

XXVII. REPORT ON EXPERIMENTS UNDERTAKEN TO
DISCOVER WHETHER THE COMMON DOMESTIC
ANIMALS OF INDIA ARE AFFECTED BY PLAGUE¹.

By Lt.-Col. W. B. BANNERMAN, M.D., B.Sc. (Edin.), F.R.S.E.,
Director, Bombay Bacteriological Laboratory,

AND R. J. KÁPADIÁ, L.M. and S., *Senior Assistant Surgeon*
Bombay Bacteriological Laboratory.

(Published by permission of the Sanitary Commissioner with
the Government of India.)

IN a report on plague in Hongkong submitted to the Secretary of State for the Colonies in December 1902, Professor Simpson² says (p. 8) "The experiments undertaken demonstrate that pigs, calves, buffaloes, sheep, hens, ducks, geese, turkeys and pigeons are, in addition to rats, susceptible to plague, and particularly so when fed with plague material." He points out that these experiments have a twofold importance for the Health Officer, viz.

(a) that the ordinary farmyard animals will have in future to be watched, and regarded as possible sources of plague, and

(b) that the eating of contaminated food may—in spite of the contrary opinion of the Indian Plague Commission—be a common way of acquiring plague, and that therefore inspection of food stuffs assumes a new and important aspect.

The opinion held in India has always been that these animals were immune to plague, and it seemed therefore necessary to reinvestigate the matter with regard to local conditions here, and measures were

¹ The original of this paper was a report submitted to the Government of India in 1904.

² *Report on the causes and continuance of Plague in Hongkong and suggestions as to Remedial Measures* by W. J. Simpson, M.D., F.R.C.P., London, Waterlow and Sons, Ltd., Printers, London Wall, 1903.

taken by Mr Haffkine to accomplish this. Accordingly, on 18th March 1904, two pigs (one boar and one sow) and two ducks (one drake and one duck) were fed with the heart, spleen, liver, lungs and kidneys of rats which had died of plague.

Owing to Mr Haffkine's departure at the end of April on furlough nothing further was done till the 12th of May, when feeding experiments were resumed and extended by us as detailed below.

The following animals were used :—

4 pigs,	2 calves,	4 fowls,
4 turkeys,	4 geese,	4 ducks.

The methods described by Professor Simpson were followed as exactly as possible, the only difference being in the fact that the germs given to the animals were in rats' bodies, not in human tissues as in the Hongkong experiments.

Rats were injected with plague germs derived from a human source and grown in broth. During the course of the experiments 97 rats were used for feeding purposes. Of these 64 died within two days of inoculation, 30 within three days, and only three lived longer than this period. There can be no doubt therefore that the plague germs used were highly virulent. After death, specimens were made from the rat's spleen for microscopic examination, and cultures on agar were taken and kept for the stalactite test. If the microscope revealed the presence of numerous plague-like bacilli the rat was used for feeding one of the experimental animals in the following manner.

The head, bladder, and intestines were removed, and the whole of the rest of the body and organs cut into small pieces. These were then mixed with the usual feed of bran which the animals were accustomed to eat. The bran was boiled and cooled down before the rat flesh was mixed with it. None of the animals refused to take this bran mash, and many of them ate greedily, picking out the rat flesh with manifest gusto. Latterly, the minced rat flesh was mixed with sterile salt solution and given to the animals without mixture with bran.

To be certain that the rats used had really died of plague, sub-cultures in broth were made from the agar tubes reserved for this purpose, and the stalactite growth watched for. The diagnosis of plague therefore was established by microscopic inspection, the characteristic appearance of the growth on agar and the formation of stalactites in broth. The character of the germs used for the scarification and injection experiments was established in a similar manner. There can be no manner of doubt

therefore that the germs used were those of virulent plague capable of killing rats, in from 2 to 3 days. The germs were derived from human sources.

The following is the result of the experiments.

Pigs.

Pigs Nos. 1 and 2, full grown male and female, were fed seven times each as above described. On each occasion one rat was given to each. The feeding took place on the following dates: 18th March, 12th, 13th, 16th, 19th and 21st May, and 8th June, 1904. The pigs at no time showed signs of illness, and are still alive and well.

Pig No. 3, full grown female, was on 18th June, 1904, scarified on the left shoulder. Into the scarifications was rubbed the juice of the liver of a rat which died of plague derived from a human source. This animal did not take its food properly for two days, but then quickly recovered.

The site of scarification was inflamed for a couple of days but after this the wounds rapidly healed.

This animal is also alive and well at date of writing, three months later.

Pig No. 4, full grown male, was on 18th June, 1904, injected subcutaneously with 2 c.c. of an emulsion of the organs of a rat which had died of plague. The emulsion was made with sterile broth and the germ was derived from a human source. This animal did not eat much for a day or two, and an abscess developed at the site of inoculation. This was opened on 30 June 1904, and had healed on 8th July 1904.

The pig is still alive and well.

Calves.

Two calves about 9 months old were fed 7 times each as above on the following dates:—24th, 26th and 28th May, and 1st, 3rd, 4th, and 11th June 1904.

Neither animal showed any signs of illness, and both are alive and well at the time of writing 3 months afterwards.

Fowls.

Fowl No. 1, a full-grown hen bird was fed 8 times on the organs of rats as above described, on 22nd, 26th, and 27th May, 1st, 2nd, 5th, 6th and 10th June 1904. This bird remained alive and well up to 20th July,

when it succumbed to an attack of chicken cholera induced in it by subcutaneous injection.

Fowl No. 2, a full-grown cock bird was fed 5 times as above, on 10th, 11th, 12th, 13th and 16th June 1904. The bird showed no signs of sickness at any time, and is still alive and well.

Fowl No. 3, a full-grown hen, was scarified on the neck and under the wing on the 16th June 1904. Into these scarifications was rubbed a piece of liver of a rat which had died of plague. No reaction was produced, and the animal is still alive and well.

Fowl No. 4, a full-grown cock, was injected subcutaneously on 18th June, 1904, with emulsion of the internal organs of a rat which had died of plague. The bird suffered no inconvenience but died on the 4th July, 16 days afterwards, of some undiscovered cause. On post-mortem examination no trace of disease could be found.

Smears from liver and heart blood were free from microbes, and cultures from liver and heart blood remained sterile.

Turkeys.

Turkey No. 1, a full-grown cock bird was fed 8 times as above described, on the 20th, 21st, 22nd, 24th, 26th and 27th May, and 4th and 9th June 1904. This bird suffered no inconvenience, and remained well up to 22nd July, when it was fed with the organs of turkey No. 2, which had died of chicken cholera. It gradually became much emaciated and died on the 19th August. Post-mortem examination revealed nothing as to cause of death. Smears from liver and heart's blood were free from microbes and cultures from the same remained sterile.

Turkey No. 2, a full-grown hen bird was fed as above described 8 times, on the following dates:—22nd, 26th, 27th May and 1st, 2nd, 5th, 6th, and 9th June 1904. This bird remained unaffected and well up to 20th July when it received a subcutaneous injection of a culture of chicken cholera, from the effects of which it died two days later.

Turkey No. 3, a full-grown hen was scarified on the neck and under the wing on 18th June 1904. A piece of liver from a rat which had died of plague was rubbed into the scarifications. The bird suffered no inconvenience, and is alive and well to this day.

Turkey No. 4, a full-grown cock was injected subcutaneously on 18th June 1904, with an emulsion made from the internal organs of a rat which had died of plague. The bird suffered no inconvenience and was well up to 20th August 1904, when it died during the rainy season

from some unknown cause. Smears of liver and heart blood were free from microbes and cultures from these remained sterile.

Geese.

Goose No. 1, a full-grown gander, was fed 7 times as above described on the following dates:—23rd, 26th and 27th May and 1st, 2nd, 5th and 10th June 1904.

This bird showed no signs of illness, and it was well up to 21st July 1904, when it was fed on organs of goose No. 2 which had died of chicken cholera, from the effects of which it died on the 29th July.

Goose No. 2, a full-grown goose was fed 7 times as above described on the following dates:—27th and 29th May and 1st, 4th, 5th, 6th, and 11th June 1904. This bird showed no signs of illness up to 20th July, when it received an injection of chicken cholera from the effects of which it died next day.

Goose No. 3, a full-grown goose was scarified on the neck and a piece of liver from a rat which had died of plague was rubbed into the place on the 18th June 1904. The bird suffered no inconvenience, and is still alive and well.

Goose No. 4, a full-grown gander, received subcutaneously on 18th June 1904 an emulsion of the internal organs of a rat which had died of plague. The bird suffered no inconvenience and is still alive and well.

Ducks.

Duck No. 1, a full-grown drake, was fed 7 times as above described on the following dates: 18th March, 16th, 19th, 21st, 22nd and 24th May and 8th June 1904. This bird showed no signs of illness at any time during this somewhat protracted period of feeding but died of some unknown cause on 24th June, 16 days after the last feeding. Post-mortem examination revealed no cause of death. Smears from liver and blood showed no micro-organisms, and the cultures from the same sources remained sterile.

Duck No. 2, a full-grown female bird, was fed 7 times as above described on the following dates: 18th March, 19th, 22nd, 26th, 27th and 28th May and 8th June 1904. The bird suffered no inconvenience, and is alive and well up to date.

Duck No. 3, a full-grown female bird, was scarified on the neck and the place was well rubbed on 16 June 1904 with a piece of liver of a rat

which had died of plague. The bird suffered no inconvenience, and is alive and well up to date.

Duck No. 4, a full-grown drake, was, on 18th June, injected subcutaneously with an emulsion of the internal organs of a rat which died of plague. The bird suffered no inconvenience, and is still alive and well.

The above experiments were, in every case, entirely negative, and it was not considered necessary further to extend the series, as the results previously obtained in this and other laboratories in India have been identical.

When Mr Haffkine in 1897 endeavoured to elaborate a curative serum for plague on the lines laid down by Roux and Yersin, the animals he used were horses, cattle, sheep, and goats; and he expressly says in his evidence before the Indian Plague Commission¹ that he used no animals that were susceptible to plague.

The German Plague Commission also experimented with various domestic animals in Bombay; 2 doves, 2 cocks and 2 geese were injected with virulent plague cultures, but showed no reaction. Two young pigs similarly injected showed no reaction. Two young pigs fed on plague rats also showed no sign of illness. Two sheep, one scarified, and one injected, showed high fever for four days, and formation of local abscess. The pus contained plague bacilli but both animals recovered. Two goats showed similar results, but the pus was sterile. Four cows, 2 scarified and 2 injected, showed a similar reaction but all recovered. They remark in conclusion, "In judging of these experiments it must not be forgotten that the infection was more intense than is met with in natural circumstances²."

Lieut. Walton, I.M.S., when working for the Indian Plague Commission, tried to infect pigeons with plague by hypodermic injection and failed³.

Beyond the confines of India, we find that numerous species of birds have been experimented with. Thus London⁴ made extensive experiments in Russia, with the common birds of that country. The birds used were pigeons, cocks and hens, ducks, crossbills, yellow-hammers, linnets and canaries. An emulsion of culture on agar of 24 hours' growth

¹ *Report of Indian Plague Commission*, Vol. I. p. 14, Q. 133.

² *Centralblatt f. Bak.* 1897.

³ *Indian Plague Commission Report*, Vol. III. p. 337, Q. 26,315.

⁴ *Les Oiseaux sont-ils sensible à la peste bubonique?* par M. E. S. London, *Archives des Sciences Biologiques*, publiées par l'Institut Impérial de Médecine Expérimentale à St Pétersbourg, Tome VI., No. 1, p. 67. St Pétersbourg, 1897.

was used in the majority of cases. Two pigeons received a culture made on pigeons' serum which had been deprived of bactericidal properties by heat. One hen was inoculated under the skin with a fragment of the spleen of a white mouse dead of plague. Not only were normal birds used, but also those whose vitality might be supposed to be lowered by such proceedings as the following: starvation, removal of the feathers from the thorax, abdomen and legs, and chilling of the body by immersion in cold water. White mice inoculated at the same time as the birds invariably died within 2 days. The conclusion arrived at by the author is that birds, either in a natural condition or when subjected to such treatment as above described, are not susceptible to plague.

Since the experiments above detailed were completed, the laboratory has received from Mr Ernest Hill, Health Officer for the Colony, a "Report on the Plague in Natal 1902-1903"¹ in Part II of which Mr H. Watkins-Pitchford, F.R.C.V.S., Government Bacteriologist, details trials he had made to test the domestic animals of South Africa in accordance with Professor Simpson's plan. He says, "Repeated endeavours have been made to induce a fatal form of the disease by the ingestion of infected materials and by the inoculation of virulent cultures and blood, as well as by close contact with an animal in a highly infectious condition." Of 20 hens used, none became ill in any way; of 10 pigs, both English and Kaffir, none died and only "one showed a transient illness due to manipulation, not to plague"; of two calves neither was affected.

It appears quite certain therefore that the farmyard animals of India and Natal are not liable to contract plague either by ingestion, scarification, or subcutaneous injection, and need not be regarded with suspicion during plague epidemics. This is quite in accordance with Indian experience up to the present.

Buffaloes were not experimented with owing to difficulty in procuring a specimen, but as these animals swarm in Bombay City and are valuable property, it is certain that plague amongst them would long ere this have been reported.

It will be interesting in conclusion to analyse Professor Simpson's experiments with the aid of the details furnished in his report, to discover if any other explanation is possible than the supposition, that the animals of China belong to a breed so different as to succumb in

¹ *Report on the plague in Natal, 1902-1903*, by Ernest Hill, M.R.C.S., L.R.C.P., D.P.H. (Cantab.), Health Officer for the Colony. Printed by Cassel & Co., Ltd., London, Paris, New York and Melbourne, 1904.

numbers to a disease against which similar animals in India and Natal are immune.

Professor Simpson used 17 pigs in all. Of these two were not inoculated with plague material because they spontaneously developed some disease and died. On post-mortem examination "plague bacilli were found in the spleen, blood and glands" (p. 72) of one of them (pig No. 16). This animal was confined "in a pen adjoining those in which pigs 8, 9 and 10 were located." The second pig No. 17 "was not in the same pen as the foregoing and died on the 14th day." No plague bacilli were found in the pig nor were any inoculation experiments performed with its tissues. Material from pig 16 however was given by the mouth to six rats, four of which "died with plague bacilli in their blood" (p. 98). *Two days before the death of this pig, Nos. 8 and 9 had died in an adjoining pen, and the organs of No. 8 when given to 6 rats caused the death of 5, in 4 of which plague bacilli were found. There was ample time for infection to spread from these pigs to No. 16, as they were confined in adjacent pens for seven days before they died.*

It seems almost certain then that these three pigs Nos. 8, 16 and 17 were suffering from the same highly infectious disease, which was communicable to rats by feeding, and caused by a bacillus indistinguishable from the plague microbe by the methods used by Professor Simpson. These methods are not clearly described in the report, all that is stated being the following (p. 50): "Cultures from the internal organs of some of the animals experimented on were made and examined, particularly from calf No. 6, sheep No. 4, hen No. 7, pigs Nos. 7 and 13, monkey No. 4 and a goose and a pigeon. The cultures correspond to characteristics of ordinary plague bacilli, while those of pig No. 13, hen No. 7, and sheep No. 4 inoculated into guinea-pigs caused their death in two or three days with plague bacilli in their blood."

The remaining 15 pigs, presumably kept in pens in the same place as the above, were fed or inoculated subcutaneously or by scarification, with emulsions of various organs of animals which had died of plague. One animal received in addition an injection of an agar culture of plague. To prove that these animals died of plague, microscopical examination seems to have been relied on, save in the case of pigs No. 7 and No. 13 noted above. In these cases cultures were made, but on what media is not stated, and no mention is made of any differentiating tests having been applied. Without such tests, mistakes might arise from the coincidence of acute diseases due to organisms of the "chicken-cholera" or "hog-cholera" groups.

In support of this idea is the case of pig 13 which, it is acknowledged, may have died of "swine typhoid." It had a high temperature at the time it was fed with plague material, and died on the fifth day after feeding. "Plague bacilli were however found in blood, spleen, glands, and kidneys. Whether this animal died of plague or swine typhoid is difficult to say" (p. 70). Six rats were fed on the internal organs of this pig, and four of them "died with plague bacilli in blood" (p. 98). Now this was eminently a case for differential cultivation, as there was doubt as to the cause of death; whether this was done or not it is impossible to say from the details given in the report.

In this laboratory we have frequently been called upon for diagnosis of diseases causing death among the animals in the Victoria Gardens of Bombay, and in many cases have found bacilli indistinguishable by microscopical inspection from plague bacilli. So like are they in form and bi-polar staining reaction, that officers who have daily worked here with plague bacilli for years have been deceived when shown such preparations. In most cases these have proved to be the microbe of chicken cholera, easily communicable by feeding or injection to birds, as already noted above (*vide* Fowl No. 1; Turkeys Nos. 1 and 2; Geese Nos. 1 and 2). This microbe was found pathogenic for guinea pigs, but not for rats, calves or pigs. In calves a local reaction with abscess formation was observed, and swelling of the nearest chain of glands; in pigs, local abscess formation and sloughing, but nothing further.

It is interesting to note incidentally, that one of the workers in the laboratory, while making a *post-mortem* examination of a swan which had died of chicken cholera, inoculated himself accidentally by running a splinter of bone into his finger, and in consequence suffered from a sharp attack of fever and formation of a local abscess. Recovery was complete in a week. Or had this worker contracted plague at the same time, which he might quite well have done, his case would have been on all fours with that of the Chinese butcher who died of plague after having scratched his hand at a *post-mortem* on a pig. The growth of the chicken cholera microbe on agar is also very like that of plague, and it is only by further cultivation and inoculation experiments that they can be distinguished with certainty.

The suspicion therefore arises that the pigs used by Professor Simpson in Hongkong, may have been suffering from "hog-cholera," or as he calls it "swine-typhoid," which appears to be very common in China, or possibly from the *pasteurellose du porc* (Sweineseuche of the Germans). Thus Staff-Surgeon Wilm of the Imperial German Navy

quoted at p. 49 of Professor Simpson's report, gives an account of the *post-mortem* appearances found in a pig which had eaten the spleen of a man that had died of plague, which corresponds almost word for word with the classical descriptions of the lesions of hog-cholera. Professor Simpson further quotes the following from the same observer:—"In the beginning of August 1896, on board of two steam-ships carrying pigs to Hongkong from the island of Hainan and from Pakhoi respectively, a large number of pigs died. A great many also died after they had been landed at Hongkong. *Post-mortem* examination of the bodies of these animals showed the same morbid appearances as in those killed by giving them plague-infected organs to eat, the appearances being most marked in the intestinal tract." Wilm found the bacillus pathogenic to "mice, rats, guinea pigs and rabbits when injected subcutaneously," but these also are just the laboratory animals susceptible to the "hog-cholera" bacillus.

At the time Wilm made these experiments and observations, the methods of differentiating these various organisms were undeveloped, and the stalactite test for plague had not been discovered by Mr Haffkine. Wilm's cultural tests would not now be regarded as conclusive and his inoculation ones as we have seen are open to another interpretation, viz. that the pigs died of hog-cholera and that the bacillus associated with that disease was mistaken for the plague microbe.

That this might easily happen is evident when we find the "hog-cholera bacillus" thus described by Theobald Smith¹:—"They are short rods with end rounded, not producing spores....They usually present a more deeply stained periphery and give the impression of a bacillus completely filled out by a feebly stained spore"; and again (p. 10) "In cover-glass preparations from the tissues of inoculated animals, the central portion of the rod is frequently only feebly stained." These are descriptions that might quite well be given of the plague bacillus.

It is instructive to place in parallel columns the description of symptoms and *post-mortem* appearances observed by Professor Simpson in his pigs, and those of the text-books describing hog-cholera.

That acute swine diseases are very common in Southern China appears from Appendix F of Professor Simpson's report. Thus, in answer to the question "Has there been any illness prevalent among pigs or cattle preceding the plague outbreak?" we find the following

¹ *Additional Investigations concerning Infectious Swine Diseases* by Theobald Smith, Ph.B., M.D., and Veranus A. Moore, B.S., M.D., published by authority of the Secretary of Agriculture, Washington Government Printing Office, 1894, p. 22.

Professor Simpson's account of the symptoms and *post-mortem* appearances in the pigs.

1. *Duration* of disease was from the 4th to 36th day after feeding: average about 3 weeks.
2. "Remained apparently quite well with no diarrhoea until a day or two before death when they appeared to be somewhat feeble on their legs and inclined to stagger" (p. 51).
"Suffered from diarrhoea" (p. 52).
"The pigs seemed to be feeble on hind legs" (p. 51).
"Unsteady gait before death" (p. 52).
3. "The eyes became congested and discharged white mucus in which plague bacilli were found" (p. 52).
"Both had congestion of eyes" (p. 52).
4. *Post-mortem* appearances: "Congestion of the organs, congestion and haemorrhagic condition of most of the lymphatic glands, specially of the throat and neck" (p. 53).
"Glands of the body were congested, but varied very much in their degree of congestion and haemorrhagic state" (p. 53).
"The lymphatic glands being congested and haemorrhagic" (p. 54).
"Patchy inflammation and haemorrhages into mucous membrane of the large intestines" (p. 51).
"The large intestines were congested and haemorrhagic" (p. 51).
"The large intestines inflamed in patches" (p. 52).
"Showed inflamed large intestines" (p. 52).
"*Post-mortem* showed lungs to be pneumonic in patches" (p. 52).
"Showed...lungs with pneumonic patches" (p. 52).
5. "Plague bacilli were present in the blood, spleen, glands, kidneys, bladder and intestinal contents" (p. 51).
Similar notes occur as to distribution of bacilli in almost all the pigs.

Text-book description of hog-cholera¹.

1. *Duration* from 1 or 2 days to 2 or 3 weeks.
2. "The animals often die suddenly without having appeared particularly ill," or "after seeming ill but a few hours" (p. 413).
"The bowels may be normal or constipated at the beginning of the attack; but later, there is generally a liquid and foetid diarrhoea" (p. 413).
"The animal becomes weak...and walks with a weak tottering gait."
3. "The eyes are congested and watery, the secretion drying and glueing the lids together" (pp. 413, 414).
4. "*Post-mortem* appearances: Extravasations of blood are common in the lymphatic glands" (p. 414).

"Extravasations of blood are common...beneath the serous membrane of the thorax and abdomen and particularly along the intestines."
"The contents of the intestines are sometimes covered with clotted blood" (p. 414).
"In hog-cholera the first effect of the disease is believed to be upon the intestines, with secondary invasion of the lungs" (p. 415).
"Occasional broncho-pneumonic changes in the lungs" (p. 415).
5. "The specific bacillus² of hog-cholera was secured by Smith from the spleens of more than 500 hogs. It occurs in all the organs and has been cultivated from the urine" (p. 415).

¹ *Text-book upon the Pathogenic Bacteria*, by Joseph McFarland, M.D., 2nd edition, Henry Kimpton, London, 1898.

² Recent observations of Dorset Bolton and McBride (*U.S. Dept. of Agriculture Bull.* 72, 1905, p. 101) have shown that the primary cause of hog-cholera will pass through a filter, and throw grave doubt on the aetiological position of the "hog-cholera bacillus."

statements: Dr A. Rennie of Canton reports "Epidemics amongst pigs are frequent in south China, but are more probably swine-fever (typhoid) than plague." Dr A. Lyall of Kwangtung says "Epidemics of pig disease are common of which they (Chinese) recognise two kinds: (a) with diarrhoea, (b) a disease in which a skin becomes 'red.'" Dr J. P. Maxwell of Changpoo says "As to pigs, what the Chinese call 'pig plague' is probably swine-fever and is certainly not usually bubonic plague." Many others to whom these questions were addressed also remark on epidemics among pigs, cattle and buffaloes in China, so that the elimination of these disturbing factors by a system of quarantine and strict segregation was important. No evidence that this was done is to be found in Professor Simpson's report, and it is possible that some at least of the results obtained may have been due to the prevalence of some sort of swine-plague among his animals.