XIV. ON THE SIGNIFICANCE OF THE LOCALITY OF THE PRIMARY BUBO IN ANIMALS INFECTED WITH PLAGUE IN NATURE.

CONTENTS.

Introduction:—Importance of the Primary Bubo as an Indication of the Path of Infection.

Almost all observers are agreed in the opinion that the primary bubo develops in the glands in connection with those lymphatics by way of which the plague bacillus has entered the body. Hunter (1904–1906), on the other hand, holds that the primary bubo is merely an enlargement of a group of glands in the course of an acute septicaemic disease and is no indication of the path by which infection has taken place.

There is abundant evidence to show that a primary bubo may develop in the glands which are in lymphatic connection with an area in which the infective material has been inoculated in a breach

of the skin surface while, at the same time, no lesion at the site of inoculation may be observed. Wyssokowitz and Zabolotny (1897) report a number of experiments in which they pricked the hands and feet of monkeys with needles contaminated with plague material. Primary buboes developed in the corresponding glands, but no local reaction was observed. The Austrian Plague Commission (1898) also showed that in the case of wild rats a prick infection in the extremities may give rise to no local reaction while a primary bubo is present in the corresponding group of glands. The Indian Plague Commission (1901) recite several instances in man in which the site of infection was apparent, and in which the bubo developed in the group of lymphatic glands in connection therewith. Further, we ourselves have made a series of experiments, in which fleas taken from septicaemic plague rats were fed on guinea-pigs, the area of skin on which they were allowed to feed being strictly limited and the same for each meal. The following was the method:—

Bombay wild rats were inoculated with a virulent culture of the plague bacillus. Next day the rats were placed separately in flea-proof cages and a number of fleas were put in with them. On the death of the rats the fleas, collected from those rats which showed a marked plague septicaemia, were placed in a glass tube, one end of which was open while the other end was closed with a single layer of fine muslin. About twenty fleas were put into each tube. The hair on a small area of a guinea-pig's skin was removed. The fleas were now allowed to feed on this area through the muslin covering the end of the tube. They were given a morning and an evening meal, the tube being applied for from 10 to 15 minutes on each occasion. In some cases the feeding only took place on a single day, while in other instances the same fleas were fed daily for several days consecutively, the same area of skin, however, being used on each occasion. The details of these experiments, along with some others in which no note was made of the area of skin on which the fleas were fed, will be published in another paper. For our present purpose we have constructed a table which shows in the case of 17 animals the relationship between the area on which the fleas were fed and through which the plague bacilli entered the skin, and the position of the primary bubo. A study of this table (Table I) makes it apparent that in practically every case the primary bubo developed in the group or groups of glands which stood in direct lymphatic connection with this area of skin. The only apparent exception to this generalisation is guinea-pig No. 2,

in which a left cervical bubo formed after the fleas had been fed on the left side of the abdomen just below the costal margin. In this case the bacilli may have passed through the lymphatic filter of the axillary glands without affecting them.

TABLE I.

Animal	Region on which fleas were fed	Position of bubo	Remarks
G.-P. 1	Below the ribs and slightly to right of middle line	Right inguinal and double axillary	Marked local reaction and oedema
G.-P. 2	Left side of abdomen just beneath the costal margin	Left cervical	No local reaction
G.-P. 3	Left side of abdomen above umbilicus	Left axillary	Local reaction present
G.-P. 4	Right side of abdomen just above umbilicus	Right inguinal	Intense local inflammatory reaction
G.-P. 5	Left side of abdomen 1 inch above umbilicus	Left axillary and double inguinal	Local reaction
G.-P. 6	Middle line ½ inch above umbilicus	Double inguinal	Local reaction present
G.-P. 7	Middle line ½ inch above umbilicus	Double inguinal	No reaction
G.-P. 8	Left side just above umbilicus	Left inguinal	Local reaction
G.-P. 9	Right side just above umbilicus	Right inguinal	No local reaction
G.-P. 10	Right side just above umbilicus	Double inguinal	Typical phlyctenule at site of feeding; subcutaneous tissues underneath oedematous and congested
G.-P. 11	Right side just above umbilicus	Right inguinal	Intense local reaction
G.-P. 12	Middle line ½ inch above umbilicus	Right inguinal	No local reaction
G.-P. 13	Middle line of neck	Double cervical	Oedema at site of feeding
G.-P. 14	Middle line of neck	Double cervical	Typical phlyctenule and oedema
G.-P. 15	Middle line, about level of umbilicus	Double inguinal	Typical phlyctenule. Local oedema and thickening
G.-P. 16	Middle line, about level of umbilicus	Right inguinal	Oedema at site of feeding
G.-P. 17	Middle line, about level of umbilicus	Left inguinal and double pelvic	Local pustule and oedema

In passing we may refer to another point of interest in these experiments, namely, the presence in most cases and the complete absence in others of a marked local reaction at the site of feeding. In the case of

guinea-pig No. 4 this local reaction was very intense, the abdominal wall and peritoneum being bound together in one mass of exudate. In the case of guinea-pigs Nos. 10, 14, 15 and 17 typical phlyctenules developed and the subcutaneous tissues underneath were markedly oedematous and congested. While in this connection it is to be remembered that the infection was concentrated on one small area of skin, still we have occasionally observed typical phlyctenules in guinea-pigs which were naturally infected. Three such instances may be given:—

1. A guinea-pig in house No. 3165 Sion Agrivada, in which a dead rat had been found on 16th March, was noticed to be sick on 24th March. Next day three phlyctenules were seen on the right side of the neck and at the same time the cervical glands were felt enlarged. The phlyctenules, situated in the centre of a small hairless patch of skin, were each surrounded by a zone of redness. They exactly resembled the vesicles seen when plague material is rubbed into the skin of a guinea-pig. Six rat fleas were taken off the animal and it was then killed with chloroform. On examination post-mortem the primary bubo was found in the submaxillary region and the organs presented all the typical appearances of plague.

2. A guinea-pig placed in house No. II, 8. 19 Sion Bhandariwada was noticed to be sick. On examination a typical phlyctenule was found on the lower lip and the cervical glands were enlarged. Four fleas were taken on this guinea-pig. On death a post-mortem examination revealed the typical appearances of plague, primary buboes being present in the submaxillary and cervical regions.

3. A guinea-pig placed in house I, 12. 26 Sion Agrivada was found sick, a small phlyctenule was noted on the neck and 26 fleas were taken on the animal. On death an examination showed that the phlyctenule contained pus, and that there were buboes in the neck and other typical signs of plague.

Having then satisfied ourselves that the primary bubo develops in those groups of glands which are in direct lymphatic connection with the area through which the plague bacillus enters the animal organism, we may now proceed to inquire if, by a study of the relative distribution of the primary bubo, on the one hand in animals naturally infected with plague, and on the other hand in animals artificially infected by different means, any evidence can be obtained which will point to any particular method as being the one by which infection takes place in nature.

Section I. Distribution of the Primary Bubo in Plague-infected Rats.

(1) *Rats naturally infected*[1].

(*a*) *Wild rats in Bombay during the plague season.* This group of rats is made up of 4000 plague-infected rats which were found in the city of Bombay during the plague epizootic of 1906. In Table II no distinction is made between *M. rattus* and *M. decumanus.*

TABLE II.

Distribution of the primary bubo in wild rats which contracted plague naturally in the city of Bombay during the plague season.

No bubo	Single bubo			Multiple buboes				Total
610	2956			434				4000
(15·2 %)	(73·9 %)			(10·9 %)				
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	2194	322	440	78	132	180	44	
	(74·3 %)	(10·9 %)	(14·8 %)	(18·0 %)	(30·4 %)	(41·5 %)	(10·1 %)	

N.B. In the animals with multiple buboes the neck glands were affected in 58·5 % of the cases. Out of a total of 3390 rats with buboes, the neck glands were affected in 2448 instances, namely, 72·2 %.

We have elsewhere given a detailed account of the post-mortem examination of these rats, so that for our present purpose we need only give the details referring to the localisation of the primary bubo. The points in this table which are more particularly to be noted are:—

(*a*) that the neck glands were involved in 74·3 per cent. of the cases with single buboes and in 58·5 per cent. of those with multiple buboes; and

(*b*) that in the total number of animals which had buboes the neck glands were affected in 72·2 per cent.

[1] There are on record very few observations relating to the distribution of buboes in naturally infected rats which are based on the examination of an adequate number of animals. Buchanan (*Seventh Annual Report of the Local Government Board for Scotland*, 1902, p. 73) notes that inguinal buboes predominate in rats as in men, while Kitasato (*Philippine Journal of Science*, I. 1906, p. 472) rightly insists that cervical buboes are the most frequent.

(*b*) *Wild rats in Bombay during the non-plague season.* This group consists of 900 rats, both *M. rattus* and *M. decumanus*, which were found plague infected in the city of Bombay during the months of June, July, August and September, that is to say, at a time when only sporadic cases of plague were occurring amongst rats and men. A reference to Table III,

TABLE III.

Distribution of the primary bubo in wild rats which contracted plague naturally in the city of Bombay in the off-plague season.

No bubo	Single bubo			Multiple buboes				Total
136 (15·1 %)	712 (79·1 %)			52 (5·8 %)				900
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	535 (75·1 %)	66 (9·3 %)	111 (15·6 %)	8	13	30	1	

In the animals with multiple buboes the neck glands were affected in 42·4 % of the cases. Out of a total of 764 rats with buboes the neck glands were affected in 557 instances, namely, 72·9 %.

which contains the details relating to this group, will show that the distribution of the primary bubo is practically the same as in the case of the previous group. This correspondence of the position of the bubo in rats during the plague season and in rats during the off-season seems to us to point to the conclusion that the same method of infection obtains during both seasons.

On comparing in tabular form the position of the primary bubo in 100 *M. rattus* and in 100 *M. decumanus*, which had become infected in nature, we find that the distribution is practically the same in both species:—

TABLE III A.

	rattus	*decumanus*
Submaxillary	54	51
Axillary	7	11
Inguinal	8	5
Pelvic	3	1
Multiple	3	7
No bubo	25	25

(*c*) *Wild rats in Punjab villages during the plague season.* We have now to consider the distribution of the primary bubo in a group of 288 rats found plague infected in the Punjab villages Dhand and Kasel during the plague epidemic of 1906. These rats were all *M. rattus*.

The details which concern the distribution of the primary bubo are set forth in Table IV. The first point to be noted is the high percentage of cases without bubo in comparison with the wild rats of Bombay. Secondly, we find the same preponderance of neck buboes both in the cases with single and in those with multiple buboes.

TABLE IV.

Distribution of the primary bubo in wild rats which contracted acute plague naturally in two Punjab villages during the plague season.

No bubo	Single bubo			Multiple buboes				Total
103 (35·7 %)	164 (57 %)			21 (7·3 %)				288
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	87 (53·1 %)	36 (21·9 %)	41 (25 %)	7	9	3	2	

N.B. In the animals with multiple buboes the neck glands were affected in 86 % of the cases. Out of a total of 185 rats with buboes the neck glands were affected in 57 % of the cases.

(2) *Rats infected experimentally by means of fleas.*

We have now to pass on to consider the distribution of the primary bubo in three groups of rats which were infected in the laboratory by means of fleas.

In every instance the rats and fleas, which had received their infection from septicaemic plague animals, were confined together in a flea-proof cage. All other sources of infection were rigorously excluded.

TABLE V.

Distribution of the primary bubo in wild Bombay rats which contracted plague experimentally as a result of being bitten by infected fleas.

No bubo	Single bubo			Multiple buboes				Total
14 (22·2 %)	43 (68·3 %)			6 (9·5 %)				63
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	29 (67·5 %)	12 (27·9 %)	2 (4·6 %)	2	2	2	0	

N.B. In the animals with multiple buboes the neck glands were affected in 66·6 % of the cases. Out of a total of 49 animals with buboes the neck glands were affected in 33 instances, namely, 67·3 %.

Three different classes of rats were used for these experiments, so that we can divide the observations into three series as follows:—

(*a*) *Wild rats of Bombay.* This group consists of 63 wild rats of Bombay, no distinction being made between *M. rattus* and *M. decumanus.*

The details concerning the distribution of the primary bubo are given in Table V.

We would draw attention to the following figures in this table:— (*a*) the neck glands were involved in 67·5 per cent. of the cases with single buboes and in 66·6 per cent. of those with multiple buboes; (*b*) out of the total number of animals with buboes the neck glands were affected in 67·3 per cent.

(*b*) *Ship rats.* This group consists of a small number, namely 25, of rats which had been caught on board ships in Bombay harbour. They all belonged to the species *M. rattus*, and had probably never been subjected to a plague epizootic. They were infected by means of fleas in exactly the same manner as the rats of the preceding experiment. Table VI contains the data which refer to the distribution of the primary bubo in these animals. From this table it is seen that both in those animals with single buboes and in those with multiple buboes the neck glands were the site of the bubo in the majority of cases, and that out of a total of 24 animals which developed buboes the neck glands were affected in 12 instances, namely, 50 per cent.

TABLE VI.

Distribution of the primary bubo in ship rats which contracted plague experimentally as a result of being bitten by infected fleas.

No bubo	Single bubo			Multiple buboes				Total
1 (4 %)	21 (84 %)			3 (12 %)				25
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	9 (42·8 %)	6 (28·6 %)	6 (28·6 %)	2	1	0	0	

N.B. In the animals with multiple buboes the neck glands were affected in 100 % of the cases. Out of a total of 24 animals with buboes the neck glands were affected in 12 instances, namely, 50 %.

(*c*) *White rats.* This group is another small group, numbering only 29, consisting of tame white rats, which had been imported from England and had never been exposed to a plague epizootic. They were infected in the laboratory by means of fleas in the same manner as the rats of the two preceding groups.

The data which refer to the distribution of the primary bubo are set forth in Table VII, a reference to which will show that the neck glands were chiefly affected, namely, in 64·3 per cent. of animals with single buboes, 55·5 per cent. of animals with multiple buboes and 61 per cent. of the total number of animals with buboes.

TABLE VII.

Distribution of the primary bubo in tame white rats which contracted plague experimentally as a result of being bitten by infected fleas.

No bubo	Single bubo			Multiple buboes				Total
6 (20·7 %)	14 (48·3 %)			9 (31 %)				29
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	9 (64·3 %)	4 (28·6 %)	1 (7·1 %)	2	3	4	0	

N.B. In the animals with multiple buboes the neck glands were affected in 55·5 % of the cases. Out of a total of 23 animals with buboes the neck glands were affected in 14 instances, namely, 61 %.

(3) *Rats experimentally infected by feeding on plague material.*

In another paper we have considered in detail the results of several series of experiments, in which plague was transmitted to rats by feeding them with plague-infected material. We have seen that as regards the pathological changes, including the distribution of the primary bubo, the result is the same whether the rats are fed on soft material, *e.g.* liver and spleen mixed with the food, or on the whole carcases of their comrades which have died of plague.

For our present purpose we reproduce a table (Table VIII) in which the data referring to the distribution of the primary bubo are given for 119 rats which became infected with plague as a result of eating the organs of plague-infected animals minced up and mixed with their food.

TABLE VIII.

Distribution of the primary bubo in wild Bombay rats which contracted plague experimentally as a result of being fed on plague-infected material.

No bubo	Single bubo		Multiple buboes		Total
10 (8·4 %)	98 (82·8 %)		11 (9·2 %)		119
	Mesenteric	Submaxillary	Submaxillary & Mesenteric	Axillary & Inguinal	
	69 (70·4 %)	29 (29·6 %)	10 (91 %)	1 (9 %)	

https://doi.org/10.1017/S0022172400033386 Published online by Cambridge University Press

The first striking fact which emerges from a study of this table is that the inguinal and axillary glands are practically never affected. The solitary instance in which buboes were present in these regions was probably an experimental accident, the animal possibly infecting its feet through cuts made by the sharp edge of the tin in which the food was placed. Secondly, it is remarkable that 70·4 per cent. of the single buboes were situated in the mesentery, the remaining 29·6 per cent. being under the chin. Lastly, it is seen that practically the only situations in which multiple buboes were found were in the submaxillary and mesenteric regions.

Summary of Section I.

In three groups of rats naturally infected with acute plague, 72, 73 and 57 per cent. of those with buboes have the primary bubo in the cervical region. In three groups of rats artificially infected with acute plague by the agency of fleas 67, 50 and 61 per cent. have cervical buboes. On the other hand, cervical buboes are found in only 36 per cent. of rats artificially infected by feeding. Mesenteric buboes occur in 72 per cent. of those infected by feeding and are not found in rats infected naturally or by the agency of fleas.

The next table (Table IX) summarises these observations as shortly as possible.

TABLE IX.

Showing the percentage frequency of buboes in different parts.

	Naturally infected	Infected by fleas	Infected by feeding
Rats examined	5188	117	119
Rats without buboes	16 %	18 %	8 %
Cervical bubo	72	61	36
Axillary ,,	23	22	1
Inguinal ,,	18	35	1
Mesenteric ,,	0	0	72·5

One may conclude that *the naturally infected rats are not infected by feeding, and that material evidence is here adduced that they are infected by fleas.*

Section II. Distribution of the Primary Bubo in Plague-infected Guinea-pigs.

We have now to consider the distribution of the primary bubo in three groups of guinea-pigs, namely, (1) a group naturally infected in plague houses; (2) a group infected in the course of the go-down experiments already described (vol. VI. p. 465), in which all the facts pointed to the infection being carried by fleas; (3) a group experimentally infected in the laboratory by means of fleas.

1. *Guinea-pigs naturally infected in plague houses.*

This group is made up of 87 animals, which had been placed in plague-infected houses either running about or in open wire cages standing on the ground. They became infected under natural conditions and the means of infection can only be surmised.

The details of the primary bubo in these animals are given in Table X. In the great majority of instances the neck glands, either submaxillary or cervical, were involved.

TABLE X.

Distribution of the primary bubo in guinea-pigs which contracted plague in infected houses.

No bubo	Single bubo			Multiple buboes				Total
1 (1·1 %)	80 (92 %)			6 (6·9 %)				87
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	73 (91·2 %)	7 (8·8 %)	0	5	1	0	0	

N.B. In the animals with multiple buboes the neck glands were affected in every instance. Out of a total of 86 animals which had buboes the neck glands were affected in 79 cases, namely, 91·8 %.

2. *Guinea-pigs which became infected in the go-downs.*

This group is made up of 253 guinea-pigs, which became plague infected in the course of our experiments in specially constructed go-downs. We have already described these observations in detail (vol. VI. p. 450) and have discussed the question of the method by means of which the animals became infected. While it is, therefore, unnecessary to repeat the arguments then brought forward, we may state that all the data

pointed to the conclusion that the rat fleas present in the go-downs were the agents by which the infection was carried from animal to animal.

The details concerning the position of the bubo in these 253 guinea-pigs are set forth in Table XI, from which it is seen that in the great majority of cases the neck glands were the seat of the primary bubo.

TABLE XI.

Distribution of the primary bubo in guinea-pigs which contracted plague in the go-downs.

No bubo	Single bubo			Multiple buboes				Total
3 (1·2 %)	232 (91·7 %)			18 (7·1 %)				253
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	212 (91·4 %)	17 (7·3 %)	3 (1·3 %)	13	3	0	2	

N.B. In the animals with multiple buboes the neck glands were affected in every instance. Out of a total of 250 animals which had buboes the neck glands were affected in 230 cases, namely, 92 %.

3. *Guinea-pigs which were experimentally infected in the laboratory by means of fleas.*

This group is made up of 108 guinea-pigs which became plague infected in the course of experiments of the same nature as those with rats already referred to.

The details concerning the distribution of the primary bubo in these animals are given in Table XII, from which it is once more seen that the neck glands were affected in the large majority of cases.

TABLE XII.

Distribution of the primary bubo in guinea-pigs which contracted plague experimentally as a result of being bitten by infected fleas.

No bubo	Single bubo			Multiple buboes				Total
1 (0·9 %)	89 (82·4 %)			18 (16·7 %)				108
	Neck	Groin	Axilla	Neck & Groin	Neck & Axilla	Groin & Axilla	Neck & Groin & Axilla	
	79 (88·8 %)	10 (11·2 %)	0	12	4	0	2	

N.B. In the animals with multiple buboes the neck glands were involved in every instance. Out of a total of 107 animals which had buboes the neck glands were affected in 97 cases, namely, 90·6 %.

Summary of Section II.

The following table (Table XIII) summarises very shortly the distribution of the buboes in these three groups of guinea-pigs.

TABLE XIII.

Showing the percentage frequency of buboes in different parts.

	Naturally infected	Infected in go-downs	Infected by fleas
Guinea-pigs examined	87	253	108
Without buboes	1 %	1 %	1 %
Cervical bubo	92	92	91
Axillary ,,	1	3	6
Inguinal ,,	14	13	22
Mesenteric ,,	0	0	0

The close correspondence between the distributions of the buboes in the three groups of animals lends support to the conclusion that the mode of infection was the same in all cases. It would follow that, both in the experimental go-downs and in the plague-infected houses, the infection was conveyed to the guinea-pigs by fleas.

General Summary.

Cervical buboes preponderate, on the one hand, in naturally infected rats and in guinea-pigs infected by being placed in plague-infected houses and also in rats and guinea-pigs artificially infected with fleas. In rats artificially infected by feeding mesenteric buboes are the most frequent, whereas in upwards of 5000 naturally infected rats in not a single case was a mesenteric bubo present. It may, therefore, be concluded that rats in nature are not infected by feeding on plague-infected material, but probably by the agency of fleas.

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