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Experimental Transmission of *Salmonella oranienburg* Through Cockroaches

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Long suspected as disseminators of human pathogens, the cockroach commensals of man have never been incriminated decisively and unequivocally as carriers of common disease agents. This is particularly true when one considers the potential role of these insects as carriers of the enteric organisms commonly referred to as food poisoning and food infection agents.

In view of the apparent need for additional and more concrete knowledge concerning the role of the cockroach as a carrier of enteric pathogens and enterotoxin producers, the investigation here described was begun in 1941.

Prior to 1947 studies related to the role of the cockroach as a disease carrier were conducted only as a side activity and were confined principally to methods for rearing and to the development of specific techniques for handling experimental insects. As a result of the rearing experiments which comprised the first work, a well-balanced food, moistened with honey and glycerine to promote its keeping qualities, was compounded. However, certain experiences with this food indicated that the honey and glycerine may have some effect on the flora of the roach intestine, and these agents were subsequently omitted. It was discovered rather early that the readily procurable dry commercial dog and fox foods could be substituted for the formula which had been worked out. Commercial products have therefore been used exclusively since that time as a matter of simplicity and convenience. A rearing cage which provided adequate ventilation and prevented fatal mold growths was also developed as a part of the basic program to provide an adequate supply of laboratory-reared insects for experimental use.

In 1943, as a part of the preliminary work involved in developing suitable methods for carrying out transmission experiments, a strain

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of *Salmonella enteritidis* was fed to a German roach and later recovered from its feces. The exact length of time between the infective feeding and the production of the infected pellet could not be determined under the conditions of the experiment, but was probably less than 2 days. Similarly, it was demonstrated that German roaches could carry living organisms in their intestines, for *Salmonella typhimurium* was recovered from the digestive tract of three experimental roaches 9 days after the original infective feeding. Unfortunately, the food in the cage was also infected at the end of this time, and it was therefore impossible to determine the exact survival time of the *Salmonella* within the alimentary tract of the insect.

Additional experiments in 1944 in which the American cockroach was used indicated that it is very difficult to sterilize the exterior of the cockroach, even when surface tension reducers and sodium hydroxide are used as adjuncts to the sterilizing agent. For example, a 10-minute exposure with agitation to a 5 percent solution of formalin containing 1 percent Triton 770 and 1 percent sodium hydroxide would not completely wash off or kill organisms which were being carried by the insects. These findings made special precautions necessary in carrying out transmission experiments, especially where it was essential to demonstrate that organisms were actually being carried within the roach rather than on its exterior.

Experimental Methods

The colonies of roaches which have been maintained for experimental purposes include four species which are common in American homes: the American cockroach, *Periplaneta americana* L., the Oriental roach, *Blatta orientalis* L., the tropical or brown-banded roach, *Supella supellectilium* Serv., and *Blatella germanica* L., generally known as the German roach, although it is often called the "croton bug" or the "water bug."

Insects used throughout the experimental work were laboratory-bred specimens. The American and brown-banded cockroaches came from stock obtained in San Antonio, Tex., in 1944, while the colonies of Oriental and German roaches are descendents of specimens originally captured at the University of Minnesota in 1946. These colonies have always been free of *Salmonella*, but as a precautionary measure specimens have been checked carefully just prior to inclusion in an experimental group to insure their freedom from natural infection. They do, however, harbor other organisms than *Salmonella*, such as *Paracolobactrum*, *Proteus* and *Pseudomonas* species. Among these, the species *Pseudomonas aeruginosa* has occurred most abundantly and has sometimes been a source of annoyance because of its tendency to overgrow less hardy forms.

Cockroaches are reared in tightly glued rectangular 24- by 32-inch

wooden boxes 9 inches high. Except for a 5-inch feeding platform running along one side, there is a false floor consisting of 14-mesh bronze wire screen set approximately 2 inches above the bottom of the box and flush with the feeding and watering area. The screen allows a considerable part of the excreta and other debris to fall through, thus removing it from the cage proper. Probably more important, it encourages air circulation through the area occupied by the insects. The top of the box is provided with a fine brass screen having 60 meshes to the inch and covering one-half of the area. In the remaining half there is a glass window for the purpose of observing the insects, and a trap door giving access to the interior of the cage. The screened top insures good air circulation and prevents mold growths which develop quickly if humidity is allowed to reach high levels. Such growths appear to be harmful to the roaches.

Water is supplied by a water-filled beaker inverted on sheets of filter paper placed in one-half of a petri dish. The paper which projects past the margin of the beaker provides a constantly moist surface from which the insects may drink, while the pouring lip of the beaker admits the small amount of air necessary to keep the fountain in operation. Water is replenished as needed—usually every 2 or 3 days.

The sole food of the roaches is fox chow which is served in self-feeders made from discarded tin cans. A can from which the top and bottom have been cut is slotted along one rim and placed in a petri dish. When the feeder is assembled, the slots, which are rectangular and large enough to allow a roach to pass through, serve as doorways along the bottom rim giving access to the food which has been placed inside. Since the can is open at the top, the feeder can be replenished readily at any time. Like the watering fountains, they can be removed and easily dismantled for cleaning.

Roaches used in transmission experiments must be kept under near-normal conditions, yet isolated to prevent contamination. After a series of experiments, two acceptable methods have been developed. In each, the wings and legs of the cockroach are immobilized to prevent contamination of feces and food. Water and normal food are supplied at all times in ample quantities, and fecal pellets are collected on a moist agar surface which prevents drying.

One of the methods, adapted from Macfie (*1*), has been used very successfully with the American roach. The insect is imprisoned within a groove in a large cork by a technique which confines legs and wings but leaves the head and posterior abdomen free. The other method involves fixing the insect by its wings and legs at the end of a wooden applicator stick, and is more applicable to the smaller German roach, although it has also been used with Oriental cockroaches.

Using an American roach as an example, the procedure employed in the first method is as follows:

A normal uninjured insect is removed from the rearing cage and anesthetized by exposing it to carbon dioxide gas for 1 to 2 minutes; other methods for immobilizing the insect, such as light chloroform anesthesia or a 5- to 10-minute chilling period in a refrigerator, have been employed, but are not as convenient or as free of after effects as the carbon dioxide method. When the insect becomes quiet and can be handled readily, it is quickly inserted in the groove of the cork. The wings are spread out on top of the cork on each side of the groove and at right angles to the body, where they are taped in place much as one would fix the wings of a butterfly on a stretching board. The legs which are left hanging free in the groove are next confined to that space by wrapping adhesive tape around the entire cork. To support the abdomen and prevent the roach from raising its hind legs over the edge of the cork, it is usually necessary to place a small piece of tape across the top of the groove. Trussed in this manner, there is complete freedom for the head and the abdomen of the insect, and the legs can be moved in a restricted space. If the cork has been properly selected for size, the head will project at one edge and the abdomen at the other, and there is no opportunity for the roach to contaminate other parts of the body while it is receiving its infective feeding, or to pass on infection from the mouth parts to the feces other than through its digestive tract. For the wingless female of the Oriental cockroach, a narrow strip of adhesive is taped across the thorax and fastened to the cork on either side. After the roach has been mounted on the cork, it is placed on a triangle of wire screen which rests on a petri dish containing agar media. By means of a carpet tack, the cork is centered along the hypotenuse of the triangle in such a manner that fecal pellets produced by the insect will fall directly on the agar surface. Food and water containers are also placed on the triangle support in positions slightly ahead of and flanking the roach. Water is provided by a medicine dropper plugged at its tip with a small cotton wick. This device is inserted in a cork and placed within easy reach of the insect. Food consisting of ground fox chow is supplied in a small cup dispenser made by soldering a short length of copper tubing, closed at one end, to a wire nail handle (six penny finishing nail). When the nail is pushed into a cork, the cup, like the water tube, can easily be adjusted to a position which enables the experimental insect to take food as desired.

This method of holding, feeding, and watering was advantageous for a number of reasons: it prevented mold growth in the food by keeping it dry; roaches could take food and water as needed; it was not as time-consuming as hand feeding by pipette or syringe; the insect was not continually disturbed by handling; and it was held im-

mobile in a position which seemed more conducive to consumption of normal quantities of food and water. The large number of well-formed and normal fecal pellets produced by cockroaches held in this manner during experimental periods is additional evidence that this method is satisfactory.

In the case of the small German roach, it has been necessary to modify the method. A wooden applicator stick is inserted under the wings of the insect, and a narrow strip of adhesive tape is applied to keep them in place. Through the application of melted paraffin the legs are also fixed to the stick, which is then inserted in the hole of a cork fastened to a triangular support as in the method described for the larger roaches.

In the final experiments food and water were sterilized before feeding, and except on weekends, the dispensers were changed daily. In order that the experimental feedings might be completely accepted and regurgitation of an infective feeding avoided, it was necessary to establish the approximate optimum size of a cockroach meal. Such information is also useful in estimating the number of pathogens an insect can acquire during one feeding under natural conditions. Experimental observations of the fluid intake of an average-sized adult American roach indicated that 0.1 ml. was readily accepted, although on occasion more than twice this amount could be taken without regurgitation. A sterile 1 ml. tuberculin syringe with a 26- or 27-gage needle was used in giving an infective feeding to the American and Oriental roaches. They usually swallowed the measured amount of broth culture eagerly and without vomiting. Since the German roaches did not take the broth readily by this method, a modification was used with this species. Broth was either mixed with ground, sterile fox chow in a sterile feeding cup and offered to the roaches as solid food, or given overnight in place of drinking water.

A strain of *Salmonella oranienburg* recently isolated from a food-poisoning episode was selected as the test organism, for this species is very commonly involved in outbreaks of food infection in the United States and Canada. The outbreak from which the culture had been recovered was a typical example of "food poisoning" and involved 16 persons who had consumed chow mein with mushrooms. They were severely ill with headache, nausea, vomiting, diarrhea, abdominal cramps, and fever up to 103°. Many were confined to bed for several days. Upon laboratory examination, *S. oranienburg* organisms were isolated from the feces of 5 of the victims. The patient from whom the test strain was derived noted his first symptoms 16 hours after ingestion of the meal in question. This strain, isolated in September 1946, has undergone only three transfers since that time.

All experiments were carried out in a temperature-controlled bacteriological isolation room which was provided with an exhaust

fan and with spun glass filters at both the air intake and outlet. A 30-watt ultraviolet germicidal lamp was installed in order that any air-borne contamination originating within the room might be reduced. Fecal pellets passed by the experimental roaches were collected on a moist agar surface, as recommended by Wedberg and Clark (*2*). A petri dish containing about 15 ml. of agar was placed beneath each cockroach to catch the feces. Various types of agar were used in the petri dishes and included desoxycholate, desoxycholate citrate, *Salmonella shigella* and desoxycholate citrate lactose sucrose agars. These media were used because they tended to inhibit molds which often became troublesome during the humid summer months. With the exception of desoxycholate citrate lactose sucrose agar, which was obtained from the Baltimore Biological Laboratories, the media used in all experiments were Difco [1] products. All cultures referred to in this paper were incubated for a period of 18–24 hours (sometimes 48 hours over weekends) at 37° C.

Pellets produced by the roaches were removed from the agar surface with a sterile loop and emulsified in 5 ml. of tetrathionate broth. Under the conditions of our experiment and with the roaches which were being used, tetrathionate broth appeared to be superior to other enrichment media. After incubation of the tetrathionate, plates of *Salmonella shigella* and desoxycholate agar were streaked successively without reloading the loop. Sometimes desoxycholate citrate or desoxycholate citrate lactose sucrose agar were substituted for the *Salmonella shigella* agar. Colorless colonies were transplanted to Kligler's iron agar slants. If the growth in this medium showed acid and gas in the butt of the tube, no change on the slant, and hydrogen sulphide production, the organism was typed with 04 *Salmonella* typing serum, using the plate technique.

In each group of test roaches the final positive result was checked by inoculation into portions of purple broth base containing lactose, maltose, dextrose, sacchrose, xylose, raffinose, arabinose, mannitol, dulcitol, sorbitol, inositol, dextrin, rhamnose, glycerol, and inulin. Simultaneously, inoculations were made into litmus milk, Simmon's citrate agar, gelatin, d-tartrate agar, nitrate agar, peptone water and MR–VP medium. If reactions were typical the cultures were typed completely for O and H antigens, using the technique outlined by Edwards and Bruner (*3*).

Experimental Results

When given a single dose consisting of 0.1 to 0.2 ml. of a *S. oranienburg* broth culture containing approximately 1,000 million organisms, the American roach has produced infected feces up to and including 10 days. Based on the passage of infected feces, one roach was

[1] Use of trade names does not represent an endorsement of the products by the Public Health Service.

infected for 10 days, two specimens for 9–10 days, three for 7 days, two for 4–5 days, and two tested for the first time on the 3d day were not infected. Usually roaches produced infected feces within the first 24 hours. A daily average of 4.3 fecal pellets per roach was passed by the infected insects during the period of observation, and 8 was the highest number of infected pellets obtained from a single roach in one day. Fecal pellets were usually 100 percent positive only during the first few days following an infective feeding. In one instance, however, all of 5 pellets passed by a roach on the 7th day were infected.

When the same organism was fed to German roaches, infection in one instance persisted for at least 12 days; one insect passed infected feces until the 12–15th day, one until the 9–11th day, one for 6 days, one for 3–4 days, and two apparently produced no infected feces, although one of these infected its water tube on the 2d day.

In feeding experiments where the Oriental cockroach was used, viable organisms of *S. oranienburg* were isolated from feces produced 20 days after an infective feeding. This species, like the others, usually passed infected pellets within 24 hours after the infective feeding. Thereafter, positive pellets were sometimes produced somewhat irregularly. The organisms were recovered from one roach on the 20th day and from one roach on each of the following days: 11–12th, the 9th, the 8th, and the 6th. Another roach found dead on the 3d day produced no feces, but it did infect its water tube during the 2d to 3d day. The roach which had passed positive feces on the 20th day was still infected when sacrificed at the end of 42 days. It would appear, therefore, that an insect can remain infected even after the period when positive feces are passed regularly.

Experiments with the brown-banded roach, *Supella supellectilium*, are scheduled. Earlier plans to test this species were changed when an epizootic of unknown etiology destroyed the stock colony of insects.

In addition to the observations made on the ability of roaches to transmit or carry infection, there have been supplementary experiments to obtain information on other points which may be of importance in considering the roach as a disease vector. For example, before the results of transmission experiments are evaluated, it is of interest to know the time required for passage of relatively inert materials and substances other than bacteria through the digestive tract of an insect. Carmine and acriflavine, a fluorescent material, were used for this purpose in tests with the American roach. Following a single feeding, carmine was retained for at least 6 days, as indicated by the color of the feces. Fluorescent feces were recovered for the same period after feeding acriflavine.

The survival time of *Salmonella* organisms in fecal pellets passed by experimental roaches is also of interest. Tests made with

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American cockroach fecal pellets held at room temperature and above saturated salt solutions to produce known humidities show that *S. oranienburg* will survive in such excrement for 199 days at a relative humidity of 31 percent or slightly less, and for 85 days at 52–56 percent. In the control where humidity was not held constant, the survival was also 85 days.

Since the cockroach frequently wanders over dishes, kitchen utensils, and through dried foods in a cupboard, it is conceivable that the surfaces represented may become contaminated with the vomitus or feces of the insect. An experiment was therefore initiated to test the survival of *Salmonella* organisms on dishes and dried foods. Glass slides were used to simulate the surface of dishes, while soda crackers and corn flakes were used as representatives of the dried foods and cereals which are commonly exposed to this type of contamination. A broth culture of the organism was applied to the test material in small drops which soon dried. During the experiment pieces of glass or of food were removed from their sterile containers at short intervals and cultured in accordance with the usual method. All experiments were carried on at room temperature in order that conditions might closely resemble the actual field situation. Results obtained show that survival of our test strain of *S. oranienburg* on glass surfaces is 34 days, on crackers at least 88 days, and on corn flakes at least 62 days.

In addition to contamination by feces and definite vomitus, there is a possibility that roaches may infect food by mere mouth contact when feeding, or by direct body contact. These methods for the spread of pathogens become particularly important if the infective organisms survive long on mouth parts or body surfaces. The following observations have been made relative to this problem. In one instance where American roaches were being tested, food was definitely contaminated for a day after an infective feeding. Otherwise results have usually been negative when the standard dry food is used. Drinking water and liquids are in another category however. The wick of the water tube was frequently contaminated, especially on the 1st and 2d days after an infective feeding. On two occasions contaminated wicks have been found on the 3d day, and once during a 4–8-day period. It is apparent, therefore, that the roach can contaminate liquids by mouth contact or regurgitation for at least 4 days. Another experiment has demonstrated that a wick once contaminated may remain positive for at least 15 days.

To test the length of survival of *S. oranienburg* on the exterior of a roach, a small colony of this organism was spread on the pronotum of each of 12 American roaches. The insects were then placed in small individual glass cages and the pronotal areas were swabbed at intervals with moist, sterile, cotton-tipped applicator sticks which

were placed in tetrathionate broth and subjected to the usual cultural procedure. By this method it has been determined that the organism can remain viable on the relatively smooth pronotal surface of a roach as long as 78 days.

Discussion

The roach, because of its dorsoventrally flattened morphology, small size, and general habits, is particularly suited to the spread of pathogens in areas not readily accessible to rats and mice. It can easily squeeze into rodent-proof rooms, relatively tight food bins and cupboards, or almost any food storage compartment. For example, large numbers have been observed in a tightly-closed ice box which was temporarily out of ice; in this instance entry was probably gained as a result of a loose door gasket or a faulty drain. In its eagerness for food the roach will consume almost anything it can chew, and frequently its voracious appetite leads to an overindulgence which can only be relieved by prompt regurgitation. It is this habit, associated with its proneness to deposit its excreta almost anywhere, which makes the cockroach a favored suspect as a food contaminator. Under optimum conditions a large number of fecal pellets are produced (frequently as many as 12 in a 24-hour period), and these are scattered at random during the various excursions of the insect. On casual inspection the pellets of the American roach are large enough to be readily mistaken for mouse droppings. It is therefore possible that some of the reports of soiled food products which have formerly been ascribed to rodents are actually referable to roaches.

It is apparent from the experimental evidence reported here that the roach, which is so admirably adapted to penetrate into various nooks and crannies of food preparation establishments, is also able to carry viable pathogens for long periods of time, and that by regurgitation or mouth contact, as well as through body contact and feces, it can contaminate foods directly or through kitchen utensils and dishes. Another factor of importance is the long survival of pathogens observed in cockroach feces under experimental conditions closely resembling those found in normal kitchens. The experiments show that *S. oranienburg* organisms remain viable for 85 to 199 days in fecal pellets. Such evidence greatly extends the sphere of influence of an infected roach, for it is evident that, even after its death, contamination of human food can occur through contact with feces, and cockroaches may actually be involved in food poisoning outbreaks where as a result of an effective extermination campaign there would ordinarily be no reason to suspect these insects as vectors.

Although it has been demonstrated that laboratory-reared cockroaches may harbor food poisoning and food infection organisms for some time, and that certain enteric organisms can survive for

extended periods in their feces, on their bodies, and on glass surfaces or dry foods which are accessible to the roach, the evidence against the insect is not yet complete. Until the actual incidence of infection in wild cockroaches is determined on the basis of a large number of specimens and for a number of geographical areas, and until this information is integrated with definite epidemiological evidence, the roach can still be regarded only with grave suspicion.

Summary

In an effort to extend the available knowledge concerning insect-borne diseases and more specifically to attempt an evaluation of the role of cockroaches as carriers of food poisoning or food infection organisms, a series of transmission experiments was undertaken with *S. oranienburg* as the pathogen and three common species of household roaches as potential carriers.

Survival of the pathogen in the insects was determined by an examination of the feces. It was found that under the conditions of these experiments the test strain of *Salmonella* could survive for 10 days in the American roach, for 12 days in the German roach, and for 20 days in the Oriental cockroach. A post mortem examination of the Oriental roach showed that it was positive 42 days after an infective feeding, even though it had passed contaminated feces only during the first 20 days.

Supplementary experiments with the American roach have resulted in the accumulation of other information which is pertinent to the problem. Carmine and acriflavine were eliminated from the digestive tract of roaches approximately 6 days after feeding, indicating roughly the time required for mechanical clearing of the digestive system. Fecal pellets from an experimental roach have remained infective for a period of 199 days at room temperature, while organisms from a culture have survived on glass surfaces for 34 days, on ordinary soda crackers for 88 days, and on corn flakes for 62 days. On the relatively smooth pronotum of the roach, the organisms have been viable for 78 days. Through mouth contact, watering tubes of the roach have been contaminated at least 4 days after the infective feeding, and organisms have survived on the cotton wick of the watering device for 15 days.

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Q Fever Studies in Southern California

IX. Isolation of Q Fever Organisms From Parturient Placentas of Naturally Infected Dairy Cows.

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Epidemiological studies of Q fever in southern California among dairy and other livestock workers and residents near dairies have shown that factors other than the personal or household use of raw milk are required to explain Q fever infections (*1*, *2*). The general hypothesis most consistent with present knowledge postulates that large numbers of *Coxiella burnetii* must be contributed to dairy and other livestock environments from known or unknown reservoirs and that some of these organisms gain entry to exposed susceptible humans and animals through contact or inhalation.

Early studies demonstrated *C. burnetii* in the milk of many cows (*3*), spinose ear ticks, *Otobius megnini*, (*4*), and in the udder and adjacent lymph glands of an infected cow at autopsy (*5*). Other studies (*6*) indicated that the urine and feces of infected cows were unlikely sources of contamination of the environment with *C. burnetii*.

The demonstration of *C. burnetii* in parturient placentas of infected dairy cows is reported here, and the significance of this finding is discussed briefly.

Procedure

Since this preliminary study was concerned with the initial demonstration of *C. burnetii* in bovine placental tissues, only heifers and cows which on the basis of previous serological tests (*7*, *8*) were believed to be infected and in which parturition seemed imminent were selected for study. Three native [1] Los Angeles County dairy herds, containing a total of 2,300 cows, with infection rates varying from 16 percent to 30 percent provided the source of specimens. Although herd practices at these dairies were generally similar, basic differences did exist.

Placentas were collected from the uterus and vagina, from the floor of calving stalls, or from the ground of corrals following normal parturition. Clean portions were excised at random, placed in sterile jars, and kept frozen until tested in the guinea pig test (*8*). At that time specimens were thawed, and 1- or 2-gram portions were removed, ground in a mortar, and suspended in sufficient saline to approximate

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[1] For the purpose of the southern California studies, a "native dairy" is one which raises its own replacements for the milking herd.

a 1:10 suspension. Each of two or three 600- to 800-gram guinea pigs were injected intraperitoneally with 3-ml. amounts of these suspensions. Normal uninjected guinea pigs were distributed among the test animals in a ratio of 1 control to 4 injected animals. All guinea pigs were bled 30 days later and the serums tested for complement-fixing antibodies against *C. burnetii*. A 3-plus reaction at 1:32 or greater dilution by the serums of one or more guinea pigs was accepted as evidence of infection and of the presence of *C. burnetii* in the specimens being tested.

A few attempts were made to establish placental strains by passage of guinea pig blood to other guinea pigs and to yolk sacs of fertile hen eggs. Titrations of placental materials were carried out to ascertain the amount of infection present in some placental tissues found positive by the guinea pig test.

Results

Placental tissues of 33 serologically positive [2] cows were tested and 13 (39 percent) found to contain *C. burnetii*. Ten were positive on initial guinea pig test, while 3 others were found positive when second portions of some placentas were tested (table 1). Similar attempts to demonstrate *C. burnetii* in placentas of 4 serologically negative cows were unsuccessful.

The presence of *C. burnetii* in the tissues was further demonstrated by production of fever and of Q fever antibodies in three groups of second-passage guinea pigs; by the cultivation of strains of *C. burnetii* from the blood of such guinea pigs in the yolk sacs of fertile hen eggs; and by the demonstration of immunity of guinea pigs recovered from Q fever, Dyer strain, (table 1).

Titrations indicated that some placental tissues diluted as high as 1 to 100,000,000 were infectious for guinea pigs (table 2).

Antibody against Q fever was found in the serums of 70 of 173 (40 percent) guinea pigs which were injected with placental suspensions, while 1 of 64 (1.5 percent) uninjected control guinea pigs was found to have developed similar antibody. It is felt that spontaneous infection in the guinea pig colony during this study was not frequent enough to interfere with interpretation of the guinea pig test results.

Discussion

The ease with which *C. burnetii* was demonstrated in small portions of placental tissues of 13 of 33 positive cows, as well as the results of the titration of portions of those tissues, makes it evident that placentas of some infected cows represent rich sources of *C. burnetii*.

[2] Serologically positive cows included those showing complement-fixing antibody (3-plus at 1:4 or greater) in the serum prior to, at, or several months subsequent to parturition, or showing similar antibodies in colostrum taken at parturition.

The frequency of contamination of dairy environments would appear to depend upon the frequency of parturition and the number of infected cows in the dairy herds. In the Los Angeles County milkshed, great numbers of infected cows are concentrated on many dairies

Table 1. *Demonstration of* Coxiella burnetii *in parturient placentas of serologically positive*[1] *Los Angeles County dairy cows*

Dairy	Cow No.	Age	Breed	Number of parturitions	Results of guinea pig tests		Immunity test in recovered (Dyer strain) guinea pigs	Cultured in yolk sacs
					First	Second		
A	851	2.5	------	1	+	------	------	------
	1659	2.5	G	1	+	+	------	------
	3759	2.5	H	1	+	+	+	+
	4019	3	H	1	+	------	------	------
	1799	3.5	H	1	+	------	------	------
B	377	3.5	H	2	+	------	------	------
	379	3.5	H	2	+	+	+	+
	165	5	H	3	0	------	------	------
	178	5	G	3	0	------	------	------
	87	6	H	4	0	------	------	------
	1221	6	H	4	+	------	------	------
	1569	6	H	4	0	0	------	------
	947	6	G	5	0	+	------	------
	569	9	G	7	0	------	------	------
	436	9	H	7	0	------	------	------
	1084	9	H	7	0	------	------	------
	1776	9	G	7	0	------	------	------
C	406	4	G	2	0	------	------	------
	2095	4	G	2	0	------	------	------
	2476	3	H	2	0	------	------	------
	1473	6	H	3	+	------	------	------
	1490	6	G	3	0	------	------	------
	1590	5	H	3	0	+	------	------
	1609	6	H	3	0	------	------	------
	1623	6	H	3	0	------	------	------
	1865	6	H	3	0	+	------	------
	1950	5	H	3	+	+	+	+
	1998	5	H	3	0	------	------	------
	1337	7	G	4	0	------	------	------
	244	8	G	6	0	------	------	------
	287	10	G	6	0	------	------	------
	316	9	G	7	0	------	------	------
	3825	11	H	8	0	------	------	------

[1] Having serum or colostrum antibodies of 3-plus at 1:4 or greater in the complement fixation test.
+Positive in guinea pig test, immunity demonstrated, or strain successfully cultured.
0 Negative in guinea pig test.
Blank=not tested.

Table 2. *Titration of placental tissues in the guinea pig test for the presence of* Coxiella burnetii

Cow number	Dilution of placental tissues in saline									
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
A 3759	------	++±	+	++0	++±	+0	+±0	+±0	00	00
A 4019	------	++	------	+0	000	000	000	------	------	------
B 121	------	+++	------	00	0	000	------	------	------	------
B 377	------	+++	------	+++	+±	+++	+0	------	------	------
B 379	------	++	++	+++	++	+	+00	+00	000	000

+ Guinea pig serum positive at 3-plus at 1:32 or greater in the complement fixation test 30 days after infection.
± Guinea pig serum showing 3-plus at 1:16 but not at 1:32 in the complement fixation test.
0 Guinea pig serum negative at 1:16 in complement fixation test.

(*1*), parturitions are frequent, and placentas are allowed to remain on the ground of open corrals. The dry climate in this area, together with the known ability of *C. burnetii* to survive desiccation, suggests that infected placentas may provide sporadic but excellent opportunities for the dissemination of viable organisms to the environments of both humans and animals. Furthermore, contamination of the hides of newborn calves by heavily infected placental membranes may occur and could be an important factor in the genesis of Q fever infection among abattoir and hide-plant workers.

Workers in Switzerland have recently reported that aborted placentas from goats produced serum antibodies against *C. burnetii* when injected in four guinea pigs (*9*). Strains were not established in the study. They presented the hypothesis that *C. burnetii* was responsible for an epidemic of abortions in goats.

C. burnetii infections of bovines under the conditions encountered in Los Angeles County dairy herds have not been associated with any apparent increased rates of abortion.

It can be seen in table 1 that *C. burnetii* was demonstrated more frequently during first parturitions than in later ones. The significance of this observation is undetermined because of the small and unequal sampling in the different herds and because of several attendant herd practices irrelevant to the main purpose of this report.

Summary

A number of parturient placentas of infected dairy cows have been shown to contain *C. burnetii*, sometimes in large quantities. This fact may be important in the spread of Q fever to susceptible persons and animals exposed to dairy and other livestock environments.

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Induced Oviposition of *Simulium* Flies by Exposure to CO_2

By Herbert T. Dalmat*

In an experiment designed to determine without doubt the vectors of onchocerciasis in Guatemala and to study the development of *Onchocerca volvulus* in the anthropophilic species of blackflies (*Simulium* spp.), it was considered necessary to establish a laboratory colony of these flies. This project was undertaken at the field laboratory in San Pedro Yepocapa, Chimaltenango, Guatemala.

During the last half of 1948 a large outdoor screen cage, 8′ x 6½′ x 5′, was constructed in the laboratory patio over a cement channel through which a stream was diverted. Some of the plants found in and about the natural haunts of the flies were planted within the cage. These were: banana, coffee, *Grevillea robusta* Cunn., *Ricinus communis* L., and *Polymnia maculata* Cav. Two herbaceous plants on which the flies commonly oviposit in this region, *Renealmia aromatica* (Aubl.) Griseb. and *Tradescantia commelinoides* R. & S., were planted along the borders of the stream in such a manner that the leaves and stems floated on the water with the current. Temperature and humidity fell well within the natural range of the region. The mottled shade and sun, usually found on the coffee plantations, was approximated by attaching lengths of rather sheer black cloth to the outside of the cage where the sun hit directly. This reduced light intensity, and the movement of the cloth in the air currents afforded additional aeration of the interior of the cage.

Many combinations of flies were introduced into the cage: wild-caught females, wild-caught females with laboratory-raised males, laboratory-raised females and males. Pupae were also introduced into the water channel so that adults could emerge naturally within the cage. The flies were permitted to feed on human subjects, defibrinated human blood, blood plasma, plant juices, and honey and sugar solutions absorbed by cotton. Although small numbers of flies lived up to 18 days in captivity, not one fly oviposited.

During the latter part of January 1950, a system was initiated whereby flies were treated with carbon dioxide gas before being re-

*From the Microbiological Institute of the National Institutes of Health, Public Health Service. This report is part of a study jointly supported by the Laboratory of Tropical Diseases of the Microbiological Institute and the Pan American Sanitary Bureau in cooperation with the Dirección General de Sanidad Pública of the Republic of Guatemala. The project was aided by a research grant from the National Institutes of Health, Bethesda, Md.

leased in the cage.[2] The flies were first placed in a museum jar into which carbon dioxide gas was introduced through a rubber tube extending from a standard gas cylinder. An oxygen manometer valve was used to control the quantity of carbon dioxide passing through the tubing to the fly chamber.

Since the actual volume of gas necessary to immobilize the flies was not measurable with the equipment used, the end-point of treatment was arrived at by observation of the fly activity. The flies were at first stimulated to greater activity, and then they would topple over as if dead. At the latter point, the treatment was halted and the jar left open until the flies revived. The actual treatment lasted for less than 20 seconds; the flies usually revived in about 3 to 4 minutes.

It appears that the gas treatment has an immediate effect upon the behavior of the flies. Upon returning to consciousness, a few flies were observed mating, and numerous females assumed a position as though they were biting. It was first believed that the position of the females, with the mouth-parts directed perpendicularly to the jar, was merely an attempt to establish their equilibrium. However, when the flies were released into the large outdoor cage, a high percentage of the flies attacked the human subject and took blood voraciously. Within 4 to 6 hours, a number of the flies that had fed also oviposited.

To date, 19 trials have been made. Of the 2,532 flies treated, 982 were *S.* (*S.*) *ochraceum* Walker; 1,112 were *S.* (*S.*) *metallicum* Bellardi; and 438 were *S.* (*Lanea*) *callidum* D. & S. Of this number, 84 took blood meals, and 21 *metallicum* and 11 *callidum* oviposited. Some of the females deposited up to a thousand eggs, but none of the eggs have developed to form larvae. This may be partly due to the frequent interruption in the flow of water occasioned by necessary repair work on the water system. It may also indicate that the eggs were sterile or that the gas adversely affected them. In the future, varying dosages of carbon dioxide will be tried to preclude possible deleterious effects of overtreatment.

Although the eggs deposited by flies treated with carbon dioxide have not developed to date, the results so far obtained are encouraging. Experimental induction of egg-laying by captive *Simulium* flies has never before been reported; thus, these results point a way toward further possible experimentation along the same line with this group of flies and with other insects that have resisted colonization.

[2] W. H. W. Komp, Laboratory of Tropical Diseases, NIH, suggested the possible use of this technique, which has previously been tried with mosquitoes (unpublished) by Dale W. Jenkins, Entomology Section, Army Chemical Center, Maryland.

New *Salmonella* Type: *Salmonella allandale*

By P. R. Edwards and G. J. Hermann*

Salmonella allandale was isolated by Mrs. Mildred M. Galton from the feces of a normal foodhandler and forwarded to the writers for identification. The organism was a motile rod which possessed the usual characteristics of the genus *Salmonella*. Upon biochemical examination the bacterium produced hydrogen sulfide and utilized d-tartrate and citrate, but failed to form indol or to liquefy gelatin. Glucose, arabinose, xylose, maltose, trehalose, mannitol, dulcitol, sorbitol, and inositol were fermented within 24 hours with the production of acid and gas. Acid was produced from cellobiose after 8 days' incubation. Rhamnose, lactose, sucrose, salicin, and adonitol were not fermented after 30 days' incubation.

Serological examination revealed that the organism was agglutinated strongly by *Salmonella riogrande* O serum (XL) and to a lesser extent by O sera derived from *Salmonella paratyphi A* and *Salmonella senftenberg*. It was shown that agglutination in the latter sera was due to the presence of antigen I in *S. allandale*, and that, as in many other types, form variation affecting antigen I occurred in the culture. *S. allandale* did not possess the whole antigenic complex represented by the symbol XL in *S. riogrande*, although it was agglutinated to the titer of *S. riogrande* serum and reduced the titer of the serum from 1:1,000 to 1:200 in absorption tests. Likewise, *S. riogrande* failed to absorb completely the agglutinins from O serum prepared from *S. allandale*, although it reduced the titer of the serum from 1:8,000 to 1:200. For diagnostic purposes the O antigens of *S. allandale* may be represented by the symbols I,XL.

The H antigens of *S. allandale* were diphasic. Phase 1 was agglutinated to the titer of *Salmonella thompson* phase 1 serum (k) and removed all agglutinins from the serum in absorption tests. Phase 2 was agglutinated by sera derived from the nonspecific phases of the genus. When tested in absorbed sera for factors 2, 3, 5, 6, and 7, it was agglutinated only in serum for factor 6. In absorption tests, *S. allandale* reduced the titer of *Salmonella anatum* phase 2 serum (1, 6) from 1:10,000 to 1:100. The antigenic formula of *S. allandale* may be expressed as I,XL:k–1,6.

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INCIDENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED APRIL 1, 1950

Influenza

Reports for the week ending April 1 indicate that the moderate influenza epidemic experienced in many areas during the past 7 weeks has passed its peak. There were 19,951 cases of influenza reported for the latest week compared with 26,505 cases for the preceding week. These figures exclude Kentucky for which data are not yet available.

The cumulative total of reported influenza cases for the first 13 weeks of this year is 190,910, which may be compared with the corresponding total of 55,545 cases for the same period in 1949 and 206,585 for 1947, the highest during the last 5 years. The corresponding 5-year (1945–49) median is 121,275.

The following States reported relatively large increases in influenza cases for the latest week over the preceding week: Ohio (3 to 41), Kansas (51 to 184), Arkansas (2,241 to 3,013), Arizona (172 to 302), and Washington (18 to 253).

Substantial decreases in reported cases are shown for the following States: Maine (1,058 to 124), New York (86 to 42), New Jersey (49 to 21), Iowa (353 to 101), Missouri (184 to 108), Maryland (116 to 67), Virginia (6,109 to 3,688), West Virginia (2,275 to 1,669), Georgia (371 to 173), Tennessee (722 to 485), Oklahoma (1,022 to 696), Texas (8,750 to 7,033), and Montana (625 to 60). In summary, 10 States reported increases, 30 States (including the District of Columbia) reported decreases, and 9 States reported no change.

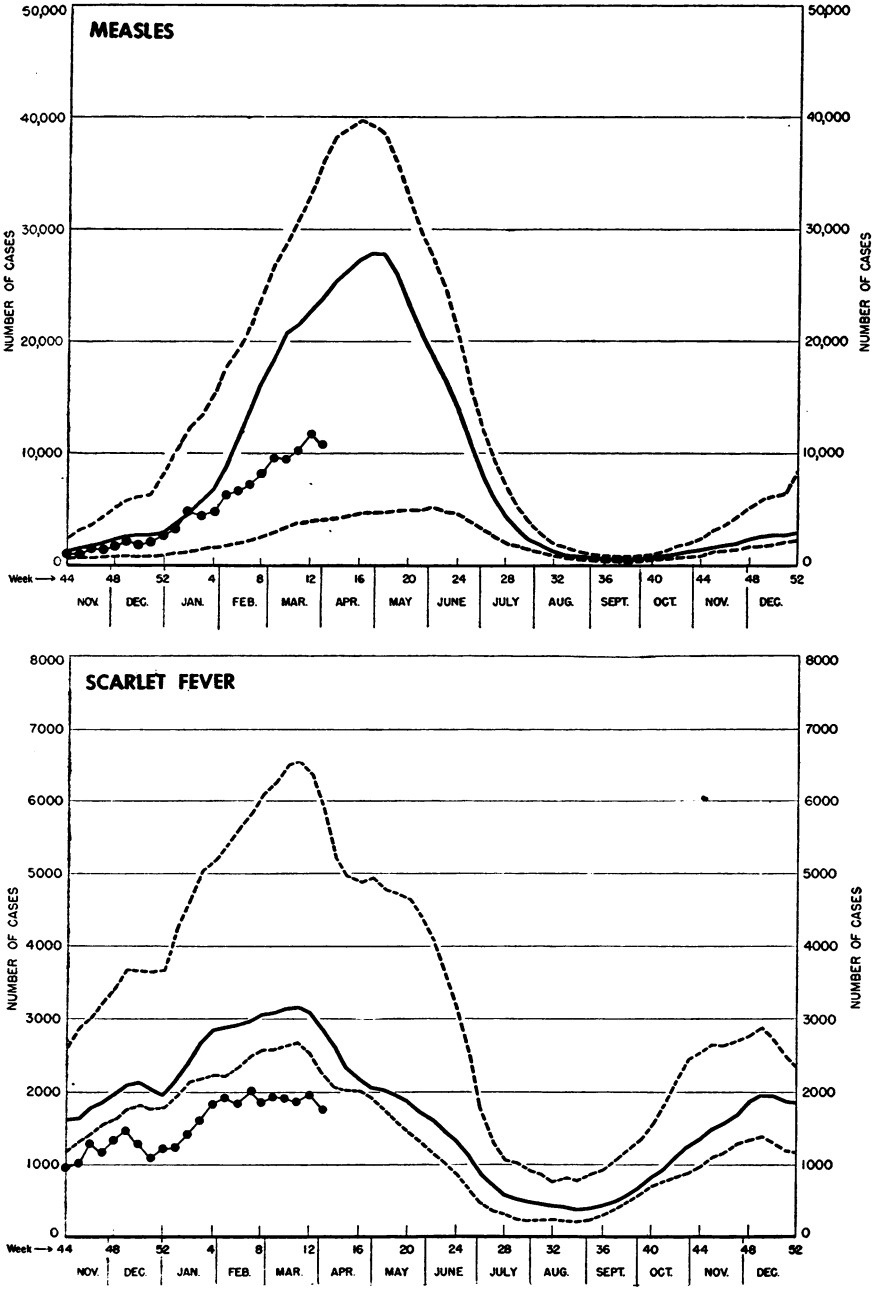
Other notifiable diseases

Increases in reported cases over the preceding week are reported for the following diseases: Anthrax (0 to 2), meningococcal meningitis (116 to 118), acute poliomyelitis (62 to 64), Rocky Mountain spotted fever (0 to 1), and smallpox (1 to 4). The 4 cases of smallpox were reported in Oklahoma.

Decreases compared with the preceding week are indicated for the following diseases: Diphtheria (114 to 110), acute infectious encephalitis (17 to 13), measles (11,940 to 10,964), pneumonia (3,147 to 3,089), scarlet fever (1,993 to 1,752), tularemia (19 to 15), typhoid and paratyphoid fever (48 to 44), and whooping cough (2,901 to 2,810).

Communicable Disease Charts

All reporting States, November 1949 through April 1, 1950



The upper and lower broken lines represent the highest and lowest figures recorded for the corresponding weeks in the 5 preceding years. The solid line is a median figure for the 5 preceding years. All three lines have been smoothed by a 3-week moving average. The dots represent numbers of cases reported for the weeks, 1949–50.

Telegraphic case reports from State health officers for the week ended April 1, 1950

(Leaders indicate that no cases were reported)

Division and State	Diphtheria	Encephalitis, infectious	Influenza	Measles	Meningitis, meningococcal	Pneumonia	Poliomyelitis	Rocky Mountain Spotted fever	Scarlet fever	Smallpox	Tularemia	Typhoid and paratyphoid fever [1]	Whooping cough	Rabies in animals
NEW ENGLAND														
Maine			124	25	1	5			16				47	
New Hampshire			2	2		3			4					
Vermont				7					2				44	
Massachusetts	2			508	3		1		130			2	169	
Rhode Island			1	14		8			8					
Connecticut			4	60	1	67			26				124	
MIDDLE ATLANTIC														
New York	6	4	[2] 42	1,243	7	425	1		[2] 181			2	176	16
New Jersey			21	1,082	8	148			79			1	165	
Pennsylvania	4			523	16	116			145			3	154	
EAST NORTH CENTRAL														
Ohio	16		41	414	6	109	2		223			1	269	12
Indiana	3		8	351	2	43	1		74		1	1	47	[4] 24
Illinois	2	1	20	436	8	233	3		86				113	3
Michigan		1	7	1,439		18	3		106				155	4
Wisconsin	1		225	449	2	33			75			1	140	
WEST NORTH CENTRAL														
Minnesota			2	60	3	10			40				21	
Iowa	1		101	546		1	1		4				22	11
Missouri			108	42	5	26	1		33			1	29	
North Dakota		3	65	2	2	85			5				3	
South Dakota				7	1	1			5				4	
Nebraska			8	167					5				1	
Kansas	3		184	24		31	1	1	23				20	
SOUTH ATLANTIC														
Delaware				4					3				4	
Maryland	5		67	27	3	57			28			4	51	
District of Columbia			1	79		18			3				4	
Virginia			3,688	79	4	134			11				49	8
West Virginia	3		1,669	219	5	39	2		12			1	45	16
North Carolina	8			175	5		3		24		1	1	71	
South Carolina	2		286	64	2	14			5			1	10	10
Georgia		1	173	245	2	177	1		4		3	2	17	7
Florida	2		20	126		9	2		1				3	

EAST SOUTH CENTRAL														
Kentucky	3			410	3	23	2		33				30	12
Tennessee	5	1	485	119	2		1		15		2		39	3
Alabama	4		686	84	5	96	2		20		3	1	33	4
Mississippi	1		142	233	1	72	1		7			1	1	
WEST SOUTH CENTRAL														
Arkansas	3		3,013	45	2	117	3		6		3	3	33	5
Louisiana	3		8	10		14	4		3		1	7	5	
Oklahoma			696	11		66			14	4		1	29	4
Texas	17	1	7,033	441	9	733	16		39		1	4	249	23
MOUNTAIN														
Montana			60	104			1		9				3	
Idaho			257	11			2		3				32	
Wyoming				13					1				2	
Colorado	6	1	89	62		19			20			1	35	6
New Mexico	2		2	63		23			6			1	16	
Arizona			302	153	1	39	4		11				85	
Utah			3	217		2			6				17	
Nevada			4	53		2			1				1	
PACIFIC														
Washington			253	93		3			61				31	
Oregon			29	10	1	27			12				54	
California	8		22	413	8	43	6		124			4	158	
Total	110	13	19,951	10,964	118	3,089	64	1	1,752	4	15	44	2,810	168
Median, 1945–49	250	9	[5] 3,067	23,784	82		25		2,892	12	16	48	1,881	2,002
Year to date, 13 weeks	2,010	[6] 167	[5] 190,910	97,105	1,231	33,798	1,263	12	23,325	18	[7] 287	602	33,845	
Median, 1945–49	3,760	100	[5] 121,308	199,206	1,119		518	6	36,972	61	267	573	28,738	
Seasonal low week ends	(27th) July 9		(30th) July 30	(35th) Sept. 3	(37th) Sept. 17		(11th) Mar. 18		(32d) Aug. 13	(35th) Sept. 3		(11th) Mar. 18	(39th) Oct. 1	
Since seasonal low week	6,281		[5] 221,417	116,235	2,144		126		39,764	38		92	55,381	
Median, 1944–45 to 1948–49	11,326		[5] 164,813	234,152	2,089		55		62,555	115		94	53,688	

[1] Including cases reported as salmonellosis.
[2] New York city only.
[3] Including cases reported as streptococcal sore throat.
[4] Report for 2 weeks.
[5] Excluding Kentucky.
[6] Addition: New Jersey, week ended Mar. 11, 4 cases.
[7] Deduction: Arkansas, week ended Mar. 11, 1 case.

Anthrax: Pennsylvania and Delaware 1 case each.
Alaska: Influenza 9, scarlet fever 3.
Hawaii: Influenza 1, pneumonia 1.

FOREIGN REPORTS

CANADA

Provinces—Notifiable diseases—Week ended March 18, 1950.—Cases of certain notifiable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox	1		28	25	380	244	44	23	24	119	888
Diphtheria					6	2					8
Dysentery, bacillary					1	6	2			3	12
German measles			114		14	885		38	207	232	1,490
Influenza			20			658	5			2	685
Measles			7	347	672	688	9	33	35	89	1,880
Meningitis, meningococcal						1	1				2
Mumps			133		224	588	11	52	150	322	1,480
Poliomyelitis								1			1
Scarlet fever	2		6	2	82	35	23	3	70	8	231
Tuberculosis (all forms)	9		11	14	113	35	18	11	20	41	272
Typhoid and paratyphoid fever					9	2		1		1	13
Undulant fever					4	1	1				6
Venereal diseases:											
Gonorrhea	4		10	18	71	51	20	13	18	(1)	205
Syphilis	2		9	4	78	30	3	2	6	(1)	134
Whooping cough	1		33		109	45	7			48	243

[1] Report not received.

JAMAICA

Notifiable diseases—4 weeks ended January 28, 1950, and 4 weeks ended February 25, 1950.—Cases of certain notifiable diseases were reported in Jamaica as follows:

Disease	4 weeks ended Jan. 28, 1950	4 weeks ended Feb. 25, 1950		Disease	4 weeks ended Jan. 28, 1950	4 weeks ended Feb. 25, 1950	
	Jamaica [1]	Kingston	Other localities		Jamaica [1]	Kingston	Other localities
Chickenpox	24	9	27	Plague	1		
Diphtheria	7		3	Puerperal sepsis	1		2
Dysentery, unspecified	5	2		Scarlet fever	3		
Erysipelas	1			Tuberculosis, pulmonary	68	29	57
Leprosy		1	1	Typhoid fever	53	4	51
Meningitis, meningococcal	3		1	Typhus fever, murine	2	1	

[1] Figures not received separately for Kingston and other localities.

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—The following reports include only items of unusual incidence or special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently.

A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

Burma.—During the week ended March 18, 1950, 1 case of cholera was reported in Rangoon.

India.—According to information dated March 29, 1950, a marked increase in the incidence of cholera has been reported in Calcutta. For the week ended March 18, 253 cases, with 105 deaths, were reported in that city (199 cases, 62 deaths during the previous week). During the week ended March 25, the number of cases reported was 312, with 114 deaths.

Pakistan.—According to information dated April 4, 1950, an outbreak of cholera was being reported currently in Dacca. Fifty cases, with a death rate of 25 percent, were stated to have occurred in that city within the week preceding the above date. An average of 6 cases daily, with the incidence increasing, was reported. Cholera has also been reported in Chittagong as follows: Week ended March 18, 1950, 6 cases (all fatal); week ended March 25, 11 cases.

Plague

China.—During the period January 13–February 24, 1950, 15 cases of plague, with 9 deaths, were reported in Kwangtung Province.

Ecuador.—During the month of February 1950, 3 cases of plague, one fatal, were reported in Loja Province.

Smallpox

Burma.—During the week ended March 18, 1950, 106 cases of smallpox were reported in Rangoon, and 47 cases in Bassein.

Chile.—Information dated April 3, 1950, states that an epidemic of smallpox has been reported in Chile, apparently centered in the South Central regions, especially the Talca and Concepcion areas. One hundred thirty-three known cases had been reported as of April 2.

China.—During the week ended March 25, 1950, 19 cases of smallpox were reported in Shanghai.

French West Africa.—During the period March 1–10, 1950, 131 cases of smallpox were reported in Ivory Coast.

India.—During the week ended March 25, 1950, 319 cases of smallpox, with 252 deaths, were reported in Calcutta.

Typhus Fever

Japan.—During the week ended March 11, 1950, 21 cases of typhus fever were reported in Yokohama, and 7 cases in Tokyo.

DEATHS DURING WEEK ENDED APR. 1, 1950

	Week ended Apr. 1, 1950	Corresponding week, 1949
Data for 94 large cities of the United States:		
Total deaths	10,328	9,819
Median for 3 prior years	9,819	
Total deaths, first 13 weeks of year	129,286	128,641
Deaths under 1 year of age	648	654
Median for 3 prior years	682	
Deaths under 1 year of age, first 13 weeks of year	8,199	8,696
Data from industrial insurance companies:		
Policies in force	69,835,573	70,499,503
Number of death claims	14,552	13,318
Death claims per 1,000 policies in force, annual rate	10.9	9.9
Death claims per 1,000 policies, first 13 weeks of year, annual rate	9.9	9.8