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TRANSMISSION OF PULMONARY AND SEPTICAEMIC PLAGUE AMONG MARMOTS[1].

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THE subject of plague transmission among marmots was brought up at the time of the historic epidemic of pneumonic plague which raged in Manchuria and Northern China during the winter of 1910–1911. The tarbagan (*Arctomys bobac*) was suspected of having played a part in the transmission of the disease, yet no experimental evidence was brought forth to show that this animal was in any way associated with plague. One of the present writers undertook an expedition to the tarbagan regions in Mongolia in 1911, and in a paper published in this *Journal*[2] produced ample evidence to show that under normal conditions plague did not exist among the marmots. It was Strong[3], working in Mukden, who first performed experiments of plague inhalation upon tarbagans, and published data concerning the possible importance of these animals in the epidemiology of this dread disease. He demonstrated, in a general way, that they could take pneumonic plague if exposed to the organisms sprayed in droplet form.

The present enquiry has been undertaken to elucidate this point clearly and to determine the part played by the marmot in the spread of infection through contact and feeding on plague corpses. The animals used were a small species related to the tarbagan and known as *Spermophilus citellus*. These ferret-like creatures are very numerous in the Mukden district and can be found in larger numbers in the summer

[1] The experiments here summarised were conducted at the Mukden Medical College. To Dr Dugald Christie, C.M.G., the Principal, and other members of the staff we are greatly indebted for their kindness and courtesy in providing us with facilities for our work.

[2] Wu Lien-Teh (1913) *Journ. of Hygiene*, XIII. 237.

[3] Strong (1912) *Philippine Journ. Sci.* VII.

season. They frequent the graveyards and burrow underground, not unlike the ground squirrel or American gopher. In size, they approximate to the rat, are very vicious and make good use of their long and exceedingly sharp teeth and claws. They can be readily trapped by pouring water into their burrows, when they rush out to escape suffocation. With care in handling, especially while trapping, they will live in captivity for a long time.

Our present work on Marmots may be conveniently grouped under experiments relating to: (A) Infection by Inhalation; (B) Susceptibility to Infection by Subcutaneous Inoculation; (C) Infection by Feeding; and (D) Histological Examination.

A. Inhalation Experiments.

The method used in these experiments conformed as far as possible to the natural conditions for infection with pulmonary plague. A 24-hour old agar slant of a moderately virulent strain of *B. pestis* was suspended in 10 c.c. of salt solution and sprayed from a graduated cylinder fitted with a very fine nozzle. Great care was taken to direct the spray in a fine cloud towards but not into the nasal passage. Although a definite amount of culture was used in the two different series of experiments, it was not possible to determine more than roughly, if at all, how much of the culture found its way into the respiratory tract. In the first series, five minims were sprayed, and in the second, ten minims. A great portion of the spray obviously failed to inoculate, because of the mechanical difficulties entailed in the spraying technique and of the position of the animals on the stage. It was necessary to clamp the neck in order to hold the animals down properly, and in many instances their normal breathing was impeded. The errors due to the loss of most of the culture spray tend to make the results obtained more striking. Before spraying, the animals were covered with a piece of absorbent cotton soaked in cresol lotion, so as to prevent droplets lodging on the fur, and after exposure to the spray the head of each marmot was carefully wiped to remove extraneous organisms. In order to minimise the danger from droplet infection, a specially constructed wooden box was used while the animal was being sprayed. This box enabled the operator to place the animal and stage in a compartment with a glass top, and permitted the spray to be introduced through an opening at the front of the box. This aperture was on a level with the animal's head as it lay fastened on the stage, and the spray, directed through the opening reached the nose quite readily.

Series I.

In this series the animals were placed in flea-proof cages, 20 ins. long by 15 ins. wide by 9 ins. high, separated from the normal animals in some instances by a single partition and in others by a complete four-walled compartment which stood in the centre of the cage. This latter type of cage was used in order to enable the sprayed animals to run about the central compartment, thus permitting them to come in freer intercourse with others outside, herein called contacts. Five minims of the culture in salt solution were sprayed in each case.

Exp. No.	Contact animal's No.	When placed in contact	Result	Sprayed animal's No.	Result
1	1	Same day	12 days. No plague	1	Killed, 20th day. No plague
	2	,,	Killed, 21st day. No plague	2	Killed, 20th day. No plague
2	1	,,	7 days. Typical plague pneumonia	1	12 days. Typical plague pneumonia
	2	,,	7 days. Typical plague pneumonia	2	Killed, 18th day. No plague
3	1	one day after	Alive, well, 22nd day	1	10 days. Typical plague pneumonia
	2	,,	Alive, well, 22nd day	2	Alive, well, on 22nd day
4	1	2 days after	15 days. No plague	1	5 days. Typical plague pneumonia
	2	,,	Alive, well, 22nd day	2	7 days. Typical plague pneumonia
				3	7 days. Typical plague pneumonia
5	1	3 days after	3 days. No plague	1	5 days. Typical plague pneumonia
	2	,,	7 days. Typical plague pneumonia	2	7 days. Typical plague pneumonia
				3	7 days. Typical plague pneumonia
Total	10		3	12	8

In Exp. 1 no changes were noted in the organs of the animals which died or which were killed. Smears and cultures from the organs and blood were uniformly negative. In Exp. 2 it is interesting to note that a "contact" succumbed five days before any of the sprayed animals died, and that out of the latter two one lived for 12 days, while a mate, confined in the same cage, failed to take plague. It is very evident that here, as with man, a difference in resistance prevails. Apart from this we must take into account the different factors which tend to modify the chances for infection even by close contact. Exp. 3 offers an example

of plague in a chronic form. This first series of experiments indicates that pneumonic plague can be readily transmitted to the marmot, and that animals suffering from plague pneumonia in turn are capable of transmitting the disease to others. In Exp. 5 it should be noted that one animal proved infective to a normal contact as early as two days after inhaling plague bacilli.

Summarising the results obtained, we may say that the chances for infection by contact were undoubtedly minimised by our method of housing the animals, therefore the few positive cases which occurred tend to render the results all the more surprising. Out of 12 animals exposed to infection by inhalation, eight (66·6 %) died of typical plague pneumonia in from three to seven days. Of the contacts, three (30 %) died and seven survived. Fifty per cent. of the animals which were exposed directly or indirectly to the disease died.

In order to ensure more natural living conditions, such as normally prevail among these animals, a second series of experiments was conducted with the contacts placed in unscreened cages. Careful examination had already revealed that fleas were very scarce on the marmots at the time of our visit (July-August) and whatever insect transmission might occur would be easily recognised from the resultant type of plague. This phase of the problem is now being studied and will form the subject of a separate report.

Series II.

Exp. No.	Contact animal's No.	When placed in contact	Died	Sprayed animal's No.	Died
1	1	1 day after	5 days. Typical plague pneumonia	1	3 days. Typical plague pneumonia
	2	,,	12 days. Typical plague pneumonia	2	9 days. Typical plague pneumonia
	3	,,	12 days, Typical plague pneumonia		
2	1	2 days after	4 days. Typical plague pneumonia	1	5 days. Typical plague pneumonia
	2	,,	6 days. Typical plague pneumonia	2	Killed, 17th day. No plague
3	1	3 days after	5 days. Typical plague pneumonia	1	5 days. Typical plague pneumonia
	2	,,	6 days. Typical plague pneumonia	2	6 days. Typical plague pneumonia
4	1	4 days after	Alive, well, 16th day	1	15 days. No plague
	2	,,	Alive, well, 16th day		
Total	9		7	7	5

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A summary of the results obtained in this series of experiments shows in a conclusive manner that pneumonic-plague-infected marmots can readily transmit the infection through the respiratory passages as in the case of man. Conditions which favour the propagation of the disease among the latter are in no way different for these animals. Close contact and moist surroundings seem to favour the rapid spread of infection from animal to animal. Out of seven marmots inoculated by inhalation, five (71 %) died after 4–6 days with acute pneumonic plague and septicaemia. Nine contacts placed with infected animals after periods varying from 1–4 days showed a mortality of 77 %. These seven marmots died after 4–6 days' contact. Here also, as in the preceding series, was noted a remarkably short incubation period, with marked infectivity on the part of the sprayed animals.

Summary.

First Series.				Second Series.			
Animals inoculated	12	Deaths	8	Animals inoculated	7	Deaths	5
Contacts ...	10	„	3	Contacts ...	9	„	7
Total	22	„	11	Total	16	„	12

Animals exposed to plague 38
Deaths 23

The autopsy findings indicate clearly that pneumonic plague among marmots is not unlike that in man. The bacilli, entering the respiratory tract, lodge in the lungs and from this primary focus enter the circulation and cause a general septicaemia. The pathological changes in the organs are most striking, they chiefly affect the lungs. The latter show extreme congestion and inflammation with fibrinous exudation, and, associated therewith, pronounced pleuritis. Enlargement of the spleen and liver is frequent, though not constant, and visceral congestion is prominent. No instance of axillary or inguinal gland involvement were observed, although in a few cases the cervical glands were enlarged, and, upon microscopical examination of smears, showed plague bacilli in good numbers. Inflammation of the trachea and bronchi occurs with marked regularity.

Bacteriological examinations demonstrated that although the lungs may contain enormous numbers of plague bacilli, yet this organ is not exclusively selective. In a fair proportion of the cases where the lung showed few organisms the spleen invariably teemed with them. This was also noted when blood smears were not particularly full of *B. pestis*. The number of organisms present in any one organ at a given time seems

to depend upon a variety of circumstances, not the least of which appears to be the individual resistance of the animal. Some animals, it was noted, may show a distinct toxaemia without marked bacteraemia. In analogous fashion, a few of the marmots may offer such low resistance to the disease that they succumb before any very marked changes appear in the organs.

Of great interest is the observation that plague may exist in a chronic form among marmots. That they can live for nine or ten days with pronounced plague and be capable of conveying infection to other animals, is of the utmost importance from an epidemiological standpoint.

B. Susceptibility to Infection.

Plague septicaemia, as seen above, results readily from plague pneumonia. In order to study the susceptibility of marmots to this type of plague, a number of animals were inoculated subcutaneously with varying doses of bacilli. All the animals died of acute septicaemic plague with slight, if any, signs of bubonic affection. This experiment shows that marmots are very susceptible to plague septicaemia. The culture used was only moderately virulent, and had been growing on agar for several generations. A 72-hour growth on small agar slants was used for inoculation.

Marmot No.	Dose (slant)	Result	Postmortem findings
1	1/20	Death, 2 days	Intense congestion at site of inoculation. Spleen and liver enlarged and congested. Smears from blood and organs showed enormous numbers of plague bacilli.
2	1/40	„ 2 days	Ditto
3	1/80	„ 6 days	„
4	1/40	„ 4 days	„
5	1/80	„ 5 days	„
6	1/80	„ 36 hours	„
7	1/80	„ 36 hours	„
8	1/80	„ 48 hours	„
9	1/80	„ 48 hours	„
10	1/80	„ 48 hours	„

Animals No. 6 to No. 8 inclusive were inoculated with the same strain after a single passage through a marmot.

C. PLAGUE TRANSMISSION BY FEEDING.

Group I. Three marmots were fed with liver and spleen taken from a guinea-pig which had died of plague after 56 hours.

Marmot 1. Died after 3 days. Liver and spleen congested. Stomach inflamed. Smears from lungs, liver, spleen and blood showed *B. pestis*. A blood culture injected into a normal marmot killed the animal within 36 hours. Acute plague with *B. pestis* in the blood and all organs.

Marmot 2. Died after 3 days. This animal was eaten by the remaining marmot before an autopsy could be made.

Marmot 3. Died after 4 days. Slight visceral congestion. Inflammation of the gastric mucosa. Smears from blood and organs showed *B. pestis*. The stomach scrapings were full of plague bacilli.

Group II. Three marmots were fed with lung, liver and spleen taken from a marmot which had died of acute plague.

Marmot 4. Died after 2 days. Congestion of liver and spleen. Marked visceral congestion and intense inflammation and congestion of gastric mucosa. Smears from organs and stomach lining showed great numbers of *B. pestis*.

Marmot 5. Died after 4 days. Liver and spleen congested and enlarged. Visceral congestion marked. Gastric mucosa greatly inflamed. Smears from blood and organs gave enormous numbers of *B. pestis*.

Marmot 6. Killed after 14 days. Autopsy: no changes in any of the organs or glands; a very slight area of old inflammation noticed in the gastric mucosa, but no plague bacilli seen in scrapings; smears from blood and organs negative. This animal had been fed twice with plague material with an interval of one week elapsing between the feedings.

This series of experiments, though small, demonstrates that marmots may transmit plague by feeding on plague carcases. The animals are carnivorous by nature, and promptly eat their dead mates. Death after infection by feeding takes place within four days and is apparently not hastened by greater amounts of plague-infected material. The most striking change in the marmot consists in the intense inflammation of the gastric mucosa. The spleen and liver show the usual changes attendant upon plague. That individual differences in susceptibility may exist is well exemplified in the case of marmot 6, which failed to take plague although it had been given a large amount of highly infective material.

The experiments upon *Spermophilus citellus* mentioned above, particularly with regard to pulmonary plague infection by contact, are perhaps the first that have been recorded. The feeding experiments are especially interesting because many workers, including Strong, have denied the possibility of plague transmission by this means.

D. Histological Examination.

The histological changes observed in the lesions of human pulmonary plague have been fully described by various writers, including Albrecht and Gohn[1], Childe[2], Strong[3], Fujinami[4], and Wu Lien-Teh and Woodhead[5]. We preserved and examined a considerable number of specimens obtained from the animals experimented upon, but as the microscopical changes in the inhalation experiments differ in no material way from those already described in the case of human pulmonary plague, we shall only refer to them briefly.

Lung. In acute pulmonary plague (*i.e.* where the animals died in 2–5 days after infection), sections of the lung showed intense congestion of the blood vessels. The part of the lung tissue adjacent to the pleura was marked by much leucocytosis and even haemorrhage, and areas of collapse could be seen. The small bronchi were filled with mucoid substance, and some were practically choked with pure cultures of plague bacilli. Around the inflamed bronchi and bronchioles were patches of pneumonia, harbouring numbers of bacilli in the capillaries and alveoli. No fibrinous lymph coagulum was noted.

In specimens obtained from two cases which died 12 days after contact with infected animals, the lung tissue showed somewhat different changes. Here haemorrhage and congestion were not so marked, and broncho-pneumonic patches were scanty. The alveoli displayed extensive signs of collapse, and around the bronchi considerable signs of inflammation and thickening were noted. Plague bacilli were not nearly so numerous as in acute plague.

Liver. In the acute form, sections of the liver showed a picture of acute red atrophy, the central lobular vein being much distended, and the portal capillaries swollen. The hepatic cells were markedly 'cloudy' and granular, but vacuolation, except in a few areas, had not set in earnestly. Haemorrhage were noted everywhere.

In specimens obtained from the chronic cases, the liver substance showed very characteristic signs of degeneration. The central lobular

[1] Albrecht and Gohn, *Centralbl. f. Bakteriol.* 1899, xxvi. 362.

[2] Childe, *Brit. Med. Journ.* 1897, i. 1215; 1898, ii. 858, and *Report of Indian Plague Comm.* London, 1900.

[3] Strong, *Rep. Intern. Plague Conference*, Mukden, 1912.

[4] Fujinami, *Ibid.*

[5] Wu-Lien-Teh and Woodhead (1913), *Journ. of Pathology and Bacteriol.* 1913.

vein was not so distended, and haemorrhage was not so marked. A large portion of the hepatic cells appeared to have lost their contents, so advanced was the vacuolation and loss of nuclear substance. In fact the whole section stained badly with haematin. Plague bacilli were seen with difficulty.

Spleen. Here also the changes observed in the acute and chronic disease were characterised by much more congestion in the former than in the latter. The number of bacilli encountered was also greater in the acute form, and the Malpighian bodies were larger and stood out more distinctly.

Kidney. As in the case of the liver, the kidney showed far more extensive signs of degeneration in the chronic than in the acute cases. There was very little thickening of the capsule in either case, but the glomeruli were swollen considerably. In the chronic cases the cells of the tubules had lost the greater part of their substance, and in several places only the basement membrane was seen, so great had been the disintegration. More haemorrhage was noted in the kidney than in other organs in the chronic cases.

Heart. The muscular tissue showed oedematous changes, the striations being more indistinct than usual, and the muscle fibres broken in places.

Lymphatic Gland. Both cervical and inguinal glands were examined, but showed no changes other than those hitherto described in ordinary plague. Plague bacilli were present in lesser numbers than in bubonic plague, and in the chronic form were sometimes not seen at all.

Stomach. So many observers have denied the existence of infection by the alimentary canal that a little more attention may be devoted to the changes observed in this organ. As stated in the preceding article, out of six marmots fed upon plague-infected viscera five died—four definitely of plague, whilst the fifth one was eaten by its fellows before an examination could be made. Only one animal survived, and after 14 days was killed, but it showed no signs of infection. Autopsies in all cases were made within a few hours after death, and the stomach of all the infected animals showed definite signs of acute inflammation, which was most marked at the pyloric end and commencement of the duodenum. Red patches denoting haemorrhage and small areas of disintegration were clearly seen. Pieces of the stomach at the pyloric end were removed from cases 4 and 5, and prepared for microscopical examination. Formalin was used as fixing agent, and the

paraffin sections were stained both with alum haematin plus eosin, and also with dilute Giemsa, as follows:

1. Stain in dilute Giemsa (1 part Giemsa solution (Grübler) in 10 parts distilled water) for 6 hours.
2. Decolorise in weak acetic acid (5 drops in 100 c.c. distilled water).
3. Wash in distilled water.
4. Blot and clear in xylol.

Plague bacilli, when present, are stained blue in the tissues by this method.

The gastric mucosa shows marked changes under the microscope. The mucous glands are intensely inflamed, and haemorrhages can be seen both inside and around them. Clots with fibrin are also encountered, sometimes firmly adherent to the underlying glands. In places large areas of glandular tissue have given way, revealing open ulcers with much leucocyte infiltration and ruptured blood vessels around the edges. Apparently the large oxyntic cells are first cast out, for here and there numbers of them are found on the surface intermixed with leucocytes. In other parts, where disintegration had been extensive, only granular débris is left. The cells of the glands are swollen and granular, and where inflammatory changes are most marked they appear broken up. Plague bacilli are met with in varying numbers amidst the glands, and are most evident on the surface of the necrotic areas. The submucous coat is thickened, the blood vessels supplying the glands being much distended and filled with corpuscles. The inner circular muscular coat is also congested, and large clumps of plague bacilli are seen distributed among the fibres, especially in the neighbourhood of blood vessels. The fibres themselves appear swollen, but no signs of disintegration can be made out. The outer longitudinal muscular coat seems also swollen, but very few bacilli are met with in this region. The peritoneal coat is slightly infiltrated in certain parts.

In the sections obtained from Marmot 5, the surface of the mucous coat seems to be largely covered with an organised coagulum of mucoid tissue of varying thickness. Where the clot had broken off, the mucous glands show necrotic changes similar to those described above, and the surrounding blood vessels are largely distended. Plague bacilli are present in large numbers both inside and outside the clot, and in the granular débris of the mucous glands.

SUMMARY AND CONCLUSIONS.

1. 52·6 % of marmots placed in contact with marmots infected with plague by inhalation developed pulmonary plague and died within 4–6 days.

2. Marmots suffering from pneumonic plague are infective at an early stage of the disease and the animals which such marmots infect acquire plague after a short incubative period.

3. Pulmonary plague can be readily transmitted to the small marmot (*Spermophilus citellus* Linn.), and these animals, when suffering from pulmonary plague, are in turn capable of transmitting the same type of plague through the respiratory passages.

4. Septicaemic plague can be developed in marmots very easily as a result of respiratory infection and also by direct subcutaneous inoculation with small amounts of culture.

5. The marmot can acquire plague by way of the alimentary tract and spread the disease by feeding on plague-infected carcases. The histological appearances observed in the lesions of these cases are characteristic.

NOTE ON ECTOPARASITES FROM MARMOTS.

Accompanying a letter dated 16th August 1916, Dr Wu Lien-Teh forwarded some specimens of ectoparasites collected by him from marmots.

1. A single flea: The specimen was determined by Hon. N. C. Rothschild as a slightly aberrant example of *Ceratophyllus famulus* Jordan and Rothschild, 1911 (*Proc. Zool. Soc. London*, 1911, p. 380, No. 7, text-fig. 115).

2. A number of ticks: The specimens consisted of but a single female accompanied by a number of nymphs, some mounted in balsam, others not. Mr C. Warburton examined the specimens and determined them as *Haemaphysalis*, one of the variants of *H. leachi*, closely resembling the form described as *H. koningsbergeri* Warburton and Nuttall, 1909 (see *Ticks, A Monograph of the Ixodoidea*, 1915, Part III, p. 468, figs. 408–410). It is desirable that more adults should be secured, including both sexes, to enable a precise determination to be made. Specimens should be preserved in 70 % spirit.

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