

THE SUSCEPTIBILITY OF SOME DESERT RODENTS TO
EXPERIMENTAL INFECTIONS WITH *SHIGELLA*
AND *BRUCELLA* ORGANISMS*

BY THOMAS M. FLOYD† AND HARRY HOOGSTRAAL‡

U.S. Naval Medical Research Unit, No. 3, Cairo, Egypt

(With 1 Figure in the Text)

Desert rodents often have been used successfully as laboratory animals in parasitology (Abdel Azim & Cowper, 1950; Adler & Feldman-Muhsam, 1952; Delanoe, 1929; Kuntz & Malakatis, 1954; Nicolle, Anderson & Colas-Belcour, 1927; Sergent & Poncet, 1951*a, b*) and virology (Durand & Laigret, 1932; Gear & Davis, 1942; Noury, 1948; Snyder, Zarafonetis & Liu, 1945; Van Rooyen & Danskin, 1944; Zarafonetis, 1945). However, with the exception of investigations into the role of some of these animals as mammalian reservoirs of plague (Ristorcelli, 1938; Tikhomirova, 1934; Wassilieff, 1933), no reports have been found of their use for experimental bacterial infections.

Among small mammals found in desert and semi-arid areas of Egypt, the gerbil (*Gerbillus pyramidum pyramidum*), the jerboa (*Jaculus orientalis orientalis*), and the jird (*Meriones shawi shawi*) are some of the most plentiful and easily obtained rodents. In an effort to find laboratory uses for these animals, other than those cited above, the susceptibility of all three to experimental infections with *Shigella*, and of the gerbil to *Brucella*, was investigated during the course of other studies on the etiological agents of shigellosis and brucellosis in Egypt.

MATERIALS AND METHODS

A. *Experimental animals*

Gerbillus pyramidum pyramidum Geoffroy, 1825. Greater Egyptian gerbil

Length (overall) 261–295 mm. (average 277 mm.); head and body 111–130 mm. (average 121 mm.). Average weight 75 g. Distributed in Egypt throughout the entire length of the Nile Valley and Delta in sandy cultivated areas, desert edge and palm groves. It is found seldom, if ever, in open desert or silt-soil cultivated areas. This gerbil is easily trapped, or, with some effort, dug from its burrow. It does not breed in captivity, but some females obtained from their nests with suckling young will care for the young. Laboratory specimens are kept alive easily for well over a year on common laboratory animal food in cages with sand bottoms.

* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

† Lieutenant-Commander (Medical Service Corps) United States Navy.

‡ Head, Department of Medical Zoology.

Jaculus orientalis orientalis Erxleben, 1777. Greater Egyptian jerboa

Length (overall) 345–395 mm. (average 359 mm.); head and body 137–150 mm. (average 144 mm.). Average weight 153 g. Distributed in Egypt only in littoral area of the Western Desert from Mariut District, near Alexandria, to Libya. It is common in this restricted area. This jerboa does not enter traps readily but is obtained easily by digging from its burrow. It does not breed in captivity but females taken with suckling young give them good care. Laboratory specimens usually thrive for many months under the conditions described above.

Meriones shawi shawi Duvernoy, 1842. Shaw's jird

Length (overall) 230–311 mm. (average 276 mm.); head and body 128–160 mm. (average 143 mm.). Average weight 122 g. Distributed in Egypt in desert areas of North Sinai and the Western Desert as far east as Wadi Natroun. Under certain conditions the jird is trapped easily but at other times it obviously avoids traps. It can be dug from its burrow with some effort, or chased at night. Under proper conditions of space, shelter and sand flooring, this jird breeds readily in the laboratory and produces one or two batches of four young each year. Laboratory specimens live for over 2 years in captivity.

B. *Experimental infections with Shigella flexneri* 3

Gerbils. Fifteen gerbils were trapped in the Nile Valley near Cairo and kept in the laboratory for 2 weeks prior to use. During this time, six rectal swab cultures were made on each animal to determine its normal intestinal flora, to rule out naturally occurring infections with *Shigella*, and to accustom the animals to handling. At the end of this observation period each animal was inoculated rectally with 1 ml. of saline containing approximately 100 million viable organisms of a recently isolated strain of *Sh. flexneri* 3. Thereafter for 23 days rectal swab cultures and observations on the general condition of the animals were made morning and afternoon.

Rectal swabs were streaked directly on to Shigella–Salmonella (SS) agar (Difco) and MacConkey agar (Difco) plates. After incubation for 24 hr. at 37° C. the plates were examined and suspicious non-lactose-fermenting colonies transferred to triple sugar iron agar (Difco) slants. Cultures that produced reactions on this medium typical of *Shigella* were tested for their biochemical and physiological properties. Final identification was made serologically, using group and type specific *Shigella* typing sera.

Jerboas. Sixteen jerboas were dug from burrows in Mariut District, near Alexandria, and kept in the laboratory for 8 days. During this time rectal swab cultures were made daily on all animals. Then each animal was inoculated rectally with 1 ml. of saline containing approximately 100 million *Sh. flexneri* 3 organisms. The animals were observed and cultured, by methods described above, for 28 days.

Jirds. Sixteen jirds were trapped or dug from burrows in the Western Desert and maintained for 8 days in the laboratory, during which time daily rectal swab cultures were made. Then they were inoculated rectally with approximately 200 million *Sh. flexneri* 3 organisms and cultured and observed for 20 days.

C. *Experimental infection of gerbils with Brucella melitensis*

This study was conducted in two phases. The general susceptibility of gerbils to infection with *Brucella melitensis*, as evidenced by recovery of the organisms from various organs and by immunological response, was tested first. Subsequently, further studies were made to determine roughly the minimum number of organisms and the minimum period of time required to establish such infections.

The first experiment utilized 140 gerbils trapped in the Nile Valley near Cairo and kept in the laboratory for 2-4 weeks. They were divided into seven groups of twenty animals each. The first group was sacrificed to determine the presence of naturally occurring *Brucella* infections among these animals. Spleens and livers were removed, measured, and cultured by methods described below. Blood was obtained from the hearts to determine the presence of naturally occurring agglutinins against *Brucella*.

The twenty gerbils in the second group were inoculated intraperitoneally with 1 ml. of saline containing approximately 100 million viable *Br. melitensis* organisms. The organisms were from a 4-day-old tryptose agar (Difco) culture of a recently isolated strain from a human case of brucellosis. The density of the inoculum was determined by comparison with a MacFarland nephelometer standard. As controls, twenty guinea-pigs, twenty white rats, and twenty white mice were inoculated intraperitoneally with the same inoculum. All controls were adult animals.

The remaining five groups of gerbils were injected by the same route with 50 million, 25 million, 1 million, 100,000 and 1000 of the same organisms respectively.

At intervals of 5, 10, 20 and 30 days after inoculation, five animals from each group of control animals were sacrificed. The animals were killed with ether and dipped into a disinfectant solution to reduce external contamination. The abdomens were opened and the animals exsanguinated by cardiac puncture. The bloods were placed in sterile tubes, centrifuged, and the serum removed and saved for serological tests. Using sterile instruments, the livers, spleens and inguinal lymph glands were removed, measured and examined for the presence of macroscopic lesions. Then the organs and glands were seared with hot spatulas and sliced. Small pieces of each were cultured, after the method of Castaneda (1946), in 4 oz. screw-capped bottles containing tryptose agar (Difco) on one wall and 10 ml. of tryptose broth (Difco). The peritoneal cavities of the animals were washed with a few ml. of sterile saline and 1 ml. amounts of this fluid were pipetted into culture bottles.

The bottles were incubated in an upright position under 10% CO₂ tension at 37° C. On alternate days the bottles were removed from the incubator and the surfaces of the slants examined under strong light. If no growth could be detected, the bottles were tilted and the surfaces of the slants bathed with the broth before returning to the incubator.

When growth was observed on the slant, typical opalescent colonies were picked and inoculated on to triple sugar iron agar slants. After 2-4 days' incubation cultures which produced no H₂S or fermentative changes on this medium were tested for their reactions in urea. Those which hydrolysed urea were streaked on to tryptose agar plates containing thionin (1:100,000) and tryptose agar plates con-

taining basic fuchsin (1:100,000). Cultures that were able to grow in the presence of both dyes were then confirmed as *Br. melitensis* by slide-agglutination tests against *Br. melitensis* antisera.

Serum agglutinin titres were determined by the macroscopic tube method using a heat-killed antigen prepared from the challenge strain of *Br. melitensis*.

In studies to determine the minimum number of organisms and the minimum period of time required to establish an infection in gerbils, 150 of these animals were used. They were obtained from the usual source and were kept in the laboratory for 2-4 weeks. A similar number of guinea-pigs served as controls.

A 4-day-old tryptose agar culture of the *Br. melitensis* strain used in the preceding experiment was suspended in sterile saline solution. This suspension was diluted until it corresponded in density with the no. 1 MacFarland nephelometer tube (approximately 300 million organisms per ml.). By serial dilutions, suspensions containing approximately 1000, 500, 250, 100, 50 and 25 organisms per ml. were prepared. The animals were divided into six groups, each containing twenty-five gerbils and twenty-five guinea-pigs. The six groups were inoculated intraperitoneally with 1 ml. of the respective inocula.

At intervals of 2, 4, 6, 8 and 10 days, five gerbils and five guinea-pigs from each group were sacrificed. The methods for autopsy and culture were the same as those described earlier, with the exception that only spleens and livers were cultured.

RESULTS

Susceptibility of gerbils, jerboas, and jirds to Shigella infections

No naturally occurring *Shigella* infections were found among the animals examined. After rectal inoculation with large numbers of *Sh. flexneri* 3, gerbils continued to excrete the organisms for as long as 16 days, jerboas for as long as 18 days, and jirds for as long as 11 days (Tables 1-3).

Table 1. *Recovery of Shigella flexneri* 3 from gerbils after rectal inoculation with 100 million of the organisms

Animals no.	Days after inoculation																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	-	+	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	Died																				
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-
6	-	+	Died																				
7	+	-	-	-	-	-	-	-	-	0	-	-	-	0	-	-	-	-	-	-	-	-	-
8	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-
9	+	+	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	+	Died																				
11	-	+	+	+	-	+	+	+	+	-	-	0	-	-	-	-	-	-	-	-	-	-	-
12	-	+	+	+	0	-	+	+	+	+	0	-	0	-	-	-	-	-	-	-	-	-	-
13	-	+	-	-	0	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
14	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	-	-	+	0	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-

+, *Shigella* recovered; -, negative; 0, unsatisfactory specimen.

Table 2. *Recovery of Shigella flexneri 3 from jerboas after rectal inoculation with 100 million of the organisms*

Animal no.	Days after inoculation																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	-	-	Died																									
2	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-
6	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	0	0	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	+	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	0	0	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	0	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	0	0	Died																								
15	-	-	Died																									
16	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, *Shigella* recovered; -, negative; 0, unsatisfactory specimen.

Table 3. *Recovery of Shigella flexneri 3 from jirds after rectal inoculation with 200 million of the organisms*

Animal no.	Days after inoculation																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	+	-	-	Died															
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, *Shigella* recovered; -, negative.

No diarrhoea was noted in any of the animals. Except for slight ruffling of the fur for a day to two following inoculation, the animals were apparently healthy throughout the period of observation. Three gerbils, three jerboas, and one jird died within 2-5 days after inoculation with the organisms. All deaths were believed to have resulted from traumatic injury during inoculation.

Susceptibility of gerbils to Brucella infections

Brucella organisms were not recovered from uninoculated gerbils and agglutinins against these organisms were not present in the sera of these animals.

Results of the inoculation of gerbils and control animals with approximately 100 million *Br. melitensis* organisms are shown in Table 4.

Table 4. *Recovery of Brucella melitensis from gerbils and control animals inoculated intraperitoneally with 100 million organisms*

Animals	Days after inoculation			
	5	10	20	30
Gerbils	5/5	5/5	5/5	5/5
Guinea-pigs	5/5	4/5	5/5	4/5
White rats	5/5	5/5	5/5	5/5
White mice	3/5	3/5	4/5	5/5

Denominator: no. animals inoculated; numerator: no. animals from which *Brucella* were recovered.

Brucella organisms were recovered as early and as regularly from the organs and glands of gerbils as they were from guinea-pigs, white rats, and white mice. *Brucella* were recovered with approximately equal frequency from spleens, livers, and glands in both gerbils and control animals. It was of interest to find that the organisms could be isolated from peritoneal washings of gerbils throughout the 30-day period.

The spleens of all inoculated gerbils appeared to be enlarged. In white rats and white mice this was noted only in the animals sacrificed on the twentieth and thirtieth days. No changes were noted in the size of the spleens of guinea-pigs. No macroscopic lesions were observed on the spleens of any animals. The livers and lymph glands of all appeared normal in size throughout the experiment and no macroscopic lesions were noted on these organs.

In Table 5 it may be seen that positive cultures were obtained from almost all of the gerbils which received inocula of 1000 to 50 million *Br. melitensis*.

Agglutinins against *Br. melitensis* were detected in high titres in the sera of gerbils and white rats, and in low titre in white mice sacrificed on the fifth day

Table 5. *Recovery of Brucella melitensis from gerbils inoculated intraperitoneally with different numbers of organisms*

No. organisms inoculated (approx.)	Days after inoculation			
	5	10	20	30
50 million	5/5	5/5	4/5	4/5
25 million	5/5	5/5	5/5	4/5
1 million	5/5	5/5	5/5	5/5
100,000	5/5	5/5	5/5	5/5
1,000	5/5	5/5	5/5	4/5

Denominator: no. animals inoculated; numerator: no. animals from which *Brucella* were recovered.

following inoculation. No agglutinins were found in the sera of guinea-pigs sacrificed at this time. From the tenth through the thirtieth days, comparable average agglutinin titres were found in all animals (Fig. 1).

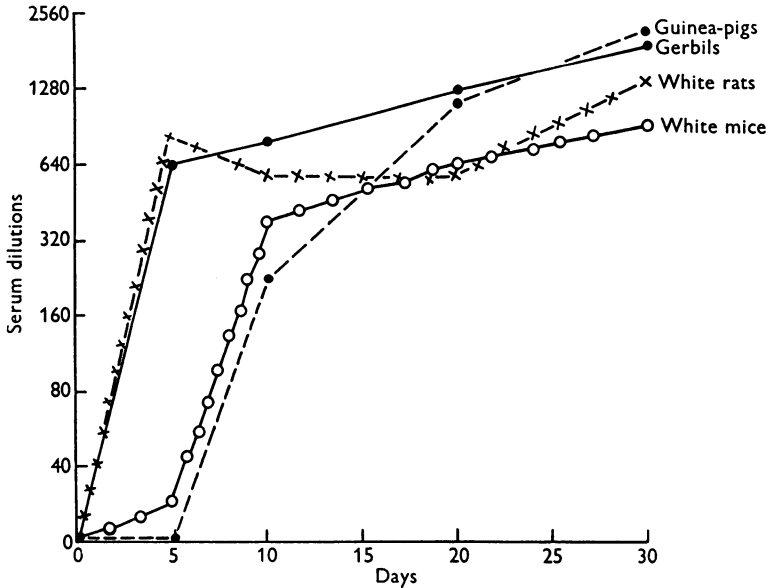


Fig. 1. Comparative serum agglutinin titres against *Br. melitensis* in gerbils and control animals inoculated with 100 million of the organisms.

Table 6. Recovery of *Brucella melitensis* from gerbils and guinea-pigs after intra-peritoneal inoculation with different small inocula of the organisms

No. organisms (approx.)		Days after inoculation				
		2	4	6	8	10
1000	Gerbils	4/5	2/5	4/5	5/5	4/5
	Guinea-pigs	0/5	0/5	1/5	1/5	0/5
500	Gerbils	3/5	4/5	2/5	5/5	5/5
	Guinea-pigs	3/5	0/5	0/5	1/5	0/5
250	Gerbils	5/5	4/5	4/5	5/5	5/5
	Guinea-pigs	0/5	1/5	0/5	3/5	1/5
100	Gerbils	0/5	2/5	3/5	0/5	2/5
	Guinea-pigs	0/5	0/5	0/5	0/5	0/5
50	Gerbils	1/5	0/5	3/5	0/5	1/5
	Guinea-pigs	0/5	0/5	1/5	0/5	0/5
25	Gerbils	0/5	2/5	1/5	3/5	2/5
	Guinea-pigs	0/5	0/5	1/5	0/5	0/5

Denominator: no. animals inoculated; numerator: no. animals from which *Brucella* were recovered.

In Table 6 are shown the results of inoculating gerbils and guinea-pigs with relatively small numbers of *Br. melitensis* and sacrificing the animals at intervals of 2, 4, 6, 8 and 10 days. *Brucella* could be recovered from a large percentage of gerbils which had been infected with approximately 1000, 500 and 250 organisms. Few recoveries were made from guinea-pigs tested under the same conditions.

With inocula of approximately 100, 50 and 25 organisms, the results were inconsistent. However, *Brucella* were isolated from twenty of the seventy-five gerbils which received these inocula, while only two of the comparable guinea-pigs yielded positive cultures.

Brucella were recovered from spleens roughly twice as often as from livers of gerbils in this experiment. Among animals receiving the three smallest inocula, *Brucella* were isolated only from the spleens.

No agglutinins could be detected in sera of guinea-pigs injected with small inocula. Among the gerbils, only sera of those that received 500 or 1000 organisms gave positive agglutination reactions. All were in low titre.

DISCUSSION

Clinical *Shigella* dysentery was not produced in gerbils, jerboas or jirds by rectal inoculation of *Sh. flexneri* 3. Although the organisms could be recovered from some animals for as long as 11-18 days following inoculation, none of the symptoms, particularly diarrhoea, that usually are associated with active clinical infections in man and monkeys were noted.

Gerbils appeared to be very susceptible to experimental infection with *Br. melitensis*. When these animals were inoculated with 100 million organisms, the immunological response and the ease and regularity with which *Brucella* could be recovered was equal to that of guinea-pigs, white rats, and white mice infected under the same conditions. It is realized that the periods of observation were too short to have allowed full development of the disease and further studies, including the pathological changes, over longer periods are indicated.

Gerbils were apparently more susceptible to infection with small inocula of *Br. melitensis* than were guinea-pigs. The numbers of organisms contained in the various inocula in this experiment are not presented as absolute, since the method used gave, at best, an approximation. However, from the dilution factor alone, the numbers probably were small. The ability to isolate *Brucella* from spleens of gerbils within 2 days after they received as few as 25 organisms suggests that the use of these animals might be of some practical value in situations where the numbers of *Brucella* organisms are small and it is desirable to recover them in as short a time as possible. To investigate this aspect, further studies are contemplated utilizing blood from suspected human cases of brucellosis as inocula.

Gerbils, jerboas and jirds are readily maintained in the laboratory and are not difficult to handle. The results of this and of earlier studies indicate that, in areas where they are plentiful and easily obtained in nature, these animals may well be used as substitutes for the more common experimental animals.

CONCLUSIONS

1. Gerbils, jerboas and jirds did not prove of value as laboratory animals for the study of *Shigella flexneri* 3 infections.
2. Gerbils are very susceptible to experimental infection with *Brucella melitensis*.

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