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An Evaluation of *Pasteurella pestis* Fraction-1-Specific Antibody for the Confirmation of Plague Infections*

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Owing to the relatively long duration of specific serum plague antibodies in the host, the serological diagnosis of plague has substantial advantages over the conventional method of isolation of the causative agent. For the performance of both the complement-fixation (CF) and passive haemagglutination (HA) tests, the use of highly purified, specific Fraction 1 antigen is essential, and its preparation is discussed in this paper. Although both tests measure the same antibodies, the passive HA test is the more sensitive and constitutes the most effective means of detecting recovery from plague infection in nature. The CF test is nevertheless invaluable for such purposes as determining the antibody content of sera from immunized or infected hosts, indicating the amount of Fraction 1 developed by strains of Pasteurella pestis *in cultures and therefore present in a vaccine, or detecting and estimating the amount of Fraction 1 antigen in extracts prepared from infected animal tissues and therefore for the field diagnosis of wild rodent plague after contamination or decomposition of rodents' carcasses.*

The most accurate diagnosis of plague involves the isolation of *Pasteurella pestis* through cultivation methods, supplemented, when necessary, by animal experiments and the subsequent identification of the organisms by serological or other methods. As a rule this entails transporting specimens of tissues, bubo fluid or blood of the organisms, which, however, may become non-viable in transit to the laboratory. Moreover, a negative finding of plague bacilli does not always mean a non-plague-infected host, as the causative agent is not necessarily present in the bubo fluid or blood. These difficulties were overcome with the development by Chen et al. (1952) and Chen & Meyer (1954) of two serological methods—the complement-fixation (CF) and passive haemagglutination (HA) tests, using highly purified Fraction 1 as antigen. These serological tests offer the advantages of greater specificity, sensitivity, and stability of the antigen, and they overcome the difficulties of detecting plague infection in human and rodent surveys. They have been used at the Hooper Foundation in experimental plague immunization studies (Chen et al., 1964) and for the measurement of antibody responses to Fraction 1 in human trials of plague-immunizing agents (Meyer & Chen, unpublished data). Moreover, owing to the longer duration of specific serum antibodies in the host, the serological diagnosis of plague has substantial advantages over the conventional method of isolation of the causative agent. Meyer (1964) confirmed this by testing sera from 83 human plague patients (including seven fatal cases); while only 36% proved bacteriologically positive, 78% were positive by serological tests.

Levi et al. (1961, 1963) evaluated the passive HA test in their laboratory and in field investigations from 1959 to 1962 of over 27 000 wild rodents. They found that only occasionally did plague-susceptible white mice, guinea-pigs and gerbils yield detectable serum antibody levels in response to inoculation of *P. pestis* antigen. Plague-resistant gerbils and susliks

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(sisels) from plague areas, however, frequently yielded antibody in high titres in response to the same virulent *P. pestis* antigens. During a plague epizootic, or shortly thereafter, 60%-100% of the rodent sera from such areas showed antibodies. Levi and associates therefore concluded that plague-resistant strains of wild rodents, which react to the passive HA test, may serve as reservoir hosts in the population and that the passive HA test is well suited to laboratory and field studies.

Hudson et al. (1964) used the passive HA test in a study of a plague-resistant *Microtus californicus* population during a 21-month period which included two successive winter-spring epizootics of *P. pestis* infection. Close correlation was found between the isolation of *P. pestis* from fleas and animal tissues and the prevalence and levels of *P. pestis* Fraction-1-specific antibody in the population.

On the other hand, results inconsistent with our findings have been reported by Cavanaugh et al. (1965) in the use of the CF test for the same purposes. In their study of 904 rodent sera, some specimens had moderate CF but no passive HA titres. A possible explanation for these results would be that these two tests measure two different kinds of antibody, since positive results were not obtained with the same highly purified *P. pestis* Fraction 1 antigen in the two procedures. An alternative hypothesis might be that the CF-positive but passive HA-negative rodents are newly infected survivors, whereas rodents having passive HA but no CF titres are from old infested areas. This supposition is partly correct and will be discussed later. In previous experiments at the Hooper Foundation, however, with sera from either man or various species of animals, including wild rodents, either CF titres have been found concurrently with passive HA titres or HA titres have occurred alone, since the passive HA test is at least 20-50 times more sensitive than the CF test employing the same antigen.

This paper describes the CF and passive HA tests for the detection of antibodies to Fraction 1 in experimentally immunized or naturally infected hosts. Adaptation of these tests to the micro-technique of Dr J. L. Sever (1962) and the method of preparation of the Fraction 1 antigen used are also described.

MATERIALS AND METHODS

Fraction 1, or the envelope antigen, was isolated in 1947 in a highly purified form at the Hooper Foundation by Baker and associates (1947, 1952) and has since been in use at this institute as specific antigen for the CF and passive HA tests. The method of preparation has remained essentially that of Baker et al., and is set forth briefly here, since the characteristics of this antigen as made for serological work in different laboratories have been variable.

Virulent *P. pestis* strain 195/P was grown on hormone-agar in Roux bottles at 37°C for 72 hours. The cells were washed with physiological saline, precipitated with two volumes of acetone at −70°C, and then allowed to stand for 18-20 hours. The sedimented plague bacilli were then collected by centrifugation (refrigerated) and washed with cold acetone three times until dehydrated. The mass of plague bacilli was then freed of acetone by placing it in a desiccator over concentrated H_2SO_4 and evacuated.

The dry plague bacilli were emulsified in sufficient 2.5% sodium chloride solution saturated with toluene to give a 5% suspension. The mixture was allowed to stand for 24 hours at room temperature (25°C). The bacilli were then removed by centrifugation at 15 000 rev/min for 30 minutes. The sedimented bacteria were again extracted, using about two-thirds of the original volume of the solution. The combined extracts were dialysed against changes of distilled water under refrigeration. The total volume of this material must be adjusted to a workable level. It may be concentrated by simple evaporation of water at 0°C-4°C.

Sufficient saturated ammonium sulfate solution at pH 7.0 was gradually stirred into the dialysed material to give a concentration of ammonium sulfate of 0.25 saturation. After 24 hours at room temperature, the precipitate was collected by centrifugation and discarded, as it contained little plague antigen. Saturated ammonium sulfate solution was then added to the supernatant fluid to give a concentration of 0.33 saturation. The mixture was allowed to stand for 24 hours at room temperature and the precipitation which formed (Fraction 1A and 1B) was collected by centrifugation. This process was repeated three times in order to obtain a highly purified preparation. The final precipitate was dissolved in distilled water and dialysed at 4°C for several days against frequent changes of distilled water to remove the sulfate ions. After dialysis, Fraction 1 was dried by lyophilization. The lyophilized Fraction 1 was dissolved in physiological saline, in a concentration of 2 mg/ml, for use as a stock solution.

The standard (macro) CF and passive HA tests for plague according to the procedures of Chen et al. (1952) and Chen & Meyer (1954) are as follows.

Both standard tests can be performed in 10 mm × 95 mm test-tubes. In the CF test, 0.2-ml volumes of twofold dilutions of inactivated serum were made in a series of tubes to which 0.2 ml of a 1 : 500 dilution of stock Fraction 1 antigen (2 mg/ml) and 0.2 ml of complement (2 units) were added. The tubes were shaken, incubated for 4 hours or overnight at 4°C and then incubated for one hour in a 37°C water-bath, after which 0.4 ml of sheep erythrocytes, sensitized 15 minutes earlier and held at room temperature, was added. The tubes were shaken, incubated at 37°C for 30 minutes, and then read in the usual manner. Final readings were taken after the tubes had been held at 4°C overnight. All sera must be tested for anticomplement activity.

For the standard passive HA test, one volume of 2.5% washed sheep erythrocytes in physiological saline was added to an equal volume of a 1 : 20 000 (weight/volume) solution of tannic acid in saline. The mixture was then incubated in a 37°C water-bath for 10 minutes and centrifuged at 1500 rev/min for 5 minutes, and the sediment washed once with saline. The tannic-acid-treated cells were then suspended in saline to a concentration of 2.5% and an equal volume of *P. pestis* Fraction 1, 25 μg/ml-50 μg/ml in saline, added. After the mixture had stood at room temperature for 15 minutes, the sensitized cells were washed twice with a 1 : 250 dilution of normal rabbit serum (1 : 250 NRS), previously inactivated and absorbed with sheep erythrocytes. Finally, the cells were resuspended in the 1 : 250 NRS to a concentration of 2.5% for the macrotechnique and 0.5% for the microtechnique.

Fresh sheep erythrocytes tanned and sensitized with Fraction 1 were used in the passive HA test as antigen. However, the erythrocytes can be preserved for a long period of time by treatment with formol as described by Csizmas (1960). In their serological survey work, Levi et al. (1964) adapted this method with great success for the diagnosis of plague. The procedure used for formolizing sheep erythrocytes to prepare the passive HA test was exactly that described by Csizmas (1960) and is set forth briefly here. Sheep blood preserved in modified Alsever's solution (Bukantz et al., 1946) was washed six times in cold 0.85% sodium chloride solution in a refrigerated centrifuge. The washed and packed cells were resuspended in eight volumes of cold 0.85% sodium chloride solution. One-fourth volume of formol solution contained in a Cellophane dialysis sac was placed into the cell suspension, which was then agitated in a shaker at room temperature for two hours. Subsequently, the contents of the Cellophane sac were poured into the cell suspension and the agitation continued for 18 hours. The suspension was then filtered through gauze and washed another six times with cold 0.85% sodium chloride solution. Finally, the packed formolized cells were suspended in 0.85% sodium chloride solution to give a 50% stock suspension. For the treatment of the formolized cells with tannic acid and sensitization with purified Fraction 1 the procedure was the same as previously described for the fresh sheep erythrocytes.

The serum to be tested was inactivated at 56°C for 30 minutes. Nine volumes of the serum under test were absorbed with one volume of washed and packed normal sheep erythrocytes for 30 minutes at room temperature to remove the heterophile antispecies antibodies.

For the standard passive HA test serial twofold dilutions of serum were made in saline containing a 1 : 100 dilution of normal inactivated and absorbed rabbit serum (1 : 100 NRS), which acts as a protective colloid for maintaining the suspension stability. To each of this series of tubes containing 0.5 ml of serum dilution was added 0.05 ml of the 2.5% suspension of tanned and sensitized sheep erythrocytes; the tubes were then shaken and held at either room temperature or in a refrigerator for two hours. Results were read immediately after these incubation periods.

Adaptation of the plague CF and passive HA tests to the microtechnique described by Sever (1962) has the advantages of rapidity of performance and the small amount (0.025 ml) of serum used.

For the micro complement-fixation test, the titrations of haemolysin, complement and complement-unit controls were carried out in tubes as usual. The serum (0.025 ml) was diluted in saline (0.025 ml) in U-bottom plates with spiral loops; 0.025 ml of antigen and then 0.025 ml of complement were added to the twofold serum dilutions. The plates were shaken, covered with plate sealer and placed at 4°C overnight, then incubated in a 37°C incubator for one hour, and the haemolytic system (0.05 ml) added (blood cells were sensitized with appropriate amount of haemolysin 15 minutes before use and kept at room temperature). The results were read after the plates had been shaken again, sealed, placed in the incubator for 30 minutes and then allowed to stand overnight in the refrigerator.

In the passive micro HA test twofold serial dilutions of the inactivated and absorbed serum were made in 1 : 100 NRS in U-bottom plates with spiral loops which deliver 0.025 ml. Then 0.025 ml of a 0.5% suspension of tanned and sensitized erythrocytes was added. The plates were shaken and read after incubation at room temperature for two hours. The first well was considered a 1 : 4 serum dilution.

RESULTS

Specificity of the tests

The antigenic complexity of *P. pestis* and its close relationship to *P. pseudotuberculosis* have been demonstrated by different investigators (Chen & Meyer, 1955; Bhagavan et al., 1956; Chen, 1965; Lawton et al., 1960). Of the 16 antigens distinguished in *P. pestis*, 13 are also common to *P. pseudotuberculosis*. The importance of the specificity of the tests for plague diagnostic work can therefore be understood. The following experiments were conducted to determine their specificity.

Ten Sprague-Dawley rats were immunized with 1 ml of Cutter plague vaccine (2×10^9 formol-killed virulent organisms per ml) by the intraperitoneal route, and 38 others infected subcutaneously with 15 000 *P. pestis* of strain 195/P. All rats were bled before and after either immunization or infection. Both the micro CF test and the passive micro HA test were made 20 days after the rats had been immunized or challenged. Table 1 shows that the rats developed passive HA and CF antibodies to Fraction 1 following infection. Three of 12 survivors of infected rats and 10 of 10 immunized rats did not react in the CF test. The passive HA titre after vaccination was of lower magnitude. Moreover, sera from rabbits hyperimmunized with the closely related organisms of different types of *P. pseudotuberculosis* tested against Fraction 1 were all negative. These data indicate that there is no cross-reaction between plague and pseudotuberculosis in the CF and passive HA tests.

TABLE 1
ANTIBODY RESPONSE OF SPRAGUE-DAWLEY RATS TO FRACTION-1-SPECIFIC *PASTEURELLA PESTIS* ANTIGEN

Rat No.	Serological reactions 20 days after immunization or infection	
	CF	HA
Immunized rats		
203	0	8
204	0	32
205	0	32
206	0	32
207	0	4
208	0	0
209	0	16
210	0	16
211	0	4
212	0	8
Survivors of infected rats [a]		
217	0	32
218	4	256
221	8	128
223	8	64
227	0	8
229	0	0
234	16	256
236	2	64
237	2	4
242	32	256
246	64	1 024
250	64	1 024

[a] Infecting dose: 15 000 *P. pestis* strain 195/P. Pre-immunization and pre-infection sera were all negative.

Sensitivity of the tests

To determine the sensitivity of these tests and to evaluate their usefulness in the confirmation of plague infections, two experiments were performed. Fourteen rats (*Rattus norvegicus*) caught in an area known to have *P. pestis* among the rodent population (Kartman et al., 1958) were obtained through the courtesy of Dr Bruce W. Hudson, US Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center, Technology Branch, San Francisco Field Station. The sera were tested by both micro CF and passive micro HA tests. As shown in Table 2, sera of four of 14 rats had passive HA titres that were taken as evidence of naturally occurring infections. Eleven of these animals were then infected with 6600 *P. pestis* organisms; three succumbed to bubonic plague. Of the eight survivors, one showed a low CF titre and four had high passive HA titres. Sera from another 17 animals (10 *R. rattus* and seven *R. alexandrinus*) captured from two known plague-free areas, Calaveras and Amador

TABLE 2

COMPARISON OF SEROLOGICAL TESTS ON *RATTUS NORVEGICUS* FROM THE SAN BRUNO AREA, A KNOWN RODENT PLAGUE FOCUS IN CALIFORNIA

Rat No.	Serological reactions					
	After capture [a]		Survivors [b]			
	HA		CF		HA	
	Serum dilution	Result	Serum dilution	Result	Serum dilution	Result
977 [c]	1 : 640	+				
978	1 : 6	0	1 : 2	0	1 : 4	0
979	1 : 6	0	1 : 2	0	1 : 1 024	+
980	1 : 6	0	1 : 6	+	1 : 1 024	+
981	1 : 10	+	1 : 2	0	1 : 128	+
982	1 : 10	0	1 : 2	0	1 : 4	0
983 [d]	1 : 10	0				
930 [c]	1 : 10	0				
931	1 : 320	+	1 : 2	0	1 : 1 024	+
955	1 : 8	0	1 : 2	0	1 : 4	0
956 [d]	1 : 6	0				
957 [d]	1 : 384	+				
958 [c]	1 : 6	0				
959	1 : 6	0	1 : 2	0	1 : 4	0

[a] The sera from the first bleeding were supplied by Dr Bruce W. Hudson.
[b] Three weeks after a subcutaneous infecting dose of 6600 *P. pestis* strain 195/P.
[c] Died after infection.
[d] Died before infection.

Counties in California, by the California State Department of Public Health at Berkeley gave completely negative reactions to *P. pestis* Fraction 1 antigen (Table 3). After a relatively small subcutaneous infecting dose of 5550 *P. pestis* organisms, one of 10 in the *R. rattus* group and four of seven in the *R. alexandrinus* group died. Three sera showed low CF titres in both groups of rats. Significantly, all the CF-positive sera also had high passive HA titres. One serum had a low passive HA titre without any CF titre. These results confirm previous findings that CF-positive sera are always passive HA-positive but the inverse is not always true, indicating that the passive HA test is much more sensitive than the CF test.

Stability of the reagents

The reliability of any serological test is determined by the stability of the serum and the antigen used. In the CF test the serum should be fresh or uncontaminated, as this test is always handicapped by anticomplement factors which produce misleading results; hence serum controls are essential. On the other hand, the passive HA test is not only more sensitive than the CF test, but it is not complicated by anticomplement factors, thereby overcoming the difficulties encountered when sera arrive contaminated from remote rural areas.

The highly purified *P. pestis* Fraction 1 is specific and stable. The soluble antigen in physiological saline may be kept at 0°C-4°C for long periods without affecting its antigenicity. One lot of Fraction 1 (1 mg/ml) in physiological saline containing 0.5% phenol, prepared in 1958 and refrigerated for seven years, was still effective when tested. A frozen antigen solution may be kept indefinitely.

DISCUSSION

As previously stated, it is critically important to use purified Fraction 1 in plague CF and passive

TABLE 3

COMPARISON OF SEROLOGICAL TESTS ON *RATTUS RATTUS* AND *RATTUS ALEXANDRINUS* FROM CALAVERAS AND AMADOR COUNTIES, KNOWN RODENT-PLAGUE-FREE AREAS OF CALIFORNIA

Rat No.	Serological reactions							
	After capture				Survivors [a]			
	CF		HA		CF		HA	
	Serum dilution	Result	Serum dilution	Result	Serum dilution	Result	Serum dilution	Result
				R. rattus				
1	1:3	0	1:4	0	1:32	+	1:2048	+
2	1:2	0	1:4	0	1:3	0	1:4	0
3	1:3	0	1:4	0	1:8	0	1:4	0
4	1:3	0	1:4	0	1:3	0	1:4	0
5	1:3	0	1:4	0				
6	1:2	0	1:4	0	1:2	0	1:4	0
7	1:3	0	1:4	0	1:2	0	1:4	+
8	1:2	0	1:4	0	1:2	+	1:32	+
9	1:3	0	1:4	0	1:6	+	1:512	+
10	1:3	0	1:4	0	1:2	0	1:4	0
				R. alexandrinus				
1	1:2	0	1:4	0				
2	1:2	0	1:4	0				
3	1:3	0	1:4	0				
4	1:3	0	1:10	0				
5	1:2	0	1:4	0	1:8	+	1:2048	+
6	1:8	0	1:4	0	1:6	+	1:256	+
7	1:2	0	1:4	0	1:16	+	1:2048	+

[a] Twenty-four days after a subcutaneous infecting dose of 5550 *P. pestis* strain 195/P.

HA tests. This fraction is soluble in a 25% saturated ammonium sulfate solution and is precipitated in a 33% saturated solution. It consists of two immunologically identical but chemically different substances (Fraction 1A and 1B). Fraction 1A is a protein-carbohydrate complex; Fraction 1B is entirely protein (Baker et al., 1947, 1952). We stated in an earlier report (Chen & Meyer, 1954) that Fraction 1B was used as an antigen to sensitize tanned erythrocytes for the passive HA test, because it was believed to be a pure protein component. Subsequent work has shown that Fraction 1A is as specific as 1B. The separation of 1B from 1A is difficult and the yield is low, about 1%. The yield of Fraction 1A may be as high as 6 g per 100 g of dry bacilli. Since both fractions are equally effective, it would seem wasteful and unnecessary to separate them when they are used for immunological and serological purposes.

Table 1 shows the specificity of these tests. Laboratory white rats having no history of contact with *P. pestis* did not reveal antibodies to Fraction 1. The same was true of wild rats captured in plague-free areas (Table 3). The appearance of antibodies specific for plague in 20 of 22 animals following immunization or infection demonstrates the value of the passive HA test. No serological cross-reaction occurred between the antigenically closely related plague and pseudotuberculosis organisms. This is borne out by observations on an Alaskan isolate (*P. pseudotuberculosis* type 1B) reported by Quan et al. (1965), who found that this isolate possessed a Fraction-1-like substance demonstrable by fluo-

rescent-antibody-staining and agar-diffusion methods. Serum from a rabbit hyperimmunized with this Alaskan strain, however, failed to reveal passive HA antibodies to Fraction 1 when tested at the Hooper Foundation. Therefore we assume that the passive HA reaction is specific and is the most dependable method of detecting plague infection in nature.

Since the passive HA test is considerably more sensitive than the CF test, haemagglutinating antibodies were often detectable when results of other tests were negative or insignificant. Tables 1 and 3 show that animals with no serological reaction before either immunization or infection subsequently showed high passive HA titres; only a few had low titres of 1 : 4 to 1 : 8, indicating the sensitivity of the passive HA test in detecting such low antibody levels. Hudson et al. (1964) considered passive HA titres below 1 : 32 as negative for rodent sera; Cavanaugh et al. (1965) considered those below 1 : 24 as negative. In so doing, they may have missed some infected rodents with very low antibody levels. Cavanaugh et al. (1965) recognized this possibility and felt that more passive HA-reacting rodent sera might have been obtained if lower serum dilutions had been employed. It is a fairly safe assumption that in an old endemic area, owing to the decline of the antibody level a few months after infection, only a few rodent sera with low passive HA titres were detected.

Follow-up studies on repeated infection of animals to simulate natural *P. pestis* infection in rodents have revealed high titres by both the CF and passive HA methods (Meyer & Chen, unpublished data); no sera tested had CF titres without passive HA titres. Davis, Hallett & Koornhof (personal communication) in the South African Institute for Medical Research infected plague-resistant wild rodents (*Arvicanthis niloticus*) raised in their laboratory with a dose of 100 000 or 1 000 000 *P. pestis* ; the Fraction-1-specific antibody among some of the survivors showed a CF titre of 1 : 256 and a passive HA titre of 1 : 2048 or a CF titre of 1 : 16 and a passive HA titre of 1 : 16 384 after 27 days. These data confirm the previous findings and suggest that the antibody response among wild rodents involves and reflects the genetic make-up of different species. Furthermore, these studies confirm that both serological tests measure the same antibodies.

Although the CF test is less sensitive than the passive HA test, its usefulness for the serological confirmation of plague should not be underestimated. We have employed it extensively since 1952 for the following purposes: (1) to determine antibody content of sera from vaccinated or infected hosts; (2) to indicate the amount of Fraction 1 developed by strains of *P. pestis* in cultures and therefore present in a vaccine; (3) to detect and estimate the amount of Fraction 1 antigen in extracts prepared from tissues of infected animals or from carcasses of animals that have died of acute plague. This test is therefore valuable for the field diagnosis of wild rodent plague, especially when the isolation of *P. pestis* or the interpretation of the pathological lesions at autopsy is rendered impossible by contamination or decomposition.

Macro and micro technique procedures of both the CF and passive HA tests have been described. The micro technique has the advantages that very small amounts of serum (0.025 ml) are used and that dilutions are easy to make. This is particularly important for plague surveys in the field, where it is impossible to perform serological tests by the macro technique (0.25 ml of serum for the passive HA test and 0.4 ml for the CF test). Because of the similar results obtained by using either the macro or micro technique, the serological studies reported in this paper were tested by one or the other of these methods, depending upon the size of the specimen available.

The use of sensitized formol-treated sheep erythrocytes instead of fresh ones for the passive HA test facilitates the procedure for field survey purposes, since this cell antigen can be prepared in any desired quantity and kept for many months either at room temperature or at 0°C-4°C without loss of capacity to react in the test. Compared by titres, the activity of formolized and non-formolized sheep erythrocytes is the same, but the results with the latter are easier to read.

Table 3 shows that *R. rattus* from Calaveras County were more resistant to infection and had less antibody response than *R. alexandrinus* from Amador County, while the latter were more susceptible to infection and the survivors gave greater antibody response. Those animals surviving infection without developing circulating antibodies are of particular interest and will be discussed in a later paper.

RÉSUMÉ

Les auteurs ont utilisé la fraction antigénique 1, très purifiée, de *Pasteurella pestis* pour la détection des anticorps au cours de l'infection pesteuse, expérimentale ou naturelle, des rongeurs. Ils ont comparé à cette occasion les avantages respectifs de la réaction de fixation du complément et de la réaction d'hémagglutination passive en utilisation normale ou en microtechniques.

L'inoculation au rat de vaccin antipesteux ou d'une souche de *P. pestis* détermine la formation d'anticorps décelables par les deux réactions; ces dernières témoignent d'une grande spécificité à condition d'utiliser l'antigène purifié. On n'observe aucune réaction croisée avec *P. pseudotuberculosis*. L'épreuve d'hémagglutination passive est la plus sensible et la mieux adaptée au dépistage de l'infection naturelle des rongeurs; sa positivité n'est jamais en défaut lorsque la réaction de fixation du complément est positive, alors que l'inverse n'est pas toujours vrai, et elle permet de ne pas tenir compte de l'existence éventuelle de facteurs anticomplémentaires. La réaction de fixation du complément garde cependant toute sa valeur pour la mise en évidence des anticorps après vaccination ou infection expérimentale, pour l'évaluation du contenu en antigène des cultures de *P. pestis* et des vaccins, ainsi que pour la recherche de la fraction 1 dans les tissus d'animaux infectés.

Les auteurs décrivent les modalités d'emploi des diverses techniques sérologiques et la préparation de la fraction antigénique 1. Ils soulignent l'avantage de la sérologie sur les méthodes habituelles d'isolement direct du bacille pesteux. Le traitement par le formol des hématies de mouton sensibilisées assure la stabilité de l'antigène et facilite l'utilisation de la réaction d'hémagglutination passive lors des enquêtes sur le terrain.

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