Onderstepoort J. vet. Res., 57, 283–285 (1990)

RESEARCH COMMUNICATION

AN ATTEMPT TO ESTABLISH AN INBRED LINE OF MICE GENETICALLY RESISTANT TO *COWDRIA RUMINANTIUM*

J. L. DU PLESSIS[1], E. H. H. MEYER[2] and L. VAN GAS[1]

ABSTRACT

DU PLESSIS, J. L., MEYER, E. H. H. & VAN GAS, L., 1990. An attempt to establish an inbred line of mice genetically resistant to *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 57, 283–285 (1990)

An attempt to establish an inbred line of mice resistant to *Cowdria ruminantium* failed. First generation couples were constituted from those mice out of 100 males and 100 females that had survived infection with the Kümm stock of *C. ruminantium* and that were serologically negative to the indirect fluorescent antibody test. An attempt to establish 10 separate inbred lines by constituting next generation brother and sister matings from predominantly seronegative survivor mice from the preceding generation, was unsuccessful because too few mice survived the challenge. The percentage seronegative survivors increased to 94 % over the first 6 generations, but then declined sharply during the next.

INTRODUCTION

The natural resistance of cattle to heartwater is a well known phenomenon. The innate resistance of young calves, independent of the immune status of their mothers (Neitz & Alexander, 1941), is distinguished from the non-specific resistance of cattle older that a year (Du Plessis & Malan, 1987a). Apart from experimental evidence that the serum protein, conglutinin, may be responsible for the resistance to heartwater of older cattle (Du Plessis & Bezuidenhout, 1979; Du Plessis, 1985), there is no explanation for this phenomenon. Uilenberg (1983) states, however, that the great differences in susceptibility, particularly apparent between resistant indigenous cattle breeds and exotic, imported European breeds, can probably be attributed to an inherited resitance acquired by the former through long, natural selection. It is not known whether the higher resistance of Persian sheep (Lounsbury, 1904), characterized by a febrile response but no other clinical signs, is genetically determined.

The only report thus far suggesting a genetically controlled resistance to heartwater (Matheron, Barré, Camus & Gogue, 1987), is based on the fact that Creole goats indigenous to the Caribbean islands, differed in their susceptibility to experimental challenge according to the time lapse between challenge and their last contact with the disease on the island of Guadeloupe, where the disease has been diagnosed with certainty only as recently as 1980 (Perreau, Morel, Barré & Durand). Matheron *et al.* (1987) found that the degree of resistance of goats removed decades ago from this island was 25 % and that of goats exposed to heartwater at the time of challenge 78 %, with that of animals not having been in contact with the disease for 10 years, 54 %. Although the authors state that one of the criteria suggesting resistance was seroconversion, it is not clear whether only seronegative animals in the group exposed to heartwater, were challenged. The inclusion of seropositive goats in this group would have further complicated the already difficult decision whether goats were resistant to the challenge or not and would question the high percentage of resistant animals in this group.

In a preliminary unsuccessful attempt to establish an inbred line of mice resistant to the Kümm stock of *Cowdria ruminantium* (Du Plessis, 1982), a male and female, the only mice in addition to one other male out of 240 mice to have survived the first 20 serial passages of the agent (Du Plessis, 1971), were mated. Their progeny and that of 16 successive generations of brother-sister matings were challenged with an infective dose of the Kümm stock. Using a combination of the parallel- and single-line systems of inbreeding (Falconer, 1972), the breeding pairs of each new generation were selected from the mice that had survived the previous challenge. Although it was found that over the first 15 generations an average of 63 % of mice were resistant, there was no substantial increase of resistance above that of the first generation, and during the subsequent generations it declined rapidly.

The highly appreciated comments on this experiment by 3 geneticists (G.W. Seifert, J. Hetzel & R. Quaas, personal communication, 1983), suggested firstly that a single or very limited number of autosomal genes were probably involved in the resistance and secondly that the gene would appear to have incomplete penetrance. They also concluded that the resistant line had probably been lost due to a loss of fitness following intensive inbreeding and that this could possibly have been avoided if more animals had been used to start the inbreeding. It was also suggested that an index of susceptibility other than mortality, which follows a binomial distribution of either dead or alive, should be applied to select new breeding couples.

In order to implement these suggestions, another attempt was made, starting several lines selected from mice that not merely survived challenge but that were serologically negative thereafter. It was hoped that, first, knowledge would be gained whether it was at all possible to increase the resistance to *C. ruminantium* in mice by inbreeding. Secondly, such an inbred line of mice could serve as a model to compare the pathogenesis and the disease in resistant with that in susceptible mice.

MATERIALS AND METHODS

Selection of 1st generation couples

One hundred 6 week-old male and 100 female conventional, outbred, Swiss mice were inoculated intraperitoneally with 0,2 mℓ of a 10^{-4} dilution of a deep frozen stabilate of the Kümm stock of *C. ruminatium* (Du Plessis, 1971), consisting of an homogenate in buffered lactose peptone of the spleens, lungs and hearts of infected mice. This represented 30 LD_{50} doses per mouse. Thirty days after the infection the sera of the mice that had survived were sub-

[1] Veterinary Research Institute, Onderstepoort 0110

[2] Animal and Dairy Science Research Institute, Private Bag X2, Irene 1675

Received 31 July 1990—Editor

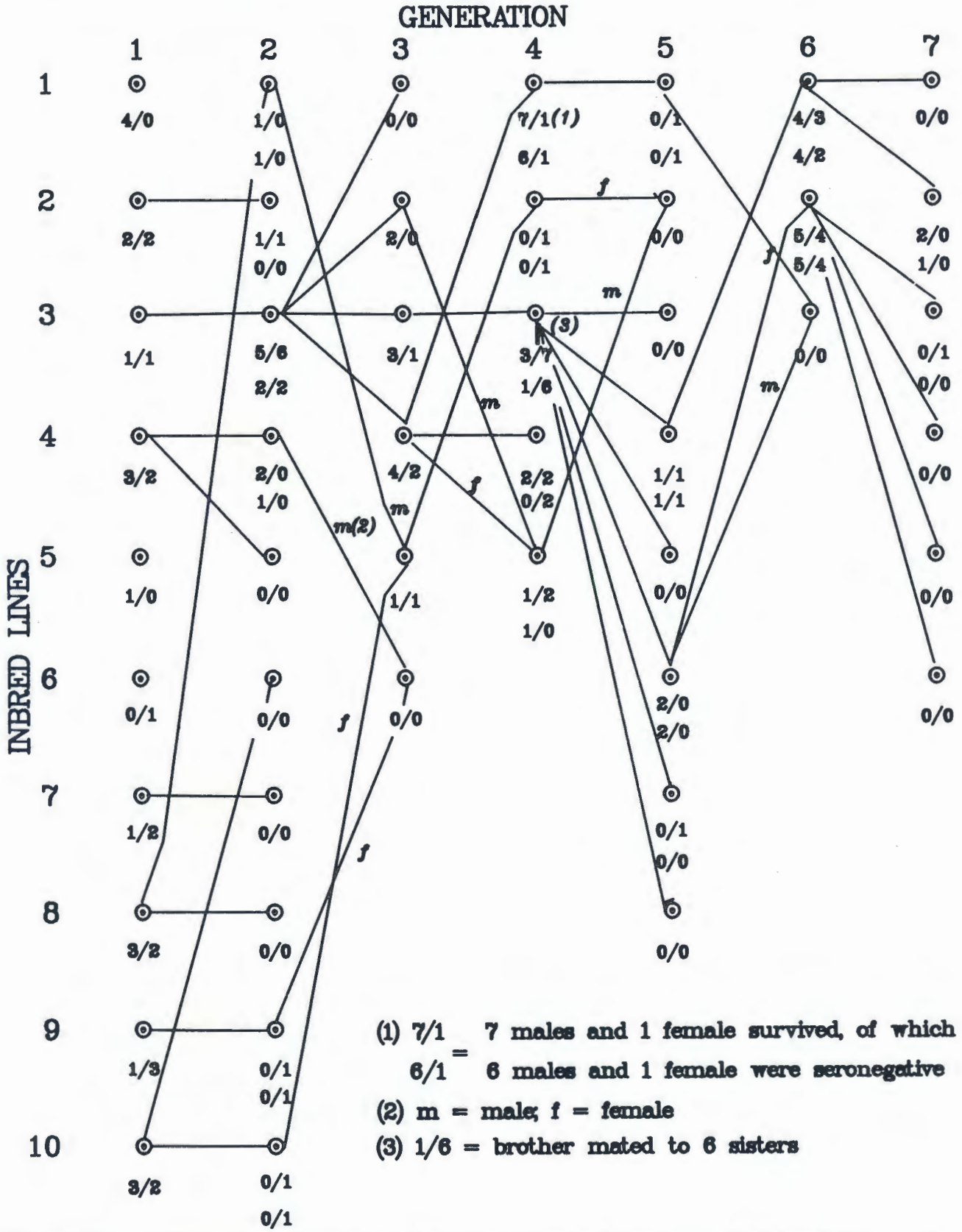

FIG. 1 Flow diagram showing mating design of mice that had survived challenge with *C. ruminantium*. Each dotted circle represents a couple

jected to the indirect fluorescent-antibody (IFA) test (Du Plessis & Malan, 1987b) at a dilution of 1:10. Since it is known that unless replication of *Cowdria* takes place there is no antibody response detectable with the IFA test (Du Plessis & Malan, 1987b), it was hoped that the absence in mice of antibody detectable with this test would be a more accurate indication of their resistance than the dead or alive rating previously used (Du Plessis, 1982). The serologically negative mice that had survived this first challenge, were used to constitute the 1st generation couples.

TABLE 1 Survival rate and serology of 7 generations of inbred mice challenged with Kümm stock *C. ruminantium*

Generation No.	No. of lines	No. of mice challenged	Survival rate		Serology of survivors		% serologically negative survivors
			No.	%	Positive	Negative	
1	10	148	34	23	ND	ND	—
2	10	126	18	14	10	8	44
3	6	106	14	13	ND	ND	—
4	5	68	26	38	7	18	69
5	8	62	6	10	1	5	83
6	3	53	16	30	1	15	94
7	6	66	3	4,5	2	1	33

Subsequent inbreeding

During the successive 6 generations one brother and sister couple for each of the 10 separate lines were selected from the progeny of the previous generation (G) that had either survived challenge (G's 1 & 3, Table 1), or from survivors that were serologically negative (G's 2, 4, 5, 6 & 7, Table 1). The progeny of G1 was challenged with 30 LD_{50} doses and that of G's 2–7 with 300 LD_{50} doses. The specificity of the mortalities of the mice that died was confirmed as having been due to *C. ruminantium* infection as previously described (Du Plessis & Malan, 1988).

The constitution of the breeding couples of G's 2–7 is represented in Fig. 1. Because so few mice survived the challenge, it was impossible to maintain more than 2 lines (3 & 4) for longer than 5 and 4 generations respectively. In an attempt to maintain as many lines as possible, Couples 1 and 2 (G3) consisted of seropositive survivors. Because they were so few, the survivors in G3 were not tested serologically. The male and female of Couples 5 and 6 (G3) were not brother and sister but originated from separate lines of G2. Couple 5 of G4, Couple 2 of G5 and Couple 3 of G6 were also not brothers and sisters. Furthermore, to increase the dwindling number of lines, a mother was mated to her son in the case of Couple 1 (G4) and Couple 2 (G6).

Results and Discussion

Out of 200 mice challenged to select the 1st generation couples, 26 males and 20 females survived (23 %) and of these 46 survivors 16 males and 10 females (56 %) were found to be serologically negative. It can be seen from Table 1 that the survival rate of some 629 mice challenged with *C. ruminantium* during the course of 7 generations varied from 4,5 to 38 %. There was no tendency for the survival rate to increase over the course of successive generations. After a peak of 38 % at G4, it dropped to 10 % at G5 and finally to 4,5 % at G7. The percentage surviving mice that failed to develop antibody levels detectable with the IFA test, increased from 44 % at G2 to 94 % at G6, but then dropped sharply to 33 % at the next generation.

It is evident from Fig. 1 that apart from Lines 3 and 4, it was not possible to maintain the other 8 lines. Couples 1 and 2 in G6 that performed best (94 % survivors seronegative), originated from Line 3, but except for 1 male their progeny in G7 had no resistance. The progeny of the few couples in G3 (Couples 5 & 6) and G5 (Couple 2) not constituted by mating brother and sister also were not resistant and these lines petered out before reaching G6.

Although an inbreeding coefficient of 80 % is achieved after 7 brother and sister matings (Falconer, 1972) and although the percentage seronegative survivors increased in this experiment, there was a sudden collapse both in the survival rate and the percentage seronegative survivors in the progency of the 7th generation. A second attempt to establish an inbred line of mice resistant to *C. ruminantium* therefore also failed. On one hand in may be argued that the challenge from G2 onwards was too severe, but on the other out of 629 mice challenged, no less than 118 survived, of which 48 were serologically negative. A substantial number of mice were therefore resistant, but it would appear that they were genetically unable to pass the resistance on to their progeny.

References

DU PLESSIS, J. L., 1971. The passage of *Cowdria ruminantium* in mice. *Journal of the South African Veterinary Medical Association*, 42, 217–221.

DU PLESSIS, J. L., 1982. A preliminary attempt to establish an inbred line of mice resistant to a rickettsia-like organism. *News Letter of the S.A. Association for Laboratory Animal Science*, 4, 5–7.

DU PLESSIS, J. L., 1985. The natural resistance of cattle to artificial infection with *Cowdria ruminantium*: The role played by conglutinin. *Onderstepoort Journal of Veterinary Research*, 52, 273–277.

DU PLESSIS, J. L. & BEZUIDENHOUT, J. D., 1979. Investigations on the natural and acquired resistance of cattle to artificial infection with *Cowdria ruminantium*. *Journal of the South African Veterinary Association*, 50, 334–338.

DU PLESSIS, J. L. & MALAN, LETITIA, 1987a. The non-specific resistance of cattle to heartwater. *Onderstepoort Journal of Veterinary Research*, 54, 333–336.

DU PLESSIS, J. L. & MALAN, LETITIA, 1987b. The application of the indirect fluorescent antibody test in research on heartwater. *Onderstepoort Journal of Veterinary Research*, 54, 319–325.

DU PLESSIS, J. L. & MALAN, LETITIA, 1988. Susceptibility to heartwater of calves born to non-immune cows. *Onderstepoort Journal of Veterinary Research*, 55, 235–237.

FALCONER, D. S., 1972. Genetic aspects of breeding methods. *In*: The UFAW handbook on the care and management of laboratory animals, 4th edn. Edinburgh & London: Churchill Livingstone.

LOUNSBURY, C. P., 1904. Persian sheep and heartwater. *Agricultural Journal of the Cape of Good Hope*, 25, 175–186.

MATHERON, G., BARRÉ, N., CAMUS, E. & GOGUE, J., 1987. Genetic resistance of Guadeloupe native goats to heartwater. *Onderstepoort Journal of Veterinary Research*, 54, 337–340.

NEITZ, W. O. & ALEXANDER, R. A., 1941. The immunization of calves against heartwater. *Journal of the South African Veterinary Medical Association*, 12, 103–111.

PERREAU, P., MOREL, P. C., BARRÉ, N. & DURAND, P., 1980. Existence de la cowdriose (heartwater) à *Cowdria ruminantium* chez les petits ruminants des Antilles Française (la Guadeloupe) et des Mascareignes (la Réunion et l'Ile Maurice). *Revue d' Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 33, 21–22.

UILENBERG, G., 1983. Heartwater (*Cowdria ruminantium* infection): Current status. *Advances in Veterinary Science and comparative Medicine*, 27, 427–480.