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*Poxvirus de type hémorragique*  
A SUSPECTED OUTBREAK OF BUFFALOPOX IN BANGLADESH

by

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During a special search for smallpox cases in the Sundarbans area in the south-west of Bangladesh, a rash case was detected on 14 July 1976 by a surveillance team in Pakhimara, Rampal Thana, one mile north of the Sundarbans forest.

1. The index case

Mujibur Rahman, a three-year-old boy, had an onset of rash on 10 July 1976. His parents could not say whether any fever had preceded the eruption of the rash. On day 4 of the illness the rash was superficial, the lesions unilocular, and there were both vesicles and pustules at different stages of development. The patient had no previous history of smallpox or chickenpox. No pock marks were visible and the child had never been vaccinated. Within the vicinity of Mujibur Rahman's house several cured cases of chickenpox had been detected and his rash was also initially diagnosed as chickenpox. A laboratory specimen was collected (vesicular fluid on filter-paper). Following instructions received prior to the search, the surveillance team vaccinated the child and his family.

2. Original laboratory findings

The laboratory specimen (Bangladesh 339) was processed in the Dacca laboratory. The gel agglutination test was negative, but, when inoculated on chick embryo, definite pock lesions appeared after 72 hours at 35°C. The pocks were similar to those produced by vaccinia, but the membrane was very haemorrhagic.

If the virus isolated was definitely not smallpox, it was possible that it was another virus of the pox group. A sample of the isolate was sent to the WHO Reference Laboratory in Moscow. There, electron microscopy confirmed the presence of a pox virus which was at first suspected to be vaccinia, but, on second examination, was thought "to be cowpox or more likely buffalopox".

3. Follow-up visits

Follow-up visits had been made to the outbreak area, and the frequency of these visits was increased when the laboratory expressed some doubts about the identity of the virus isolated.

On day 13, after the onset of rash, Mujibur Rahman had fully recovered, but 20-30 superficial pock marks were left on his face and trunk. He had a visible vaccination scar. The last scab left, on one of his toes, was collected, but the laboratory did not isolate any virus from this inadequate specimen. No further rash case had occurred in the village.

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Increased attention was given to the detection and investigation of any diseases that had affected the large number of cows and buffaloes in the area. It was reported that since the beginning of the year, many of these animals had died of an unidentified disease, but none had had any skin eruption.

The proximity of the Sundarbans forest, where wildlife is abundant, added to the complexity of the situation, as a possible epidemiological link between wild and domestic animals could be postulated.

The members of the surveillance team which had collected the first laboratory specimen were repeatedly asked to describe in detail the procedures followed during their first investigation. It was, however, impossible to ascertain whether a vial of vaccine had been open at the time of specimen collection.

A more comprehensive study of this outbreak was scheduled for October, when the monsoon would be over and the rivers more navigable. The study was planned with reference to the investigation of diseases similar to smallpox in African regions where monkeypox had occurred. The operational phase in the outbreak area was followed by laboratory investigations in the Moscow WHO Reference Laboratory.

#### 4. Operations

The study, which was to last 30 days, started on 11 October 1976.

##### 4.1 Activities

A systematic house-to-house search was organized for the area within an eight-mile radius of the infected house. During this search the following procedures were carried out:

- (1) all members of each household were enumerated, their name, age, sex, family relationship and vaccination status were recorded in a specially designed booklet;
- (2) any occurrences of rash-with-fever or rash-death over the six months preceding the search were recorded;
- (3) persons found with more than five facial pock marks were listed;
- (4) markets, schools and village practitioners were visited and interviews conducted;
- (5) blood specimens were collected from rash-with-fever cases, and also from a certain number of people who had not reported any illness but were to be included in a control group;
- (6) all domestic animals owned by the listed households were enumerated with a record of any recent illness or death, and blood specimens were taken from a number of sick and healthy animals.

Wild animals were collected by local villagers who had been offered a monetary incentive for their capture. The collection included: different species of birds, rats, snakes, frogs and other animals which could possibly have represented an epidemiological link between wild and domestic animals. In addition to a blood sample, kidneys and liver were collected from each animal.

##### 4.2 Personnel

The personnel included:

- 3 epidemiologists
- 1 veterinarian officer
- 1 operations officer
- 2 paramedical assistants
- 17 surveillance team leaders and members
- 1 laboratory technician
- 4 speed-boat drivers and general messengers

#### 4.3 Logistics

The logistical problems were difficult to solve. From Khulna, the district headquarters, Pakhimara village can only be reached by boat. It was necessary to establish a base which had adequate space for a field laboratory, staff accommodation, and storage. Therefore, the Niramoy, a hospital ship which provided a suitable base for operations, sailed from Dacca to the outbreak area. The ship carried all necessary equipment including refrigerators, cold boxes, speed-boat engines, petrol drums and many other items required for conducting the search and preparing the laboratory specimens for transportation. A radio communication system was established which linked the ship with Khulna, Dacca, and all other radio stations of the smallpox programme network.

#### 4.4 Progress of the operations

The house-to-house search and the survey of animals progressed more slowly than was planned. Major transport problems were encountered because of unfavourable weather conditions or significant tidal changes. The area to be searched was reduced to that within a four-mile radius of the infected house. The study protocol was, however, followed with no major difficulty, owing to the active support of the local authorities, particularly the forest officers, and the public.

### 5. Results of field survey

#### 5.1 Population surveyed

|                             |        |
|-----------------------------|--------|
| Number of villages searched | 26     |
| Number of houses visited    | 9 894  |
| Number of persons listed    | 52 337 |
| Number of persons absent    | 10 295 |
| Number of persons examined  | 42 042 |

#### 5.2 Pock mark and rash detection

FIG. 1. POCK MARK SURVEY RESULTS

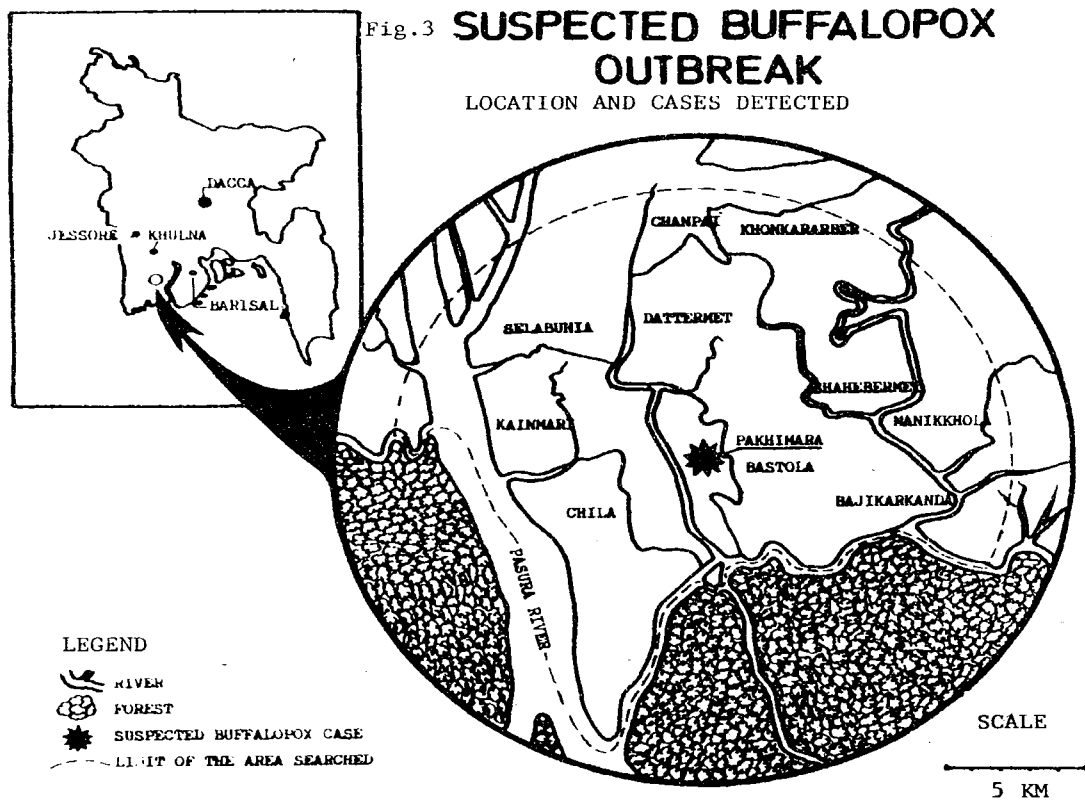
|                               |        |
|-------------------------------|--------|
| Persons examined              | 42 042 |
| Persons with pock marks*      | 612    |
| Reported year of pox disease: |        |
| Before 1970                   | 103    |
| 1970                          | 13     |
| 1971                          | 0      |
| 1972                          | 421    |
| 1973                          | 69     |
| 1974                          | 6      |
| 1975                          | 0      |
| 1976                          | 0      |
| Total                         | 612    |

\* Five or more facial pock marks.

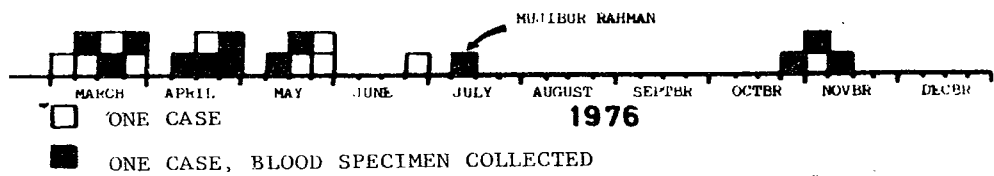
FIG. 2. RASH-WITH-FEVER AND RASH-DEATH DETECTION

|  |    |
|--|----|
| Rash-with-fever cases in previous six months | 27 |
| Rash-deaths in previous six months           | 0  |
| Diagnosis of rash-with-fever cases:          |    |
| Chickenpox (including four active)           | 19 |
| Measles                                      | 4  |
| Infected scabies                             | 4  |
| Total  | 27 |

The chickenpox and measles cases are shown on the epidemic curve (Fig. 3).



CHICKEN POX AND MEASLES CASES DETECTED



A blood specimen was collected from 10 of these chickenpox cases and one measles case. An additional 141 blood specimens were collected from persons who had not reported any illness. Scabs or pustular fluid were collected from three out of the four patients who had had an onset of chickenpox rash in October-November.

5.3 Enumeration of domestic animals: sickness and deaths

FIG. 4. ENUMERATION OF DOMESTIC ANIMAL POPULATION  
IN INVESTIGATION AREA - OCTOBER 1976

| Species                         | Total         | Over last six months                   |     |      |     | Blood specimens collected |
|---------------------------------|---------------|--|-----|------|-----|---------------------------|
|                                 |               | Sick                                   |     | Died |     |                           |
|                                 |               | No.                                    | %   | No.  | %   |                           |
| <b>Livestock:</b>               |               |  |     |      |     |                           |
| Buffalo                         | 1 729         | 65                                     | 3.7 | 65   | 3.7 | 48                        |
| Cow                             | 18 118        | 473                                    | 2.6 | 711  | 4.0 | 75                        |
| Goat                            | 4 855         | 12                                     | 0.2 | 76   | 1.5 | 7                         |
| Sheep                           | 158           | 1                                      | 0.6 | 3    | 1.9 | 0                         |
| Pig                             | 1 336         | 0                                      | 0   | 0    | 0   | 0                         |
| Horse                           | 25            | 0                                      | 0   | 0    | 0   | 0                         |
| Poultry                         | 11 608        | 14                                     | 0.1 | 120  | 1.0 | 1                         |
| Ducks                           | 17 508        | 0                                      | 0   | 134  | 0.7 | 1                         |
| Geese                           | 85            | 0                                      | 0   | 0    | 0   | 0                         |
| Pigeon                          | 2 040         | 0                                      | 0   | 8    | 0.4 | 0                         |
| <b>Animal pets:</b>             |               |  |     |      |     |                           |
| Dog                             | 2 024         | 3                                      | 0.1 | 5    | 0.2 | 1                         |
| Cat                             | 4 330         | 6                                      | 0.1 | 23   | 0.5 | 0                         |
| Monkey                          | 14            | 0                                      | 0   | 0    | 0   | 0                         |
| Bear                            | 7             | 0                                      | 0   | 0    | 0   | 0                         |
| Bird (pet)                      | 66            | 0                                      | 0   | 0    | 0   | 0                         |
| <b>Total animals enumerated</b> | <b>59 579</b> | <b>Total blood specimens collected</b> |     |      |     | <b>133</b>                |

The reported illnesses were often related to an outbreak of foot-and-mouth disease in cattle and buffalo, and possibly in some goats, during the early part of the year. Parasitic infestation with round worms and flukes was very common in the area. Heavy infestation combined with additional stress factors may have caused a great many of the deaths among cattle.

The veterinarian attributed the deaths to an epidemic of haemorrhagic septicaemia, anthrax, or Black Water. The deaths of buffaloes, specifically, were due to Pasteurella multacida infection.

Three calves were found with active skin disease, however, neither these nor any other animal was found to have a pox type rash.

5.4 Collection of wild animals

Specimens were collected from selected species; where possible blood, kidneys and liver.

FIG. 5. SPECIMENS COLLECTED FROM WILD ANIMALS

| Species   | Number collected |
|-----------|------------------|
| Frog      | 27               |
| Birds     | 43               |
| Snakes    | 13               |
| Monitor   | 3                |
| Mongoose  | 3                |
| Crocodile | 1                |
| Deer      | 1                |
| Rats      | 67               |

#### 6. Laboratory results

The laboratory specimens were sent to the Dacca laboratory in ice boxes. The chief of this laboratory carried all specimens with her to the Moscow reference laboratory where the study was to be conducted. This study comprised two parts:

- (1) A comparison of the isolate "Bangladesh 339" was made with a reference buffalopox strain, supplied from Glasgow, and also with some other vaccinia and cowpox strains.
- (2) The isolation of any pox virus from organs of animals, and detection of antibodies against the pox group of viruses in human and animal blood.

#### 6.1 Comparison with reference viruses

A comparison of the isolate BD 339 was made with: a reference buffalopox (Ref. BP), a Lister strain of vaccinia from Dacca (BD vaccine), an Indonesian vaccinia strain (Ind. vaccine), a cowpox 188 strain (CP 188), and cowpox Brighton strain (CP Brighton). The tests for identification were as follows:

The pock morphology on CAM of chick embryo at 37°C indicated that the pock of BD 339 appeared similar to that of BD vaccine, although more haemorrhagic. There were secondary pocks after 72 hours. Ref. BP also looked somewhat like the above two strains but without secondary pocks. Ind. vaccine gave grey and haemorrhagic pocks. Pocks of CP 188 and CP Brighton were different from that of BD vaccine, BD 339 and Ref. BP.

All the strains were inhibited at 40.5°C except Ref. BP which showed no growth at 38.5°C.

At 37°C all the vaccinia strains, Ref. BP and BD 339 produced the same titre of haemagglutinin, but cowpox produced a lower titre.

In the test of gel precipitation the vaccinia, BD 339 and Ref. BP all showed identical antigenic patterns but CP 188 and CP Brighton were found non-identical with the above group.

Chick embryo virulency was tested by observing the death of a chick embryo within four days after inoculation with a known virus dose. BD 339, BD vaccine and Ref. BP were of similar virulent nature. Ind. vaccine, CP 188 and CP Brighton were less virulent.

The mice lethality test showed that, when mice were inoculated intracerebrally, BD 339 appeared to be most virulent.

Rabbit skin inoculation showed similar lesions for all the strains.

In the test of cytopathic effect (CPE) on pig kidney cell, BD vaccine and BD 339 showed similar CPE, while Ref. BP, CP 188 and CP Brighton showed a different type of CPE.

In the test of immunofluorescent foci on tissue culture, BD 339 and BD vaccine showed similar types of immunofluorescent foci which were different from Ref. BP.

After comparing the results of all the tests it was evident that BD 339 was very similar to BD vaccine. It was not at all similar to Ref. BP. The conclusion of the study was, therefore, that BD 339 belonged to the vaccinia group.

## 6.2 Examination of animal sera

The second part of the study was an examination of animal sera for the presence of antibodies of the pox virus group. The sera of cows, buffaloes, birds, crocodiles, mongooses and goats were processed for haemagglutination inhibition (HI) testing. These tests, and where appropriate testing of emulsified liver and kidney specimens, are under way.

An effort was made to isolate pox virus in specimens from animals in the area surrounding the patient's house. There was no positive virus isolation. The presence of HI antibody in the sera of the cows, buffaloes, and other animals suggests, however, that there might be a natural infection among the animals. It would be of interest to look for the antibody in the animal sera, which, if found, would suggest previous infection with pox group viruses. It would also be interesting to study the organs of animals, whose blood contained high titres of HI antibody, for isolation of pox group virus.

## 7. Conclusions

The epidemiological findings and the laboratory investigations were in agreement: the child had not been attacked with buffalopox or cowpox but presumably with chickenpox. The initial specimen had been contaminated with vaccinia at some stage between collection and laboratory testing but it was not possible to determine where and when this contamination had occurred.

The study also revealed a positive titre of antibody of pox group viruses in the animal sera processed for haemagglutination inhibition test and, in some samples, neutralization test. This suggests that there might have been a natural infection among cows and buffaloes, and also birds and mongooses.

Further studies on human sera, and animal organs and sera, are being carried out and may bring additional information to the study of pox group diseases and their transmission patterns.

As mentioned it was necessary to reduce the radius of the search area to four miles (compared with the proposed eight miles) because of the high population density.

However, from all other operational points of view the protocol formulated by "An Informal Group for Monkeypox and related viruses" proved entirely suitable to this study in a rural area of Bangladesh.

## ACKNOWLEDGEMENTS

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