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# A NEW POTENTIAL VECTOR OF *SCHISTOSOMA HAEMATOBIUM* IN PORTUGAL*

por

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The increase of human relations in recent times between tropical and temperate regions gives more and more significance to the problem concerning the possibility of import and fixation in the latter regions of the infectious diseases typical of the former.

Meanwhile, according to this aspect we must consider the tropical diseases due to agents which are transmitted, either directly or indirectly, from individual to individual, such as, for example, cholera and amebiasis, the diseases due to agents that must have an evolution period in the soil, such as ankylostomiasis and strongyloidiasis, and those that must have a vector or intermediate host, such as malaria and bilharziasis, among others.

In order to evaluate the possibility that the diseases of this third group might eventually originate new foci in temperate regions, it is necessary to check the presence of possible local vectors and then ascertain whether the climatic and populational living conditions are also favourable to those vectors.

As a contribution to the study of the possible creation of foci of vesical bilharziasis in continental Portugal, we subjected *Bulinus B. truncatus* of the continent to infection by *Schistosoma haematobium* from Portuguese Guinea, as described below.

* Paper presented at the Academia das Ciências de Lisboa on 18th February 1965.

## MATERIAL AND METHODS

The miracidia of *S. haematobium* were obtained from eggs from acute human bilharziasis cases from Portuguese Guinea. The urine of the patients was collected and allowed to settle at environment temperature (18°C to 20°C) in conical glass containers of 50 cc., for about half an hour. The urine was then decanted and water was added to the sediment (about 4 times its volume), a mixture was made and we waited for the elimination of the miracidia, which took place at the end of about half an hour.

*Bulinus truncatus* is reported by Nobre as *B. contortus* (1) in the North of the Country (fig. 1); we tested just one strain, collected near Coimbra in 1962, classified by Medeiros (2) as *Bulinus (B.) contortus* and kept in the Laboratory ever since that date, and so we are quite sure that the specimens tested were not infected by any other trematode species. The shell of this strain (fig. 2) is like the shell of the *Bulinus truncatus* typical (3), but according with Mandal-Barth (4) that has observed the our species... «the Portuguese snails differ so much from Egyptian *B. truncatus*, esp. in size and shape of the radula teeth, that I believe it justifiable to separate them as a distinct subspecies or perhaps even as a distinct species from which the name *contortus* is available». So, according with this observation the portuguese species is being studied by us at the same time the species of *B. truncatus* from Iran, kindly offered by Dr. F. Arfaa and from Irak, kindly offered by Dr. Wadji, but in this work we with considered it as *Bulinus (B.) truncatus*.

For the infection of the snails we have employed, up to the present, five lots (table I) with a total number off 83 specimens which were subjected to an average of 10 to 50 miracidia per snail from December 3, 1964 to March 11, 1965. The snails were kept at temperatures ranging from 22°C to 25°C and as control we used 49 specimens which were kept under the same conditions of the infected ones.

In order to appreciate the susceptibility of the snails to infection, we waited for the final term of the parasite's develop-



TABLE I

**Results of the experimental infection of *Bulinus (B) truncatus* from Portugal Continental by the *Schistosoma haematobium* from Portuguese Guinea.**

| Number of the lots | Number of snails | | Number of miracidia for each snail | Pre patent period (days) | Period of elimination of cercariae (days) | | | Snails that eliminated cercariae | | Mortality of the snails till 90 days after infection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Submited to the infection | Control | | | Minimum | Maximum | Average | Number | % | Submited to the infection | | | | Control | |
| | | | | | | | | | | Positives | | Negatives | | | |
| | | | | | | | | | | Number | % | Number | % | Number | % |
| 1 st. | 20 | 10 | 10 | 42 | 6 | 51 | 27 | 7 | 35 | 6 | 86 | 4 | 31 | 3 | 30 |
| 2 nd | 20 | 12 | 15 | 35 | 4 | 50 | 38 | 6 | 30 | 6 | 100 | 3 | 20 | 2 | 18 |
| 3 rd. | 15 | 10 | 20 | 30 | 3 | 62 | 32 | 7 | 47 | 6 | 86 | 4 | 50 | 3 | 30 |
| 4 th. | 12 | 7 | 30 | 31 | 2 | 64 | 26 | 8 | 67 | 6 | 75 | 1 | 25 | 1 | 14 |
| 5 th. | 16 | 10 | 50 | 30 | 8 | 53 | 21 | 7 | 44 | 7 | 100 | 2 | 22 | 2 | 20 |
| Total | 83 | 49 | — | | — | — | — | 35 | 42 | 31 | 88,5 | 14 | 29 | 11 | 22 |

ment - elimination of cercariae, after the snails had been placed for half an hour in an incubator at 37° C.

Simultaneously, histological sections were made of 8 snails, that had been infected as said above and that had been killed between 30 and 45 days, for the observation of the parasite's development in the snails tissues.

## RESULTS

Concerning the susceptibility of the snails to the infection, evaluated by the elimination of cercariae we have observed that for a total of 83 experimental infected snails, 35 become positives, that is 42 por 100 (table I). In the five studied lots the average period of cercariae elimination has oscilated between 21 and 38 days and the period of elimination had as minimum 2 days and as maximum 64 days in the 4th lot. The mortality of the infected snails till 90 days after the infection was 88,5 % in the 31 where the cercariae elimination occurred, and 29 % in the negatives ones ; in the 49 snail controls was 22 %.

According with these results we can arrive at the conclusion that the strain of Portuguese *Bulinus truncatus* is too susceptible to *S. haematobium* of Portuguese Guinea. In fact, in comparison with the snail species considered as being the ones most susceptible to schistosomes, the species now tested shows a considerable infection rate, thus leading us to include it, according to the criterion of one of us (5), in the good vectors group (infection rate of 25 to 50 por 100). If we take in consideration the infection rate of 42 por 100 obtained with *Bulinus (Ph) africanus* from Portuguese Guinea in relation to this Province's own strain (6), we may conclude that the Portuguese strain now tester behaves as a good vector.

The pre-patent infection period in snails (30-42 days) corresponded to the habitual period which is experimentally observed (4-8 weeks) at the same temperature of the test.

The observation of the histological sections under the above mentioned conditions revealed in 5 of the snails observed the evolution of the parasite in the hepatopancreas to the cercaria phase (Fig. 3).



## DISCUSSION

Though laboratorial data should always be transported to the field with the utmost prudence, it is undeniable that the Portuguese strain of *Bulinus truncatus* proved to be susceptible to *S. haematobium* from Guinea ; accordingly, it is to be expected, under natural conditions, that this snail might become infected and thus behave as a biological vector.

In fact, in order that this may happen, it is necessary that the climatic and the human living conditions of the areas where the snail occurs contribute for such a role.

Climatic conditions are adequate to the breeding of the mollusk, whose favourable temperature is from 18° C to 28° C (7), surely also favourable to the development of the parasite in it, at least during the summer in certain areas such as, especially, the Coimbra region, where annual medium temperature is 15° C, ranging from the medium maximum of 21,23° C to the medium minimum of 10.4° C according to Table II.

**TABLE II**

**Monthly mean temperatures and maximum and minimum mean temperatures of the air of Coimbra from 1921 to 1950 (O clima de Portugal, fasc. IX, Serviço Meterológico Nacional, Lisboa, 1956).**

| Months | Temperatures | | |
|---|---|---|---|
| | Maximum | Minimum | Average |
| January | 14,0 | 5,4 | 9,1 |
| February | 15,6 | 5,9 | 10,1 |
| March | 18,0 | 8,1 | 12,4 |
| April | 20,2 | 9,2 | 14,0 |
| May | 22,0 | 10,9 | 15,7 |
| June | 26,3 | 13,8 | 19,2 |
| July | 28,6 | 15,0 | 20,8 |
| August | 29,6 | 15,2 | 21,2 |
| September | 27,2 | 14,3 | 19,6 |
| October | 23,0 | 12,0 | 16,5 |
| November | 17,3 | 8,4 | 12,3 |
| December | 14,2 | 6,0 | 9,5 |
| Year | 21,3 | 10,4 | 15,0 |

Thus, at least from May to October, the parasite may develop in the snail, since only below 14°C, according to Gordon and coll., 1934 (8), is this development threatened. However, it must be said that De Wit (9) reported that the *S. mansoni* miracidium is immobilized only at 10°C, at which temperature he was unable to infect a single snail.

In analogy with what was reported in Egypt by El-Gindy and Rushdi (10), it is to be assumed that snails may be infected in Portugal only during the summer and that other areas in the North and South of the Country, besides Coimbra, may also offer favourable conditions for human infection, at least as far as temperature is concerned, as may be deduced according to the graphicof fig. 4 which concerns the monthly medium temperatures of Porto (North), Coimbra (Center) and Praia da Rocha (South) of Portugal and Cairo, the latter included as control of an endemic region near Portugal.

The human living conditions of the continent are also surely favourable to the development of the parasite in Nature, since the populations, being essentially rural, urinate freely on the ground, perhaps often in any small stream or body of water where the snails may occur, thus possibly infecting them.

It mus also be kept in mind that *Bulinus (B.) truncatus* behaves as a vesical bilharziasis vector in several regions where the epidemic is present, such as (Fig. 5) Egypt, Sudan, North Africa, Palestine, Syria, Irak, Cyprus, etc. Besides, it is not only a vector of *S. bovis*, a species included in the *haematobium* complex, in Corsica, Sardinia, Irak, Tunisia and Morocco, but it was also possible to infect experimentally some strains of several geographical areas with *S. haematobium*, as reported by Brumpt and Werblunsky (11), Cram and coll. (12), Wright (13), and others.

Accordingly, it should be assumed that the Portuguese strain might behave as a vector; such a conclusion would, however, be valid only if it had been possible either to find the infected snail in Nature or to infect it experimentally, as we now achieved.

Indeed it must be remembered that the same mollusk species does not always behave similarly towards a given schistosome species; De Witt (9) has even reached the conclusion that in the same colony of *Australorbis glabratus* there may appear des-

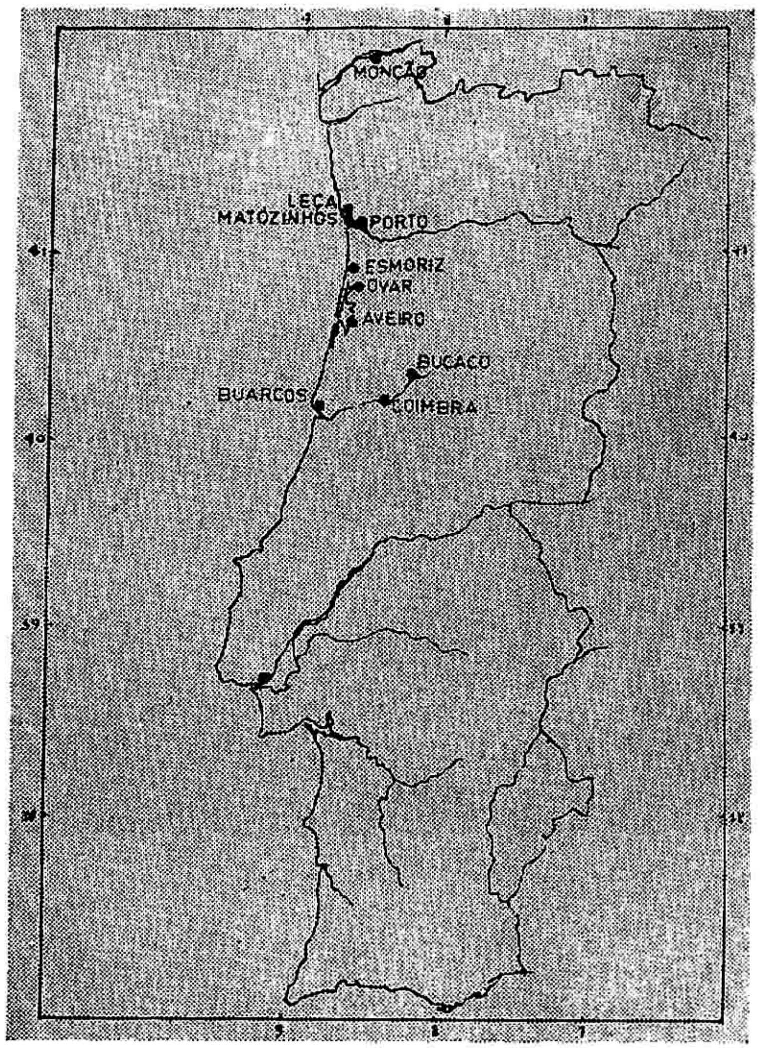



Fig. 1.—Localities (•) of Portugal were the *Bulinus contortus* was foudend. (According with Nobre, 1941.)

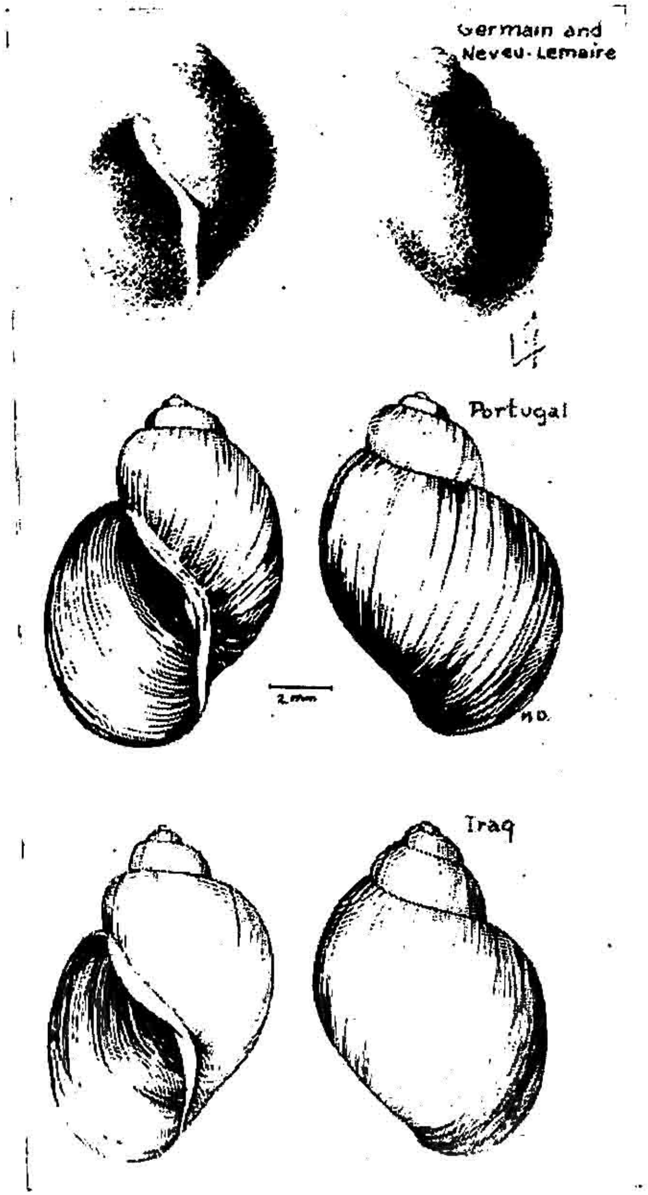



Fig. 2.—Shells of *Bulinus (B.) truncatus* of Portugal and Irak (specimens kindly offered by Dr. Wadji) compared with the shell of *Bulinus (Isidora) contortus* presented by Germain et Neveu-Lemaire (1926).

Fig. 3.—Development of the miracidium of *S. haematobium* from Portuguese Guinea till the cercariae stage in the hepatopancreas of *Bulinus (B.)* truncatus from Coimbra (center of Portugal). 200 ×

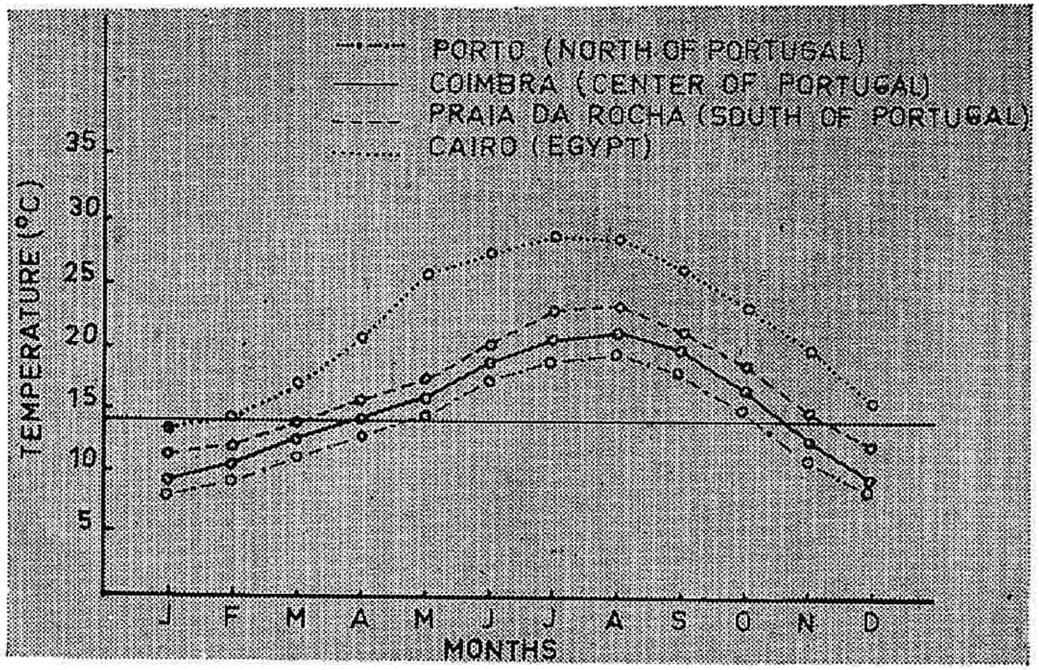



Fig. 4.—Graphic of the monthly mean temperatures of the day in Porto (North), Coimbra (Center) and Praia da Rocha (South) of Portugal, from 1921 to 1950 (Clima de Portugal, fasc. IX, Serviço Meteorológico Nacional, Lisboa, 1956) and in Cairo (Almaza), Egypt, from 1945 to 1950 (World Weather Records, 1941-1950, Washington D. C., 1959).

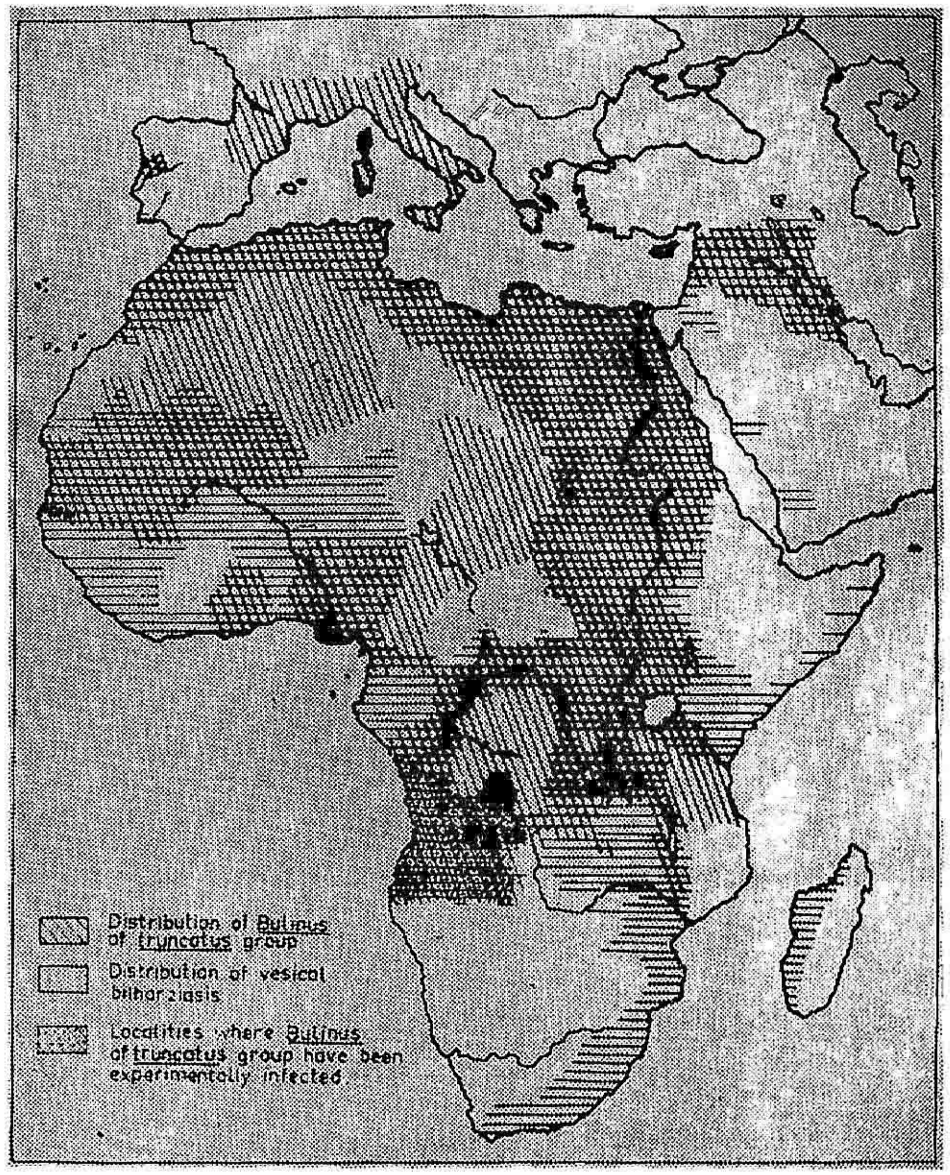
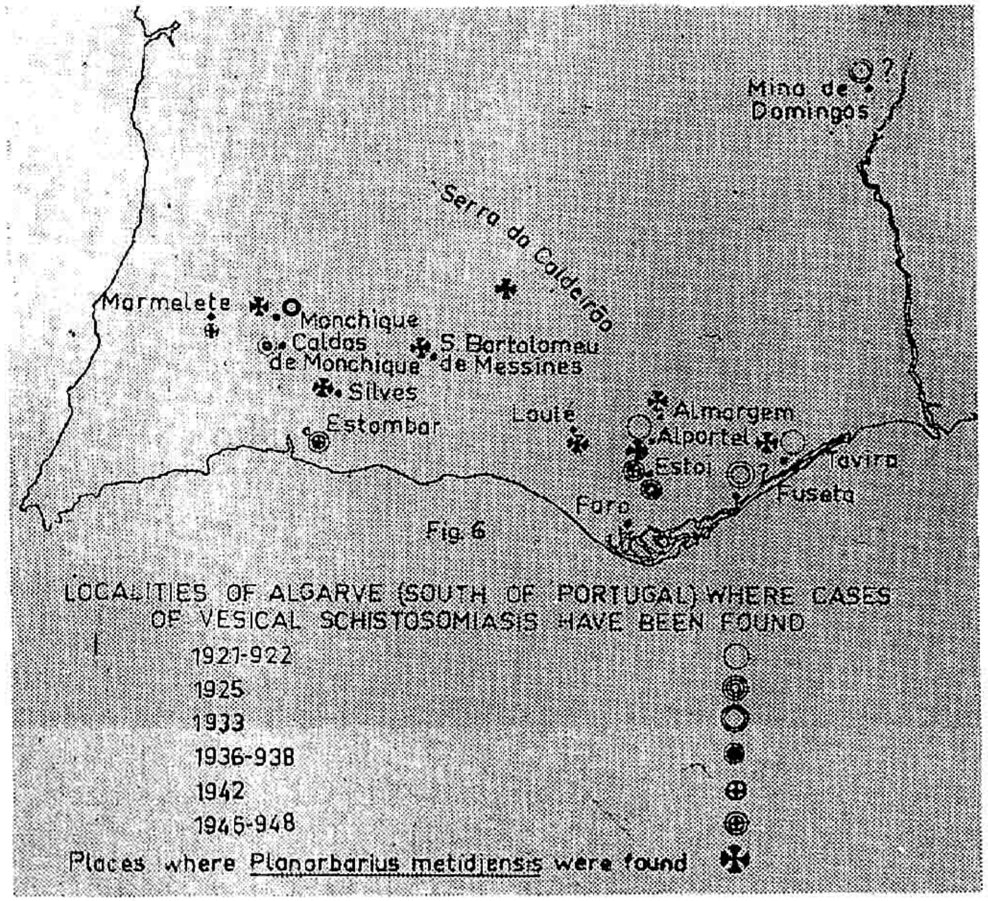



Fig. 5.—Distribution of the vesical schistosomiasis and *Bulinus truncatus*.






cendants which behave differently, as far as susceptibility to infection by *S. mansoni* is concerned.

Meanwhile, the first results now obtained by us concerning the susceptibility of *Bulinus (B.) truncatus* from Portugal to *S. haematobium* from Portuguese Guinea and, possibly, to the same schistosome species from other areas of the African Continent should lead us to consider that snail as a permanent potential vector and should compel us to take adequate defensive steps in order to stop it from becoming such a vector. According, precautions should be taken not only regarding *Planorbarius metidjensis*, which is admittedly a vector of *S. haematobium* (Bettencourt and coll. (14), França (15), Fraga de Azevedo and coll. (16, 17) in South of Portugal (Fig. 6), but also regarding the *Bulinus (B.) truncatus* of our Country.

## SUMMARY

When subjecting to infection by miracidia of *S. haematobium* from Guinean patients five lots of *Bulinus (B.) truncatus* from continental Portugal, with a total of 83 specimens and kept at temperatures from 22°C to 25°C, it was noticed that this mollusk species became infected in a global rate of 42 %, which proves its high susceptibility to this parasite.

Considering, on the other hand, that the temperature of some areas where *Bulinus (B.) truncatus* of Portugal has already been reported stays above 14°C during some months of the year, and that the rural living conditions of the respective populations are favourable to the infection by the parasite's cercariae, we conclude that there is the possibility that new foci of vesical bilharziasis may appear in the areas where the mollusk occurs; according to present knowledge, these areas correspond to the North of the Country.

## SUMARIO

Con ocasión de la infectación de 5 lotes de *Bulinus B. truncatus* de Portugal continental con miracidias de *S. haematobium* procedentes de enfermos de Guinea, con un total de 83 muestras mantenidas en temperaturas de 22° a 25° C, se observó que esta especie de molusco llegaba a infectarse en un porcentaje global de 42 por 100, lo que prueba su alta susceptibilidad a este parásito.

Considerando, por otro lado, que la temperatura de algunas áreas donde *Bulinus B. truncatus* ha sido ya comprobado que se mantiene a 14° C durante algunos meses del año, y que las condiciones de la vida rural de las poblaciones respectivas son favorables a la infección por las cercarias

del parásito, se concluye que hay la posibilidad de que puedan aparecer nuevos focos de bilharziosis en las áreas donde los moluscos existen; de acuerdo con el presente conocimiento, estas áreas corresponden al norte del país.

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