* * * Extract from "Revue d' hygiène et de Police sanitaire, Vol. XXV. No. 5.", 1903, **pages 426-437.**
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EXPERIMENTAL RESEARCH

ON THE ROLE OF PARASITES IN THE TRANSMISSION OF THE PLAGUE (1)

By MM. Doctors
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Note 1. An analysis of this work was presented to the Academy of Medicine by Professor Proust, Inspector General of Health Services. (Bull. Acad. Med., December 16, 1902.)

Simond's theory on the parasitic transmission of rat to rat plague and of the rat to man was, from the outset, adopted very generally by the French authors and also by some foreign scholars. It was pointed out, however, that no control studies had come to confirm the experiments so well known (2). The latter were, on the other hand, with their conclusions, subjected to severe criticism in a few neighboring countries. But the experimental data on which the refutation of the theory in question is based seem insufficient.

Note 2. Annals of the Pasteur Institute, October 1898, p. 674.

The diagnostic research requested of us in certain cases concerns animals which are always suspect and generally parasitic; they require a special device against any danger of propagation. Finding us in particularly favorable conditions to verify Simond's theory on its main points, we did not think we should neglect this opportunity.

The problem contained several experimental data that could, in short, be brought to these two heads:

1° Is the plague transmitted from rat to rat by the bite of certain parasitic insects?

2° Do these insects attack humans under conditions similar to those which, by hypothesis, carry out animal-to-animal contagion?

I. Transmission of the Plague from rat to rat.

In our experience, parasites commonly found in rats are either insects of the pulicidal family or some types of mites. Very rare pediculidae, found sometimes also, do not seem to us to have to be taken into account.

Flea transmission tests.

For our experiments on fleas, we tried to place ourselves in conditions as close as possible to what must happen in practice. Collecting at once a number of fleas from healthy captured rats, we artificially parasitized them with laboratory animals previously inoculated with pure cultures. We then sought to produce parasitic infestation and subsequent infection of new animals.

We have adopted an experimental device which makes it possible to eliminate any cause of transmission other than the passage of parasites from one animal to another and which, moreover, gives perfect safety during these manipulations precisely considered as delicate, even by the opponents of Simond's theory.

We used a cylindrical cage of iron wire, about 20 centimeters in diameter, divided in the middle by a vertical partition formed by two metal grids 2 centimeters apart. The inoculated animal being placed in one of the compartments, the healthy animal, introduced after its death into the other, has no contact with the corpse, the double partition even preventing it from passing the muzzle into the neighboring part. On the other hand, the fleas can jump from one side of the cage to the other and we have, in each experiment, observed their rapid emigration.

It is absolutely necessary to prevent the fleas from being able to leave the experimental cage and carry the dangerous infection with which they are loaded on the laboratory staff and animals. For this, we enclose our metal cage in a large glass jar, closed with a cork stopper covered with paraffin to close the orifices and carefully lapped on its edges. The inner cage must be sufficiently high and flush under the cap which thus serves as a cover for it, so that the rats cannot, by climbing against the walls, pass from one compartment to the other. Two large diameter glass tubes, passing through the cap, open onto the two compartments of the cage; through these chimneys, it is possible to introduce the food, the new fleas and the rats themselves, without there ever being any communication between the inside and the outside; it is sufficient to place the animals or objects between two cotton pads, the first of which, previously used as a stopper, falls with them into the cage, the other remaining to close from

behind the orifice of the tube. Two other tubes of smaller diameter and also plugged with wadding make it possible to ventilate the jar by connecting them to any vacuum cleaner or wind tunnel.

To remove, during the experiment, the first corpse, it is possible to introduce through the glass chimney a long clamp wrapped in a cloth, soaked in sublimated solution, forming a tent; from the opening, the rat is wrapped in this linen and the whole is immediately immersed in an alcoholic solution of sublimate that would kill any stray flea.

Once the experiment is complete, just pour a few cubic centimeters of ether into the inside of the jar to kill all the remaining fleas. The entire system is then easily disinfected by filling the jar with antiseptic solution.

Under such conditions, it is possible, by paying strict attention to it, to carry out these searches for transmission by the fleas without fear of spreading the plague around itself.

Experiment A. On September 16, 1902, a white rat was inoculated, by subcutaneous injection, with a culture of human plague. About ten fleas, collected from healthy rats captured on board various vessels, are placed on the animal in experiment a few hours after the inoculation.

This rat dies on the third day. A new healthy rat is introduced into the compartment adjacent to the cage. The corpse of the first is removed only after about ten hours, when all the fleas seem to have emigrated to the new rat and appear several times on the surface of its hair.

This second rat dies after seven days. The remaining fleas are killed.

The autopsy of the first rat showed an intense inflammatory reaction at the point of inoculation, multiple adenitis, diffuse infiltration of the subcutaneous tissue, but a discreet general infection; liver and spleen smears did not contain markedly characteristic bacilli; however, inoculation of heart blood and liver pulp gave a typical culture of heavy bacilli.

At the autopsy of the second rat, we found a much more massive sepsis. Subcutaneous cell tissue was hyperaemic and inguinal ganglia were clogged to the left. Spleen, liver, and lung smears had large numbers of morphologically typical heavy bacilli. Tubes seeded with heart blood, liver pulp and urine collected aseptically in the bladder gave pure plague cultures.

Experiment B. - On September 27, a white rat is inoculated with a very virulent plague culture; a few hours later, twenty fleas collected on rats of ships are thrown into his cage. The inoculated animal succumbs in 48 hours. A new white rat is introduced into the adjacent compartment a few hours before the corpse of the first animal is removed. This second rat dies after 5 days.

The autopsy of the first animal showed the usual signs of experimental heavy infection, verified by positive inoculation of heart blood and liver pulp.

At the autopsy of the second, there was a diffuse injection of subcutaneous cell tissue, without adenitis. Liver smears showed a few rare bacilli; the seeding of the

liver pulp gave impure cultures, but the culture obtained with the blood of the heart provides quite typical forms of plague.

Experiment C. - On October 3, a white rat inoculated with plague is parasitized using about twenty fleas collected from healthy rats taken in the city or from various ships. This rat succumbed in 36 hours; as in the previous experiments, a healthy white rat is introduced into the second compartment of the cage. The latter died after 6 days.

Autopsy of the first rat revealed typical sepsis with bacilli in liver smears and pure cultures obtained by seeding liver pulp and heart blood.

The autopsy of the second rat gave smears of organs devoid of bacilli, but, in cultures seeded with the blood of the heart and the pulp of the liver, it developed from the typical heavy bacillus.

In the following experiments, we did not take the precaution of separating the new animal from the infected corpse.

Although single-contact transmission was reported as possible, we were led to hold it to be zero in cases of sepsis in non-parasitic animals, such as the rats and mice in our laboratory. We never managed to contagious these animals by simply placing them in the same jar with infected and non-parasitic white rats. In those circumstances, I consider the latter observations to be as rigorously conclusive as the former.

Experiment D. - On 29 June 1902, six fleas, collected from sewer rats captured in the city, are placed on a white rat inoculated with post. This animal succumbs 30 hours after inoculation. Immediately after his death, a new white rat was introduced into the same jar; the corpse of the former is removed only after about 15 hours to allow the fleas to migrate to the healthy animal. He died 10 days later.

At the autopsy of the first rat, we found intense sepsis, with bacilli in the smears of organs and pure cultures by seeding the blood of the heart and the pulp of the liver.

The second rat was also infected. There was diffuse congestion of subcutaneous cell tissue; the organ smears showed typical bacilli, although slightly less abundant than in the smears of the first rat; cultures seeded with heart blood and liver pulp were characteristic.

Experiment E. Following one of the previous experiments (B), we placed a white mouse in the cage where the second rat had died, before destroying the fleas. This white mouse died in 24 hours and the inoculation of heart blood gave typical plague cultures (1).

Note 1. We have only had one experiment on mice, because these animals seem to us to be useless in these trials, because of their ability to destroy the fleas. Indeed, under different circumstances, we had the opportunity to place six dog fleas on an inoculated white mouse; after his death a new mouse was introduced into the jar; but she quickly managed to get rid of the parasites and remained unharmed.

During the experiments described above, we did not attempt to grasp the passage of Yersin's bacillus into the flea organism. Other authors have made this point clear before us (2).

Note 2. G. Zirolia. He Polielinieo, Suppl, seltim., April 12th, 1902, p. 139.
Let us note by passing that we had several times opportunity to determine on the animals in experience, the existence of hematic spots which speckled their fur; Zirolia has shown that fleas leave these traces during suction, filling and emptying their digestive tract several times from their host's blood.

But, on the way, we have sometimes smears or sowed fleas collected from septicaemic animals. All recently collected insect smears had morphologically typical heavy bacilli. In two cases, where we have recently cultured killed insects, characteristic Yersin bacillus has developed. In one of the cases, this bacillus has proved to be devoid of virulence, a fact which can be well explained after the passage of an insect of as small volume as a flea into antiseptic solutions, the use of which is nevertheless necessary to destroy the germs existing on its external patch. In the other case, the culture obtained killed the mouse in 48 hours.

The absolutely consistent results of this series of experiments allow us to conclude that the fleas of the rat are capable, in a constant way, of transmitting the plague from animal to animal, rat or mouse. The animal inoculated by the fleas succumbs in 5 to 10 days with a generalized weighty septicaemia. One mouse even died exceptionally in 24 hours, already septicaemic.

In one case, the animal infected through the fleas showed us a more massive infection than the inoculated rat; in others, infection was less intense in flea-inoculated animals. Finally, on occasion, we were even given to highlight the heavy bacillus in its passage through the organism of the flea.

Transmission tests by parasitic rat mites.

In addition to fleas, there are other parasites from the Mites group on city and ship rats, often with a fairly high abundance. These parasites, very small and very agile, were submitted to the examination of Dr. Bordas, head of zoological work at the Faculty of Sciences of Marseille, who kindly made the determination. They belong to the Gama family, of the genus *Hœmomyson*; they are *musculi (Megnin*).

Their sometimes very considerable number, led us to think that the study of their possible role in the contagion should be the subject of some

experimental research, similar to those instituted for pulicidae. These mites do not jump like fleas, it is easier to protect against their outcome from the jar experiments; a rather thick layer of vaseline at the top of the jar and a bath of sublimate around this container are sufficient to prevent any exodus.

In this research we did not take the precaution of separating the animals either; we have already indicated in connection with the experiments on fleas that this condition does not seem indispensable to us.

Experiment F. - On June 29, a white mouse inoculated with plague is loaded with a dozen mites taken from a sewer rat captured in town. The next day, two healthy mice are placed with the inoculated mouse. The latter dies seven days after the inoculation, of weighty sepsis, verified by examination of the smears of organs and the development of typical cultures.

The healthy animals, on which we could see a few mites pass, remain in good health and, by sacrificing them eighteen days after the death of the first mouse, we can see the complete absence of infection.

Experiment G. - On August 10, a white rat is inoculated under the skin with a plague culture. After 48 hours, when the animal seems already sick and less able to defend itself against parasites, 8 mites are placed on it, caught on a sewer rat captured in town. The experimental rat dies 36 hours later, of weighty sepsis, verified by the smears and the seeding of organs.

A new white rat is placed all night near the corpse. It remains healthy and after having sacrificed it after 20 days, one can check at the autopsy the complete absence of infection.

Experiment H. - On September 11, a white rat, inoculated with plague, parasitized about thirty mites collected from sewer rats captured in the city. This rat dies after four days of serious sepsis, verified bacteriologically at the autopsy.

A healthy white rat, left with the corpse for a few hours, is then kept under observation for 23 days; sacrificed at the end of this time, it is found free of any infection. However, it still carried 28 mites.

One of these parasites was collected on the corpse of the first rat in this experiment, washed with absolute alcohol and crushed to provide smear and seeding. Neither on direct examination, nor in culture, we found in this parasite the heavy bacillus. The same negative finding was made on several parasites of this kind collected in a ship rat which had succumbed to spontaneous plague and whose fleas, on the other hand, provided virulent cultures of Yersin bacillus.

We can conclude from these experiments that parasitic mites in rats do not appear to be capable of infecting an animal under ordinary septic conditions and cannot then carry the infection to a new host.

In these tests, we had not separated our animals and the absence of transmission would already show that contact alone cannot be sufficient to

determine the heavy infection. We have however undertaken other experiments to study this mode of contagion already revoked in doubt by Simond.

Simple contact transmission tests.

We have multiplied these trials by frequently placing healthy animals in the same jar where we locked inoculated rats or mice during diagnostic or systematic research. In no case have we seen the non-parasitic animal, simply exposed to contact with a heavy congener, become infected in its turn. We will not recount all these various tests, some twenty in number, all identical and negative. We will only indicate, in their detail, the most typical experiences.

Experiment J. A healthy white mouse is placed, from 18 to 30 September, in successive contact with six heavy mice.

These inoculated in batches of two, are replaced by four in four days, as they succumb to the bacteriologically verified infection.

The mouse thus exposed is then kept under observation for one month; it remains healthy and after sacrificing it, it is found to be healthy.

Experiment K. - On November 19, three adult rats, inoculated with plague by subcutaneous injection and a white spleen with two young, aged six to eight weeks, are placed simultaneously in the same jar.

The inoculated animals die successively in two and three days of typical sepsis. One of the corpses is removed on the third day for bacteriological examination but the other two are left in place until November 27.

Despite this prolonged contact, the spleen and the two young rats remained strictly free.

These two experiments, confirming our other observations and made more rigorous by the long duration of contact with septicaemic animals or their corpses, the number of inoculated animals successively or simultaneously tested, by the particular receptivity of the young undergoing the second test, seem to demonstrate that mere contact is not sufficient to transmit infection from an animal with severe sepsis to a healthy animal.

II. - Do the fleas of the rat bite the man?

According to the experimental studies that are the subject of the first part of this work, we were allowed to admit that the fleas transmit the weighty sepsis from one animal to another. We then had to investigate

whether these same rat fleas can bite humans under conditions that seem similar to those that carry out contagion between animals.

Using the same method as in the transmission experiments, fleas were collected as such from captured grey rats. After making sure that there was no infection in the host, we placed the parasites, which had been fasting for a few hours, on the subject's arm or leg.

Experiment I. - A flea collected on a rat captured in town is placed, after 6 hours of fasting, on the forearm of subject A which it does not spit.

Experience II. - 2 insects of the same origin, fasting for 24 hours, are placed on the forearm of subject B, which feels stinging but has only dubious marks.

After the experiment, the contents of the abdomen of the flea are clearly seen, by transparency, in red. One of the insects escapes, the other provides a glittering smear where the blood cells have their characteristic appearance after staining with eosin.

Experience III. - A flea, of similar origin, fasted for 24 hours, is placed unsuccessfully on the forearm of subject C, in a sleeve of gummed canvas. Half an hour later, this same insect is placed on the forearm of B, with the same device. After about 10 minutes, the insect very visibly starts to sting and soon drops large drops of gushing blood on the arm. Traces of stings are not very visible. The flea escapes and gets lost.

Experience IV. - 2 other fleas, being in the same fasting conditions, are still placed on the forearm of B, which feels stitched and yet offers only dubious marks, although both crushed insects are clearly sipped with fresh blood, recognizable by microscopic examination.

Experiment V. - 3 sewer rat fleas, left fasted for 48 hours, are placed on the forearm of C. It rises and immediately stitch with the magnifying glass they are; see reddening and swelling. The bite was clearly felt and we can see three very distinct marks.

One sample escape, the other two can be determined, they are *pulex fasciatus.*

Experience VI. - 1 insect of the same origin, fasted for 36 hours, is placed on the forearm of C, under a glass cylinder. The flea stings several times, leaving three sharp marks; when it is removed half an hour later, its stomach is seen well drawn in dark brown. The insect is then transported on the forearm of B; it sits and seems to stitch, without leaving a visible trace; he is killed during these manipulations.

The stomach is extracted by magnifying glass dissection and its frankly gleaming content is spread over the blades. The eosin coloration shows very distinctly red blood cells.

Experience VII. - On a rat captured in the courtyard of the laboratory there are three pulex fasciatus and one non-pectinated flea differentiating from p. Irritans. (We will return later to the characters of this variety.) These 4 insects are tested after 24 hours of fasting; placed on the forearm of A, they all bite for a short time, leaving a single punctiform mark, without peripheral areole or petechiae. The unspectinated flea dies at night, the three fasciatus are stored in tubes respectively 2, 4, 8 days doing each day l or 2 meals, the duration gradually

increasing between 2 and 9 minutes. The traumas, inevitably exerted during these successive manipulations, caused the death of the insects. The stings which, in the first days, left only barely perceptible traces, then determined on the skin very sharp and pruriginous petechias in all the points where the fleas had been placed.

Experience VIII. - A pulex fasciatus fasted for 24 hours, placed in the inner region of the leg of B, thus makes several meals during the day, - four applications, four stings; then is accidentally killed.

Experience IX. - A small pulex fasciatus, found with five others on a house mouse, is fed from 25 November to 15 December by daily or bi-daily meals lasting an average of 2 to 6 minutes and a maximum of 13 minutes. In the meantime, the insect is kept away from the cold in a glass tube placed in the pocket of a garment worn only during the day. Usually fed on the forearm of C, it takes from time to time without difficulty a meal on a second person (subject D). The sting marks are not constant; when they appear only after a few minutes; they are more accentuated on the skin of subject D than on C; they are always not very pruritic.

It can be seen that, in sum, of 9 experiments carried out using 16 fleas, a single test, attempted after only 6 hours of fasting of the insect, remained entirely negative (subject A) (1). However, fleas of the same origin but after a longer fasting, very clearly stitched subject B.

Note 1. It may be useful to note that subject A was undergoing intensive treatment with subcutaneous injections of quinine salts at that time.

Similarly, in the third experiment, we see a sample that refused to stitch C, attack half an hour after the subject B. This one, boy of the laboratory, is moreover, of the various experienced subjects, the one that offers the appearance of the most vigorous health.

Apart from these two failures, one total, the other partial, involving only two of the 16 insects tested, all attempts were successful. Once disdained subjects could be bitten in the aftermath. All meals offered were carried out with full success; the same insect was able, quite often, to bite its human host several times in the same day. The flea of experiment IX survived 20 days despite its exclusively human diet.

Only part of the fleas tested were determined during these tests. We found seven pulex fasciatus and one non-pectinated flea. In several cases, the insect escaped or was immediately used for histological preparations this determination was impossible.

We did not believe, moreover, that there was a need to take the investigation - entomological as the basis and principle of our study, the main point being to prove that the fleas that commonly live as parasites on the rat are capable of sting man.

But it seemed to us that the zoological determination of the parasites of these rodents is not without interest and we practiced it, during our research, on about 300 samples.

Flea varieties found in experiments with rats. - Fleas usually exist only in limited numbers on • the healthy rat we often find two or three, sometimes none; in other cases, these insects exist in considerable numbers (2).

Note 2. A rat, which was brought to the laboratory by Dr.Dupuy (med. san. Mar.) and which was the initial occasion of a series of our research was literally covered with parasites. On another, we collected 160 fleas, of which 188 belonged to the same species, typhlopsylla musculi.

Parasitic fauna, in our observations, was found to be very different from those of ships.

In earth rats and a few mice, we find, on 32 samples:

Pulex fasciatus	45
Unpaid fleas other than p. Irritans	3
Typhlopsylla musculi	2
Pulex serraticeps	2

In rats from ships, from very different sources, we found, on 230 samples:

Pulex irritans type	2
Unpaid fleas other than p. Irritants	64
Typhlopsylla musculi	178
Pulex fasciatus	6

We met on a ship rat two fleas of the man; but we were unable to infest white rats with this type (*Pulex irritans*), while we made the species commonly found in the grey rat live easily on these same animals.

The other unspectinated fleas that we have encountered and that belong to a single variety, are very similar to the flea of man in its main features, in particular the absence of combs, the shape of the head and antenna, the respective formulae of the segments of the tarsus, to the different legs. They seemed to us to deviate from it always notably by their size which is much smaller, by their lighter color, by the absence of the dark striation which, in *Pulex irritans*, shows the top of the antenna dig and finally by the shape of the genital reinforcement. This type of flea, by these characters, is very similar to the variety described by Taschenberg as *Pulex pallidus*. These fleas, in our research were quite

special- to the rats of ships; we made sure that they could nest and complete all their development on these animals.

CONCLUSIONS

Our experiences allow us, we believe, to answer - in a precise way - the double question we asked at the beginning of this work.

Parasitic transmission of plague is possible. We have seen it happen from rat to rat *through the fleas* of these animals, but not through the mites with which they are sometimes infested. It is not carried out by simple animal-to-animal contact when any parasite is excluded from the experiment.

The fleas thus implicated in the spread of epizootic diseases must be feared as possible agents of transmission from the rat to the man, since we have seen that *the fleas collected on the rats bite the man* without difficulty.

This study therefore seems to us a full confirmation of the theory built by Simond. It thus constitutes a new indication to direct against this special mode of transmission an adequate prophylaxis strictly applied.