the southern part of the country, an area where no FMD has been recorded for more than 20 years and where vaccination is not practised. The rate of specific antibodies of these animals is checked and only those which show complete negative reactions to usual seroneutralization methods are retained.

These animals are isolated in the Testing Centre of Motopi, located in the North of the country, under conditions of safety which avoid any spread of the virus. At the end of the test, after slaughtering, they are incinerated on the same premises.

4. Vaccines.

Vaccines are made from industrially produced viruses (using the Frenkel technique adapted by IFFA-Mérieux) and inactivated with formaldehyde; saponin and aluminium hydroxide are incorporated as adjuvants.

Once having satisfied the different safety tests, they are used in immunological studies: for monovalent vaccines, the vaccine dose is 1 ml.

B. METHODS

1. Subtyping serological diagnostic method.

The complement fixation method at 50% Osler’s haemolysis in its adaptation by Roumiantzeff with spectrophotometric reading was used for sera titrations. All sera titres were obtained at the optimum antigen concentration and the values retained for calculation were always means made from at least 3 or 5 titrations.

The relationship between two strains A and B is calculated from reciprocal reactions between each strain and the respective sera a and b. The study of each pair of strains results in establishing the unilateral relationships $r_1$ and $r_2$ which show respectively the relations of heterologous serum titres over homologous serum titres.

$$r_1 = \frac{a \cdot B}{a \cdot A} \quad \text{and} \quad r_2 = \frac{b \cdot A}{b \cdot B}$$

The relationship $R$ is expressed in percentage and is calculated according to the following formula:

$$R = 100 \sqrt{r_1 \times r_2}$$
2. Direct immunological method by cross vaccination virulent challenge in cattle.

The method with a challenge 21 days after vaccination with 10,000 50% Bovine Infectious Doses (= BID 50) was retained. When it is applied to cattle receiving varying doses of vaccine, it is the method of the 50% Vaccinating Bovine Dose or VBD 50 which enables the determination of the bovine potency of a vaccine. It is the only method retained as a reference by the European Pharmacopoeia for direct potency testing of FMD vaccines.

3. Indirect immunological method by cross serum-neutralisation in cell culture.

This is a micro-serum-neutralisation method on « Microtest » (N.D.) plates using variable amounts of vaccinated cattle sera against a constant amount of virus (320 CPED$_{50}$/ml) obtained in cell culture. The cell system used was a pig kidney cell line close to IBRS$_2$.

The serum-neutralizing titres are expressed as the logarithm of the reciprocal of the serum dilution, before mixing with the virus, which protects 50% of the wells.

Immunological relationships can then be calculated according to the same principles as for serological relationships.

RESULTS

A. SEROLOGICAL RESULTS

1. SAT 1 type.

Fourteen different strains of SAT 1 type virus were studied and the results of these studies can be found in Tables I and I a (unilateral serological relationships) and Table II (serological relationships).

SAT 1 Rho. 12/72, SAT 1 Bot. 1/77 and SAT 1 Bot. 8/79 were the sera used in routine work for typing tests when new field strains were received.

It should be noted that the strains which appeared in Southern Africa (outside Botswana) between the end of 1979 and the beginning of 1981, are nearer to Moz. 3/77, Rho. 12/78 and Bot. 8/79 strains than to the Bot. 1/77 strain, and do not seem to be very different from each other.

However, the study of serological relationships shows that the relationship between two strains only seldom reaches the value of 50% (SAT 1 Rho. 12/78 - SAT 1 Moz. 3/77 - value 52.5%) which leads to the assumption that different sub-types exist in this area of Southern Africa. This resulted in us including two different vaccine strains in our SAT 1 type vaccines.
<table>
<thead>
<tr>
<th>Antigens</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
<th>SAT 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 1 Bot. 1/68</td>
<td>1</td>
<td>0.22</td>
<td>0.50</td>
<td>0.30</td>
<td>0.54</td>
<td>0.13</td>
<td>0.16</td>
<td>0.43</td>
</tr>
<tr>
<td>SAT 1 Moz. 3/77</td>
<td>0.30</td>
<td>1</td>
<td>0.38</td>
<td>0.46</td>
<td>0.25</td>
<td>0.06</td>
<td>0.33</td>
<td>0.57</td>
</tr>
<tr>
<td>SAT 1 SAR 4/74</td>
<td>0.14</td>
<td>0.34</td>
<td>1</td>
<td>0.18</td>
<td>0.13</td>
<td>0.14</td>
<td>0.11</td>
<td>0.43</td>
</tr>
<tr>
<td>SAT 1 Rho. 12/78</td>
<td>0.27</td>
<td>0.60</td>
<td>0.23</td>
<td>1</td>
<td>0.31</td>
<td>0.10</td>
<td>0.19</td>
<td>0.97</td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>0.25</td>
<td>0.23</td>
<td>0.11</td>
<td>0.31</td>
<td>1</td>
<td>0.07</td>
<td>0.14</td>
<td>0.34</td>
</tr>
<tr>
<td>SAT 1 Iran 62</td>
<td>0.20</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td>0.14</td>
<td>1</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>SAT 1 Bot. 8/79</td>
<td>0.18</td>
<td>0.29</td>
<td>0.13</td>
<td>0.31</td>
<td>0.44</td>
<td>0.13</td>
<td>1</td>
<td>0.39</td>
</tr>
<tr>
<td>SAT 1 Ken. 7/79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
<td>0.26</td>
</tr>
<tr>
<td>SAT 1 Zam. 1/81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
<td>0.54</td>
</tr>
<tr>
<td>Sera</td>
<td>Antigens</td>
<td>SAT 1 Rho. 5/80</td>
<td>SAT 1 RSA 7/80</td>
<td>SAT 1 Nam. 10/80</td>
<td>SAT 1 Ken. 7/79</td>
<td>SAT 1 Zam. 11/80</td>
<td>SAT 1 Zam. 1/81</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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<td>-----------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>SAT 1 Moz. 3/77</td>
<td>0.71</td>
<td>0.38</td>
<td>0.52</td>
<td>0.37</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Rho. 12/78</td>
<td>1</td>
<td>0.77</td>
<td>0.48</td>
<td>0.34</td>
<td>0.60</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>0.42</td>
<td>0.35</td>
<td>0.18</td>
<td>0.12</td>
<td>0.20</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Bot. 8/79</td>
<td>0.35</td>
<td>0.51</td>
<td>0.51</td>
<td>0.29</td>
<td>0.43</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Ken. 7/79</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Zam. 1/81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table II

*Serological relationships of SAT 1 strains: Relation R*

<table>
<thead>
<tr>
<th></th>
<th>SAT 1 Bot. 1/68</th>
<th>SAT 1 Moz. 3/77</th>
<th>SAT 1 SAR 4/74</th>
<th>SAT 1 Rho. 12/78</th>
<th>SAT 1 Bot. 1/77</th>
<th>SAT 1 Iran 62</th>
<th>SAT 1 Ken. 7/79</th>
<th>SAT 1 Zam. 1/81</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 1 Bot. 1/68</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Moz. 3/77</td>
<td>26%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 SAR 4/74</td>
<td>26.5%</td>
<td>36%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Rho. 12/78</td>
<td>28.5%</td>
<td>52.5%</td>
<td>20%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>36.5%</td>
<td>24%</td>
<td>11.5%</td>
<td>31%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Iran 62</td>
<td>16%</td>
<td>10%</td>
<td>16%</td>
<td>14%</td>
<td>10%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 1 Ken. 7/79</td>
<td>17%</td>
<td>31%</td>
<td>12%</td>
<td>24.5%</td>
<td>25%</td>
<td>19.5%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>SAT 1 Zam. 1/81</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>33%</td>
<td>17.5%</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T. 100%</td>
</tr>
</tbody>
</table>

SAT 1 Bot. 1/68  Moz. 3/77  SAR 4/74  Rho. 12/78  Bot. 1/77  Iran 62  Bot. 8/79  Ken. 7/79  Zam. 1/81
2. SAT 2 type.

Twenty-four different strains of SAT 2 type were studied and results of these studies can be found in Tables III, IIIa and IV.

SAT 2 Bot. 8/78, SAT 2 Rho. 2/79 and SAT 2 SAR 4/79 sera are now used in routine work for our typing tests for the identification of new strains.

Among the strains studied, some showed themselves to be the same as others and do not appear on the tables. These are:

SAT 2 Bot. 8/78 = SAT 2 Bot. 8/78
SAT 2 Bot. 9/79 = SAT 2 Bot. 7/79
SAT 2 SAR 6/79 = SAT 2 Bot. 11/79
SAT 2 SAR 10/79 = SAT 2 Bot. 11/79
SAT 2 SAR 12/77 = SAT 2 Moz. 2/77

It should be pointed out that most of the strains studied (apart from the Kenyan ones) have always shown unilateral relationships at 0.5 with one or other of the SAT 2 Bot. 8/78 or SAT 2 Rho. 2/79 strains. On the other hand, these ‘r’ values are constantly lower (0.3 at most) with the SAT 2 SAR 4/79 strain.

We can say that since the beginning of 1980, the different strains that appeared (from Botswana, Rhodesia, Mozambique or South Africa) are all very close to the SAT 2 Rho. 2/79 strain.

As for the SAT 1 type strains, the study of the serological relationships led us to include two vaccine strains in the SAT 2 type vaccines.

3. SAT 3 type.

Four different strains were studied and the results of the unilateral studies can be found in Table V below:

<table>
<thead>
<tr>
<th>Antigens</th>
<th>SAT 3</th>
<th>SAT 3</th>
<th>SAT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1/65</td>
<td>1/74</td>
<td>1/80</td>
</tr>
<tr>
<td>Rho. 1/74</td>
<td>0.15</td>
<td>1.00</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The fourth strain confronted with SAT 3 Rho. 1/74 serum gave a result of ‘r’ = 0.17; this strain was from South Africa, January 1980: SAT 3 SAR 1/80 (Homu). It is thus very close to the SAT 3 SAR 1/80 (Lebowa) strain.

A homologous serum of this latter strain was produced and studies of serological relationships are being undertaken to see if the new 1980 field
### TABLE III

*Unilateral serological studies, SAT 2 strains: Values of r*

<table>
<thead>
<tr>
<th>Antigens</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
<th>SAT 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 3/77</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT 2 Ken. 183/74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
</tr>
<tr>
<td>SAT 2 Bot. 8/78</td>
<td>0.59</td>
<td>0.18</td>
<td>0.42</td>
<td>0.42</td>
<td>0.43</td>
<td>1</td>
<td>0.39</td>
<td>0.48</td>
<td>0.53</td>
<td>0.25</td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79</td>
<td>0.18</td>
<td>0.28</td>
<td>0.49</td>
<td>0.67</td>
<td>0.58</td>
<td>0.44</td>
<td>1</td>
<td>0.55</td>
<td>0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>SAT 2 SAR 4/79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

The table above provides the values of r for unilateral serological studies on SAT 2 strains. Each row represents different antigens, and the columns represent sera from various samples.
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 8/78</td>
<td>0.48</td>
<td>0.54</td>
<td>0.58</td>
<td>0.33</td>
<td>0.47</td>
<td>0.60</td>
<td>0.30</td>
<td>0.39</td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79</td>
<td>0.65</td>
<td>0.81</td>
<td>0.83</td>
<td>0.91</td>
<td>0.78</td>
<td>0.61</td>
<td>0.31</td>
<td>0.81</td>
</tr>
<tr>
<td>SAT 2 SAR 4/79</td>
<td>0.21</td>
<td>0.30</td>
<td>0.26</td>
<td>0.23</td>
<td>0.19</td>
<td>0.34</td>
<td>0.16</td>
<td>0.21</td>
</tr>
</tbody>
</table>
**TABLE IV**

*Serological relationships of strains SAT 2: Relation R*

<table>
<thead>
<tr>
<th></th>
<th>SAT 2 Bot. 3/77</th>
<th>SAT 2 Ken. 183/74</th>
<th>SAT 2 Bot. 8/78</th>
<th>SAT 2 Rho. 2/79</th>
<th>SAT 2 SAR 4/79</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 3/77</td>
<td>100%</td>
<td>N.T.</td>
<td>41.5%</td>
<td>14%</td>
<td>N.T.</td>
</tr>
<tr>
<td>SAT 2 Ken. 183/74</td>
<td>N.T.</td>
<td>100%</td>
<td>38%</td>
<td>48%</td>
<td>N.T.</td>
</tr>
<tr>
<td>SAT 2 Bot. 8/78</td>
<td>100%</td>
<td>100%</td>
<td>41.5%</td>
<td>41.5%</td>
<td>25%</td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>37%</td>
<td>37%</td>
</tr>
<tr>
<td>SAT 2 SAR 4/79</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

SAT 2 Bot. 3/77  SAT 2 Ken. 183/74  SAT 2 Bot. 8/78  SAT 2 Rho. 2/79  SAT 2 SAR 4/79
strains resemble the SAT 3 Bec. 1/65 strain. In any case, it would seem that they are quite different from the SAT 3 Rho. 1/74 strain.

B. IMMUNOLOGICAL RESULTS

1. Direct immunology.

a) SAT 1 type.

Studies were concentrated on SAT 1 Rho. 12/78 and SAT 1 Bot. 1/77 strains and only one test has been done:

Vaccination of 4 cattle with 1 dose of SAT 1 Rho. 12/78 vaccine with challenge 21 days later with 10,000 BID 50% SAT 1 Bot. 1/77 virus.

The rate of protection observed was 50%. It is highly likely that the two strains under study belong to different subtypes.

b) SAT 2 type.

Present results concern the two following strains: SAT 2 Bot. 8/78 and SAT 2 Rho. 2/79.

In each quantitative cross safety test, 5 cattle were vaccinated with 1 dose of vaccine and challenged 21 days later with the other strain. The reading was done 7 days later.

Results can be found in Table VI and lead to the conclusion that these two strains belong to different subtypes (60% protection observed both ways).

2. Indirect immunology.

a) SAT 1 type.

Cross studies were made with the same strains used in the direct cross immunology test, i.e. SAT 1 Rho. 12/78 and SAT 1 Bot. 1/77.

All results of cross seroneutralization are shown in Table VII. It is interesting to note the slightly contradictory results obtained with the sera of cattle vaccinated with vaccines Nos. 0401 and 0405.

Unilateral immunological relationships are shown in Table VIII and the values expressed are the means obtained in two series of tests.

It is thus possible to calculate the immunological relationship ‘R’ between these two SAT 1 strains:

\[ R = 100 \sqrt{0.41 \times 1.39} \]

\[ R = 75\% \]

b) SAT 2 type.

The two SAT 2 Bot. 8/78 and SAT 2 Rho. 2/79 strains were bilaterally studied and in the same way as the studies of the SAT 1 strains; results are found in Tables IX and X.
### Table VI

**Immunological quantitative cross studies, SAT 2 strains**

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>SAT 2 Bot. 8/78 (10,000 BID 50%)</th>
<th>SAT 2 Rho. 2/79 (10,000 BID 50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 8/78</td>
<td>not tested*</td>
<td>60%</td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79</td>
<td>60%</td>
<td>not tested*</td>
</tr>
</tbody>
</table>

* * Average of minimum protection rates observed during the homologous activity tests (bovine potency test):
  - SAT 2 Bot. 8/78 strain: 96%
  - SAT 2 Rho. 2/79 strain: 88%

### Table VII

**Seroneutralization cross test results, SAT 1 strains**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sera</th>
<th>SAT 1 Rho. 12/78</th>
<th>SAT 1 Bot. 1/77</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 1 Rho. 12/78</td>
<td>SAT 1 Bot. 1/77</td>
<td>m = 1.63 - N = 3 - Sd = 0.252</td>
<td>m = 1.17 - N = 3 - Sd = 0.472</td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>Rho. 12/78</td>
<td>m = 1.82 - N = 5 - Sd = 0.259</td>
<td>m = 1.50 - N = 5 - Sd = 0.529</td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>SAT 1 Vaccine No. 0401</td>
<td>m = 1.93 - N = 3 - Sd = 0.550</td>
<td>m = 1.53 - N = 3 - Sd = 0.351</td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>SAT 1 Vaccine No. 0405</td>
<td>m = 1.15 - N = 4 - Sd = 0.300</td>
<td>m = 1.72 - N = 4 - Sd = 0.386</td>
</tr>
</tbody>
</table>

### Table VIII

**Immunological unilateral relationships, SAT 1 strains**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sera</th>
<th>SAT 1 Rho. 12/78</th>
<th>SAT 1 Bot. 1/77</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 1 Rho. 12/78</td>
<td>SAT 1 Bot. 1/77</td>
<td>1</td>
<td>0.41</td>
</tr>
<tr>
<td>SAT 1 Bot. 1/77</td>
<td>SAT 1 Rho. 12/78</td>
<td>1.39</td>
<td>1</td>
</tr>
</tbody>
</table>
TABLE IX
Seroneutralization cross test results, SAT 2 strains

<table>
<thead>
<tr>
<th>Sera</th>
<th>Virus</th>
<th>SAT 2 Bot. 8/78</th>
<th>SAT 2 Rho. 2/79</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 8/78 Vaccine No. 91504</td>
<td>$m = 0.95 - N = 4 - Sd = 0.129$</td>
<td>$m = 1.33 - N = 4 - Sd = 0.206$</td>
<td></td>
</tr>
<tr>
<td>SAT 2 Bot. 8/78 Vaccine No. 91511</td>
<td>$m = 1.10 - N = 2 - Sd = 0.424$</td>
<td>$m = 1.50 - N = 2 - Sd = 0.566$</td>
<td></td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79 Vaccine No. 0511</td>
<td>$m = 1.27 - N = 3 - Sd = 0.351$</td>
<td>$m = 2.10 - N = 3 - Sd = 0.360$</td>
<td></td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79 Vaccine No. 0516</td>
<td>$m = 1.37 - N = 3 - Sd = 0.321$</td>
<td>$m = 2.00 - N = 3 - Sd = 0.265$</td>
<td></td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79 Vaccine No. 0517</td>
<td>$m = 1.50 - N = 3 - Sd = 0.361$</td>
<td>$m = 2.40 - N = 3 - Sd = 0.200$</td>
<td></td>
</tr>
</tbody>
</table>

TABLE X
Immunological unilateral relationships, SAT 2 strains

<table>
<thead>
<tr>
<th>Sera</th>
<th>Virus</th>
<th>SAT 2 Bot. 8/78</th>
<th>SAT 2 Rho. 2/79</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT 2 Bot. 8/78</td>
<td></td>
<td>1</td>
<td>2.46</td>
</tr>
<tr>
<td>SAT 2 Rho. 2/79</td>
<td></td>
<td>0.17</td>
<td>1</td>
</tr>
</tbody>
</table>

The immunological relationship between these two strains can thus be calculated:

$$R = 100 \sqrt{2.46 \times 0.17}$$

$$R = 65\%$$

It can be stated that the SAT 2 Bot. 8/78 strain predominates over the SAT 2 Rho. 2/79 strain.

DISCUSSION

We do not intend to discuss the methods themselves which were used in these studies. It is obvious that other methods exist which can also show some advantages, but those used by us have been known for a long time and their advantages widely discussed elsewhere (Foot-and-Mouth Disease Symposium, Lyon, 1976). It can be said that they make a coherent and representative set of methods in the performance of compared studies of different strains of FMD virus.
1. The importance of each method in the selection of vaccine strains.

It is obvious that the serological diagnostic method of subtyping is the quickest method to use when the laboratory receives a new strain. It enables the epidemiological risk to be evaluated but this risk is probably exaggerated. So immunological studies are also necessary. In the instance of bilateral studies performed on SAT 2 type strains, the following was observed:

- serological relationship: \( R = 41.5\% \);
- immunological relationship: \( R = 65.0\% \);
- 60% rate of cross-protection after primary vaccination in both ways.

It is obvious that for pluri-vaccinated animals, this cross-protection rate would be higher than 70% and it is usually considered that the 70% rate obtained after a vaccination campaign is enough to stamp out the disease in the vaccinated area. However, taking the conditions of habitual extensive breeding in most African countries into account, the vaccination coverage is not always as high as could be hoped.

The epidemiological risk represented by the appearance of a new strain (even if it is not further from the vaccine strain than the two SAT 2 strains studied between each other), remains high, particularly for animal populations which are not regularly vaccinated. This is the reason why it was thought necessary to widen the spectrum of activity of the vaccines used for each valency and to include two vaccine strains.

Serological studies led us to the following choice:

- SAT 1 valency: SAT 1 Rho. 12/78 strain
  - SAT 1 Bot. 1/77 strain
- SAT 2 valency: SAT 2 Bot. 8/78 strain
  - SAT 2 Rho. 2/79 strain

The SAT 3 type is of lesser epidemiological importance and a current strain has been retained: SAT 3 SAR 1/80 (Lebowa).

It is thus possible to limit risks of immunity breakdowns in regularly vaccinated populations, a risk which may appear either due to a lack of sufficient vaccine protection or by the threat of a new virus strain different from our vaccine strains.

2. Use of vaccines in the field.

Once the vaccine strains had been chosen, it proved interesting to take stock of the results obtained in the field. As shown by serological studies, we have been able to study recent virus strains from different countries of Southern Africa, all of which were responsible for epidemic outbreaks of the disease. Our vaccines were used in different countries (Zimbabwe, Zambia, Namibia, South Africa) and allowed these epidemic outbreaks of the disease to be stopped after a primary vaccination, sometimes after only a very short
time. The composition of the vaccines was the same (for each valency) for the different countries concerned.

These combinations of different vaccine strains can be presumed as being satisfactory, but for a more efficient use, the following vaccination schedule can be advised:

- primary vaccination (preventive or in case of an epidemic) : 2 vaccine injections at a one-month interval (when conditions permit);
- routine medical prophylaxis : 1 booster injection every six months.

It is obvious that taking into account the epidemiological context of Southern Africa, the best we can do is to recommend the use of the SAT 1/SAT 2 bivalent vaccine, or even the SAT 1/SAT 2/SAT 3 trivalent vaccine which will be ready in 1982, in order to get routine vaccination campaigns going.


Thanks to strict animal movement control and the implementation of massive vaccination campaigns, Botswana, a country where the disease was still rampant in 1980 (SAT 1 and SAT 2 virus types), has been able to rid itself of the scourge and declare itself free of foot-and-mouth disease by September of the same year.

The use of the bivalent vaccine (SAT 1 and SAT 2) since the beginning of 1980 has certainly been one of the most important elements of this success. Two vaccination campaigns in 1980 enabled the disease to be virtually wiped out; this system of medical prophylaxis has been maintained in 1981 and will be pursued in future years with trivalent vaccines (SAT 1 + SAT 2 + SAT 3). A booster injection every 6 months seems absolutely necessary for success on a country-wide scale.

The immediate consequence of this success was the authorisation granted to Botswana to start exporting meat again to countries of the European Economic Community, as of June 1st, 1981.

CONCLUSION

The serological and immunological studies of FMD virus undertaken at the BVI show that marked differences exist between strains of a same type, especially in the Southern African region. It is thus important to be very careful in choosing the vaccine strains used to make up the vaccines for use in the field, and not to limit the judgement criteria merely to serological studies.

Immunological studies should also be done as soon as important differences appear between a new strain and the vaccine strains. Results obtained through vaccination must be analysed and risks of epizootic disease well considered before considering any alteration in the choice of vaccine strains.
It is thus obvious that constant epidemiological control (not only in the country where the production laboratory is located, but also in neighbouring countries) must be organised in order to allow the laboratory to adapt its vaccine formulae to the epidemiological context of the region.

Nothing but cooperation between the different countries concerned will enable these aims to be reached. This was in fact the conclusion of the Lusaka Meeting in April 1980: the BVI must have a regional vocation (spreading even to other countries in the future) and must be the cornerstone of the organisation of FMD control in the countries of Southern Africa, for it can both ensure constant epidemiological surveillance and produce efficient FMD vaccines, the quality of which is regularly and officially tested on the susceptible species.

ACKNOWLEDGMENTS

We would like to thank Dr. J. Falconer, Director of Veterinary Services, Republic of Botswana, for his permission to present results achieved at the Botswana Vaccine Institute in Gaborone.

REFERENCES

(See page 366)