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# A METHOD FOR ASSESSING THE RESPONSES OF MOSQUITOS TO RESIDUAL INSECTICIDE DEPOSITS IN NORTH BORNEO

by

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## INTRODUCTION

To carry out entomological field trials employing the tentative operational method for assessing the effects of insecticide dosage, it is essential to choose an area provided with the maximum density of vector anophelines in human dwellings throughout the transmission season. During our selection of areas and houses for test in North Borneo, in most of the areas (both coastal and interior) the density of vector anophelines inside human dwellings was found to be very low. It is possible, therefore, that there were not sufficient vector anophelines in human dwellings to show up room-mortality after spraying. In order to overcome this, a method has been devised, as described below. It may serve as additional evidence of effectiveness of a given dosage and as a supplement to the tentative method described in document WHO/Mal/285.

North Borneo includes the whole of the northern portion of the island of Borneo. The China Sea washes its western shore and the Sulu and Celebes Seas its eastern coast. The heavily-indented coastline measures some 800 to 900 miles. A. sundaicus, distributed patchily along the whole coastal area, is responsible for malaria there. Since the report of DDT-resistant A. sundaicus in Java, dieldrin has been employed for controlling this vector and found very successful. For safety, it was suggested that dieldrin be used in the coastal areas of North Borneo, and dosage trials of dieldrin, therefore, were undertaken mainly against A. sundaicus and also A. barbirostris, which happens to breed in the rice fields next to the tidal belt.

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The interior of North Borneo is more or less hilly and covered with dense forest. A. balabacensis appears to be widely distributed and has shown consistently the highest rates of infection. This vector is certainly responsible for high hyperendemicity of malaria throughout the interior part of North Borneo. DDT has been proved as an effective insecticide against this vector. Dosage trials of DDT therefore were undertaken against A. balabacensis.

Unfortunately, since it was difficult and expensive[1] to collect a large number of A. balabacensis for facultative bio-assay, it was necessary to use A. barbirostris as a routine assessment for the experiment in the interior area.

1. Description of method used

Two unsprayed areas have been selected for the experiments:

(a) Kampong[2] Kuala, district Papar - this is the coastal area (approximately five feet above sea level) where the main vector is A. sundaicus with the secondary vector A. barbirostris.

(b) Kampong Kilimu, district Ranau - this is the interior area (about 2200 feet above sea level) where the main vector is A. balabacensis with the secondary vector A. barbirostris.

For each area, three groups of houses were selected: group I with four houses sprayed with higher dosage (dieldrin 0.6 $g/m^2$ for Papar area and DDT 2 $g/m^2$ for Ranau area); group II also with four houses but sprayed with lower dosage (dieldrin 0.3 $g/m^2$ for Papar area and DDT 1 $g/m^2$ for Ranau area); and group III with two houses serving as a check.

2. Construction of cubicle

Ten unsprayed houses in each area were chosen, numbered and marked on a sketch map. To use these 10 houses in each area, permission had been obtained from the house owners that one cubicle (6' x 6' x 6' at Papar and 7' x 7' x 7' at Ranau) could be built in

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[1] According to our experiences in North Borneo, it was necessary to hire at least 20 human baits to sleep the same night in one unsprayed local house to get about 50 A. balabacensis for one test.

[2] "Kampong" means village.

one corner of the house. Also assurances were obtained from the owners that the cubicles would be available for seven months for a weekly evaluation lasting one night. For this service, payment was offered to each house owner.

The materials for constructing the cubicle were exactly those adopted in the local houses. At Papar, the cubicles were constructed of wooden posts with kajang (ready-made piece of dry palm leaves) for the walls and roof. At Ranau, they were made of bamboo posts with split bamboo for the walls and attap (thatch) for the roof. It must be noted that the cubicles built with these local materials were not at all mosquito-proof. They were more or less similar to the walls and roof of the local houses, with many slits and little openings. The mosquitos released inside the cubicles for the test therefore had many chances to escape. Each cubicle was provided with a door for entering and a window-opening facing the outside of the house to which a rectangular trap, as described in Annex 3, Appendix 1, page 17, WHO/Mal/285, could be fitted. The furniture inside the cubicle was arranged so that floor-sheets could be laid down overnight to permit morning collection of the mosquitos killed during the night.

## 3. Operating procedure

### 3.1 Collecting vector anophelines by night from outdoor animal baits

Several domestic buffaloes were used as baits. Just after sunset they were tethered to the stalks of bushes close to the breeding places. Usually the mosquitos, attracted by the baits, were resting on the twigs and leaves of bushes nearby. They were conveniently located with an electric torch and collected with a sucking-tube or test-tube from 7-10 p.m. Blood-fed vector anophelines of the same species were kept together for releasing inside the cubicle prepared for the test.

At Papar, the vector anophelines used for the routine work of facultative bio-assay were *A. sundaicus* and *A. barbirostris*, while at Ranau only *A. barbirostris* was used.

3.2 Night releasing of anophelines in the cubicle

The cubicles ready for test were installed with a night trap. Floor-sheets were laid down inside cubicles for the night. All animals and insects inside the cubicles, i.e. ants, spiders, lizards, cockroaches, cats, ground beetles, etc. were removed or kept away from the cubicles. Occupants who usually slept inside cubicles were moved out during the period of test. Before releasing the mosquitos, the window-openings for the window traps were masked for 20 minutes so that the mosquitos would settle down on the walls instead of going directly to the trap. The door and the big openings of the cubicle were closed and a moderate safety lamp was hung in the centre of the cubicle so that the mosquitos could not very easily find their way out.

The time for the release of the vector anophelines in the prepared cubicle was 10 p.m. and usually 50 mosquitos were released in the centre of each cubicle.

3.3 Observation of behaviour of vectors after releasing

In order to observe the normal behaviour of the vector species and their reactions to the presence or absence of insecticide, the entomologist, as a routine, made a stay inside a cubicle once a month after the releasing to find out certain data, i.e. (a) the movements of the mosquitos from one type of resting place to another, (b) the preference for non-sprayable types of surface such as clothing, (c) time required for the mosquitos to knock down with peak time for knock-down action, and (d) time for the mosquitos to fly into the window trap.

3.4 Morning check in cubicle and in window trap

At 6 a.m. the following morning, the floor collections and the dead mosquitos on the movable tray of the window trap were carefully recorded and transferred to a container. The walls and roof of both the cubicle and the window trap were carefully examined. Live mosquitos resting on the cubicle and on the trap were recorded and collected for a further 16 hours' survival test.

3.5 Application of residual insecticide

The above-mentioned procedures (from 3.1 to 3.4) were carried out as a routine both at Papar and at Ranau for a month before spraying and also for a period of seven months after spraying. The residual spraying in cubicles was carried out by experienced men and was directly supervised by the WHO engineer. Care was taken to see that the operation conformed closely to the standard demanded in an eradication campaign. The cubicles at Papar were sprayed for the first time on 25 October 1961, while those at Ranau were done on 16 November 1961.

4. Results, confirmations and interpretation

After eight hours' exposure to treated cubicles and 16 hours of survival tests, counts of dead and alive mosquitos were recorded. It must be noted that the observed uncorrected mortalities (in percentages) were measured only according to those mosquitos maintained in the cubicles and traps. Those mosquitos which escaped from the cubicles (usually more than 50% of those released) were discarded. The mortality for one treatment was recorded every 30 days after spraying. Where average control mortality for a month exceeded 20%, the series of tests was considered unsatisfactory. Those between 5-20%, the average observed mortalities, were corrected by Abbott's formula.

A marked drop in the average mortalities (in the cubicle and trap) in a sprayed cubicle indicated the loss of continued effectiveness of vector control by a given dosage and frequency of application of certain insecticides. Less than 70% was considered as an unsatisfactory mortality rate under all but the severest conditions of malaria endemicity.

RESULTS

1. Results before spraying

As shown in the following table, the percentages of mosquitos collected after releasing for eight hours in a cubicle were 33.3% at Papar and 44.7% at Ranau. The recovery rates of mosquitos after releasing indicated a good yield of mosquitos for the test.

| Area | Date (1961) | Total no. released | Total no. recovered | % recovery | % M. | In cubicle | | | In trap | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | No. dead | No. alive | % M. | No. dead | No. alive | M. |
| Papar | 22.9 -19.10 | 825* | 275 | 33.3 | 5 | 4 | 54 | 7 | 11 | 206 | 5 |
| Ranau | 10.11, 15.11 | 85** | 38 | 44.7 | 3 | 0 | 3 | 0 | 1 | 34 | 3 |

* About one third were A. sundaicus, the others were A. barbirostris, A. lesteri and A. baezai.

** All the anophelines released were A. barbirostris.

2. Results obtained after spraying

As shown in Tables I and II, in dieldrin-sprayed cubicles at Papar the mortality rates of A. sundaicus and A. barbirostris fell only very slightly from the first to the third month after spraying for both the lower and higher dosages. However, they fell abruptly and became less than 70% from the fourth month after spraying. The difference in mortality between the lower and higher dosage of dieldrin during the period of five months after spraying was 15% against 20% for A. sundaicus and 35% against 50% for A. barbirostris.

In DDT-sprayed cubicles at Ranau, the mortality rates of A. barbirostris for both the lower and higher dosages were high from the first to the third months after spraying, as shown in Table III. It started to fall from the fourth month after spraying for the lower dosage and from the fifth month for the higher dosage. The difference in the mortality rate of A. barbirostris between the lower and higher dosages of DDT during the five-month period after spraying was 63% against 85%. The lower dosage during the six-month period after spraying was not effective at all, with only a 56% mortality rate, while that of the higher dosage was still effective with a 74% mortality rate. However, both lower and higher dosages were not effective from the seventh month after spraying.

According to the above findings, for dieldrin it is necessary to spray four rounds a year for the lower dosage and three rounds a year for the higher dosage in North Borneo, while for DDT three rounds a year are required for the lower dosage and two rounds a year for the higher dosage.

3. Observation of activity of the mosquitos released in the cubicle

Observations were made each month after spraying in both control and sprayed cubicles, with the following findings.

DDT caused a lot of activity for A. barbirostris at Ranau, whereas dieldrin did not appear to irritate either species at Papar. Moreover, the activities of A. barbirostris in a DDT-sprayed cubicle six months after spraying were found almost the same as those of the first month after spraying.

Results of observations on preference of mosquitos for non-sprayable types of surfaces, such as hanging clothing, indicated that more A. barbirostris rest on non-sprayable surfaces at Ranau (DDT-sprayed area) than at Papar (dieldrin-sprayed area).

Observations on the time required for the released mosquitos to knock down indicated that the peak of knock-down was the second hour after releasing, one to three months after spraying. However, the peak shifted to the third hour, four to six months after spraying, for both higher and lower dosages of DDT and dieldrin.

Observations on the time for the released mosquitos to fly inside the trap in both DDT-sprayed and unsprayed cubicles indicated that the peak time of exit from the unsprayed cubicle to the trap was early morning, as shown below:

| Time after releasing | Per cent. mosquitos entering trap | |
|---|---|---|
| | From unsprayed cubicle | From DDT-sprayed cubicle |
| 24.00 | 2.5 | 8.6 |
| 2.00 | 7.5 | 11.4 |
| 4.00 | 20.0 | 8.6 |
| 6.00 | 70.0 | 0.0 |

DISCUSSION

This method has been devised to determine the optimum cycle and dosage of insecticides under natural and local conditions with a great number of mosquitos for test. It may give evidence of the effectiveness of a given dosage and period after spraying in North Borneo where vector density inside human dwellings is not always high.

Results of this investigation indicate that using dieldrin, it is necessary to spray four rounds per year for lower dosages and three rounds per year for higher dosages. As for DDT, it needs three rounds per year for lower dosages and two rounds per year for higher dosages. These findings were confirmed by bio-assay carried out on the well-sprayed walls of a cubicle which showed a notable drop in the lethal effect of dieldrin (0.6 $g/m^2$) four months after spraying, and of DDT (2 $g/m^2$) six months after spraying.

In the DDT-sprayed cubicles at Ranau, mortality rates of A. barbirostris one to three months after spraying for lower dosages were high, up to 100%, as shown in Table III. The fact that DDT at 1 $g/m^2$ gave 100% kill in a large species like A. barbirostris for a period of three months after spraying is somewhat unusual. However, as mentioned in the second paragraph (Construction of cubicle) under "Description of method used", the cubicles built with local materials with many slits and little openings were not at all mosquito-proof. The mosquitos released inside the cubicle had many chances to escape and the observed uncorrected mortalities (in percentages) were measured only according to those mosquitos maintained in cubicles and in traps; those which escaped from cubicles (usually more than 50%) were not counted. The mortality rate of this method is not supposed to be compared with that of a susceptibility test.

~~Since a great number of mosquitos~~ escaped through the slits and little openings of the cubicle after releasing and thus affected the true mortality rate of the test, it is suggested that the cubicle should be constructed with local materials in such a way as to be more or less mosquito-proof in order to get a real mortality rate.

Again, in DDT-sprayed cubicles at Ranau, it was found that very few A. barbirostris entered the window traps attached to the cubicles for the period of one to three months after spraying for both lower and higher dosages. This phenomenon is self-explained by the fact that most of the mosquitos went to the traps because they were attracted by the morning sunlight, as shown in paragraph 3.3 (Observation of behaviour of vectors after releasing). In a newly DDT-sprayed cubicle, the mosquitos released either escaped from the cubicle or were knocked down by DDT one hour after releasing. Seldom was one seen remaining on the wall waiting for the morning sunlight to appear. Of course, there is the possibility of A. barbirostris getting into the trap due to its irritability to DDT. However, due to the small size of the trap opening (only 40 $cm^2$) and the fast knock-down action of DDT, this chance is very small without the help of the morning sunlight.

Judging from the fact that mosquitos, after being released in the cubicle, seldom fly to the trap during the night, it is suggested that there should be two traps for one cubicle. And as the cubicle is always with two walls facing the outside of the house, it is suggested that one trap be installed on each wall facing the outside of the house.

SUMMARY

The response of A. sundaicus and A. barbirostris to dieldrin deposits at Papar (coastal area) and of A. barbirostris to DDT at Ranau (interior area), North Borneo, has been investigated by means of releasing a great number of mosquitos in sprayed locally-made cubicles installed with exit window traps.

In dieldrin-sprayed cubicles at Papar, mortality rates in A. sundaicus and A. barbirostris fell only very slightly from the first to the third month after spraying, for both lower and higher dosages. However, mortality rates fell abruptly from the fourth month after spraying and became less than 50% from the fifth month. The difference in mortality between the lower and higher dosages of dieldrin during the fourth month after spraying was 53% against 70%.

In DDT-sprayed cubicles at Ranau, mortality rates of A. barbirostris were high for both lower and higher dosages during the first to the third months after spraying. They started to fall from the fourth month after spraying for the lower dosage and from the fifth month for the higher dosage. During the period of six months after spraying, the lower dosage was not effective at all with only 56% mortality rate, while that of the higher dosage was still effective with 74% mortality rate. However, both lower and higher dosages were not effective from the seventh month after spraying.

According to the findings by this method used in North Borneo, for dieldrin it is necessary to spray four rounds per year for lower dosages (0.3 $g/m^2$) and three rounds per year for higher dosages (0.6 $g/m^2$), while for DDT it needs three rounds per year for lower dosages (1 $g/m^2$) and two rounds per year for higher dosages (2 $g/m^2$).

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TABLE I. RESULTS OF FACULTATIVE BIO-ASSAY AT PAPAR USING <u>A. SUNDAICUS</u>

| Treatment | Control | | | | | | Dieldrin (0.3 $g/m^2$) | | | | | | Dieldrin (0.6 $g/m^2$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. day after spraying with date of test | 1-30 (4,17/ 11/61) | 30-60 (13/1 /62) | 60-90 (10/2 /62) | 90-120 (10/3 62) | 120-150 (12/4/ 62) | 150-180 (16/4/ 62) | 1-30 (10-21 /11/61) | 30-60 (30/11- 23/12/61 | 60-90 (29/12/61- 19/1/62) | 90-120 (25/1- 15/2/62) | 120-150 (24/2- 23/3/62) | 150-180 (28/3- 14/4/62) | 1-30 (15-23 /11/61) | 30-60 (2/12/ 61) | 60-90 (17/1 /62) | 90-120 (4-10/ 2/62 | 120-150 (5,15/3 /62) | 150-180 (30/3- 13/4/62) |
| Total no. of mosquitos released | 80 | 28 | 25 | 42 | 28 | 120 | 148 | 151 | 90 | 128 | 155 | 212 | 126 | 25 | 77 | 81 | 72 | 182 |
| Total no. of mosquitos recovered | 33 | 9 | 10 | 16 | 14 | 24 | 45 | 50 | 33 | 53 | 69 | 91 | 42 | 12 | 25 | 20 | 29 | 69 |
| % recovery | 41 | 32 | 40 | 38 | 50 | 20 | 30 | 33 | 37 | 41 | 45 | 43 | 33 | 48 | 32 | 25 | 40 | 38 |
| Number dead in cubicle | 1 | 0 | 0 | 0 | 0 | 0 | 37 | 39 | 19 | 27 | 6 | 0 | 32 | 11 | 9 | 12 | 7 | 10 |
| Number alive in cubicle | 15 | 8 | 7 | 13 | 10 | 20 | 2 | 2 | 0 | 0 | 19 | 37 | 1 | 1 | 0 | 0 | 5 | 21 |
| Number dead in trap | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 7 | 11 | 1 | 4 | 1 | 9 | 0 | 12 | 2 | 8 | 2 |
| Number alive in trap | 17 | 1 | 3 | 3 | 4 | 3 | 0 | 2 | 3 | 25 | 40 | 53 | 0 | 0 | 4 | 6 | 9 | 36 |
| % mortality | 3 | 0 | 0 | 0 | 0 | 4 | 95 | 92 | 91 | 53 | 15 | 1 | 97 | 92 | 84 | 70 | 52 | 17 |

TABLE II. RESULTS OF FACULTATIVE BIO-ASSAY AT PAPAR USING **A. BARBIROSTRIS**

| Treatment | Control | | | | | | | Dieldrin (0.3 g/m²) | | | | | | | Dieldrin (0.6 g/m²) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number day after spraying with date of test | 1-30 (4,11/11/61) | 30-60 (4-23/12/61) | 60-90 (4,17/1/62) | 90-120 (24/1,13/2/62) | 120-150 (28/2,21/3/62) | 150-180 (10/4/62) | 180-210 (12,19/5/62) | 1-30 (30/10-23/11/61) | 30-60 (30/11-24/12/61) | 60-90 (24/12/61-18/1/62) | 90-120 (24/1-13/2/62) | 120-150 (28/2-21/3/62) | 150-180 (10-15/4/62) | 180-210 (9-19/5/62) | 1-30 (4-21/11/61) | 30-60 (30/11-23/12/61) | 60-90 (24/12/61-19/1/62) | 90-120 (25/1-15/2/62) | 120-150 (24/2-21/3/62) | 150-180 (10-15/4/62 | 180-210 (12,20/5/62) |
| Total number of mosquitos released | 108 | 203 | 100 | 95 | 79 | 45 | 50 | 367 | 170 | 245 | 274 | 124 | 58 | 138 | 271 | 454 | 401 | 200 | 105 | 45 | 57 |
| Total number of mosquitos recovered | 38 | 68 | 34 | 21 | 15 | 22 | 18 | 157 | 61 | 89 | 79 | 37 | 18 | 79 | 154 | 171 | 176 | 72 | 38 | 16 | 21 |
| % recovery | 35 | 33 | 34 | 22 | 19 | 49 | 36 | 43 | 36 | 36 | 29 | 30 | 31 | 57 | 57 | 38 | 44 | 36 | 36 | 36 | 37 |
| Number dead in cubicle | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 143 | 48 | 35 | 33 | 6 | 1 | 3 | 137 | 146 | 117 | 37 | 15 | 3 | 4 |
| Number alive in cubicle | 18 | 17 | 8 | 6 | 5 | 10 | 7 | 3 | 0 | 1 | 3 | 7 | 6 | 29 | 1 | 6 | 1 | 2 | 3 | 1 | 4 |
| Number dead in trap | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 | 7 | 29 | 5 | 7 | 2 | 5 | 16 | 13 | 38 | 7 | 4 | 4 | 5 |
| Number alive in trap | 20 | 48 | 24 | 13 | 10 | 12 | 11 | 7 | 6 | 24 | 38 | 17 | 9 | 42 | 0 | 6 | 20 | 26 | 16 | 8 | 8 |
| % mortality | 0 | 4 | 6 | 10 | 0 | 0 | 0 | 94 | 90 | 70 | 42 | 35 | 17 | 10 | 99 | 93 | 87 | 57 | 50 | 44 | 43 |

TABLE III. RESULTS OF FACULTATIVE BIO-ASSAY AT RANAU USING **A. BARBIROSTRIS**

| Treatment | Control | | | | | | | | DDT (1 g/m²) | | | | | | | | DDT (2 g/m²) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number day after spraying with date of test | 1-30 (5,10/12/61) | 30-60 (23,30/12/61) | 60-90 (12-13/2/62) | 90-120 (3-13/3/62) | 120-150 (17/3-14/4/62) | 150-180 (21/4-9/5/62 | 180-210 (29/5-12/6/62) | 210-240 (19-20/6/62) | 1-30 (19/11-14/12/61) | 30-60 (16/12/61-13/1/62) | 60-90 (15/1-12/2/62) | 90-120 (17/2-13/3/62) | 120-150 (14/2-17/4/62) | 150-180 (21/4-10/5/62) | 180-210 (12/5-12/6/62) | 210-240 (15-16/6/62) | 1-30 (19/11-15/12/61) | 30-60 (16/12/62) | 60-90 (15/1-12/2/62) | 90-120 (17/2-13/3/62 | 120-150 (17/3-14/4/62) | 150-180 (17/4-11/5/62) | 180-210 (12/5-9/6/62) | 210-240 (15-22/6/62) |
| Total number of mosquitos released | 125 | 65 | 90 | 180 | 350 | 205 | 250 | 98 | 396 | 570 | 437 | 666 | 720 | 1 260 | 890 | 140 | 562 | 185 | 471 | 795 | 600 | 1 560 | 1 325 | 505 |
| Total number of mosquitos recovered | 68 | 32 | 44 | 65 | 113 | 74 | 102 | 44 | 105 | 158 | 111 | 185 | 176 | 362 | 319 | 74 | 108 | 46 | 102 | 200 | 141 | 436 | 443 | 143 |
| % recovery | 54 | 49 | 49 | 36 | 32 | 36 | 41 | 45 | 27 | 27 | 25 | 28 | 24 | 28 | 36 | 53 | 19 | 25 | 22 | 25 | 24 | 28 | 25 | 28 |
| Number dead in cubicle | 0 | 2 | 1 | 0 | 0 | 2 | 1 | 0 | 101 | 152 | 104 | 147 | 96 | 198 | 141 | 30 | 104 | 46 | 102 | 175 | 110 | 306 | 260 | 70 |
| Number alive in cubicle | 13 | 17 | 10 | 20 | 20 | 17 | 28 | 4 | 0 | 0 | 0 | 0 | 5 | 82 | 143 | 27 | 0 | 0 | 0 | 2 | 5 | 64 | 131 | 34 |
| Number dead in trap | 0 | 0 | 2 | 0 | 5 | 2 | 0 | 0 | 4 | 6 | 7 | 19 | 14 | 10 | 17 | 5 | 4 | 0 | 0 | 22 | 13 | 17 | 33 | 15 |
| Number alive in trap | 55 | 13 | 31 | 45 | 88 | 53 | 73 | 40 | 0 | 0 | 0 | 19 | 61 | 72 | 18 | 12 | 0 | 0 | 0 | 1 | 13 | 49 | 19 | 24 |
| % mortality | 0 | 6 | 7 | 0 | 4 | 3 | 1 | 0 | 100 | 100 | 100 | 84 | 63 | 56 | 50 | 47 | 100 | 100 | 100 | 99 | 85 | 74 | 66 | 59 |