CURRENT MICROBIOLOGY, Vol. 4 (1980), pp. 225–229

Current Microbiology
An International Journal

Intra- and Interspecies Relatedness of *Yersinia pestis* by DNA Hybridization and Its Relationship to *Yersinia pseudotuberculosis*

Hervé Bercovier,† H. H. Mollaret,† Jean Michel Alonso,† J. Brault,† G. Richard Fanning,‡ Arnold G. Steigerwalt,§ and Don J. Brenner§*

†Centre National des Yersinia, Institut Pasteur, Paris, France
‡Division of Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20012, USA
§Enteric Section, Center for Disease Control, Public Health Service, U. S. Department of Health and Human Services, Atlanta, Georgia 30333, USA

Abstract. The biochemical characteristics of *Yersinia pestis* are presented and compared with those of *Y. pseudotuberculosis*. Motility at 28°C, urease, fermentation of rhamnose, and growth rate on nutrient agar are the best means of separating these organisms. DNA hybridization studies demonstrated that *Y. pestis* strains are 90% or more interrelated and that *Y. pestis* and *Y. pseudotuberculosis* are indistinguishable by DNA relatedness. On the basis of DNA data and biochemical and antigenic similarity, these organisms should be treated as two separate subspecies of the same species. *Y. pseudotuberculosis* was described before *Y. pestis* and therefore has priority. *Y. pseudotuberculosis* subsp. *pseudotuberculosis* and *Y. pseudotuberculosis* subsp. *pestis* are recommended as new designations for *Y. pseudotuberculosis* and *Y. pestis*. For medical purposes, *Y. pestis* and *Y. pseudotuberculosis* can and should continue to be used.

Yersinia pestis was first described in Hong Kong by Yersin in 1894 [29]. Since then, the "plague bacillus" was frequently transferred from one genus to another until, with general agreement, it was finally placed in the genus *Yersinia* Van Loghem [28]. It is described as such in the eighth edition of *Bergey's Manual* [21]. The isolation of *Yersinia pseudotuberculosis* in Europe in 1883 is credited to Malassez and Vignal [15]. *Y. pseudotuberculosis* has also undergone frequent nomenclatural changes [18].

Taxonomists considered these two organisms to be separate species; the main problem for the bacteriologists who isolated *Y. pestis* from throughout the world during the third pandemic was to differentiate it from *Y. pseudotuberculosis*. The consequence for a country in which the plague bacillus was isolated—i.e., in terms of quarantine, vaccination, prophylaxis, etc.—were so important that between 1910 and 1950 an enormous amount of scientific literature was devoted to that topic [24]. *Y. pestis* and *Y. pseudotuberculosis* are still considered separate species, but in the field, it is difficult to differentiate between them, especially with nonhuman isolates.

Since it was difficult to separate these organisms, our approach was to determine the extent to which they were similar. In this report, we present biochemical and DNA relatedness data, and review antigenic and epidemiological findings to define the relationship between *Y. pestis* and *Y. pseudotuberculosis* and the relatedness of these two organisms to other *Yersinia* species.

Materials and Methods

Organisms. The *Yersinia pseudotuberculosis* and *Y. pestis* strains used for DNA hybridization are listed in Table 1. Other *Yersinia* species [7] and species of Enterobacteriaceae [26] used for DNA hybridization have been described previously. Some fifty strains each of *Y. pseudotuberculosis* and *Y. pestis* from the collection of the National Yersinia Center (Institut Pasteur, Paris) were studied biochemically.

Biochemical and genetic characterization. Biochemical reactions were done at 28°C, unless otherwise indicated. Tellurite reduction was done according to Brzin [8]. Reference to the other methods used has been previously cited [1]. Methods used for the separation of single- and double-stranded DNA on hydroxyapatite (HA), and thermal elution chromatography on HA have been described [2,3,4,6]. DNA from *Y. pseudotuberculosis* and *Y. pestis* strains was labeled in vitro with tritium using John Johnson's modification of the methods described in references [10] and [23]. G+C content was determined by the optical thermal denaturation method [16].

*To whom offprint requests should be addressed.

0343-8651/80/0004-0225 $01.00

Table 1. Bacterial strains.

Species	Strain designation[a]	Serogroup	Source	Country of origin (sender)
Yersinia pseudotuberculosis	IP P1	II	Human	France
Y. pseudotuberculosis	IP P3 (55.85), NCTC 10275, Thal 14-1	I		Sweden (Thal)
Y. pseudotuberculosis	P62			
Y. pseudotuberculosis	P105		Mink	Denmark (Thal)
Y. pseudotuberculosis	P320		Human	Germany (Thal)
Yersinia pestis	IP P1, Harbin			USSR
Y. pestis	IP P2, PKR 288		Rodent	Iran
Y. pestis	IP P3, Kenya 129			Kenya
Y. pestis	IP P4, Kenya 102			Kenya
Y. pestis	IP P5, Congo Belge Lita			Zaire
Y. pestis	IP P6, PKR 292		Rodent	Iran
Y. pestis	IP P7, Madagascar 6/69		Human	Madagascar
Y. pestis	IP P8, Dalat 286		Human	Vietnam
Y. pestis	IP P9, Madagascar 8/70		Human	Madagascar
Y. pestis	IP P10, Dalat 317		Human	Vietnam
Y. pestis	IP P11, Kenya 147			Kenya
Y. pestis	IP P12, Turquie 10/5			Turkey
Y. pestis	1122			(Corwin)

[a] IP, Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, Colindale, England.

Results and Discussion

Conventional biochemical tests as well as API 20E and API 50E tests were done on about 50 strains each of *Yersinia pseudotuberculosis* and *Y. pestis*. The API tests were done according to the manufacturer's instructions (Analytab Products Inc.)[1] except that incubation was at 28°C. Conventional biochemical tests were also carried out at 28°C. Good agreement was obtained with all three methods. The one exception was with D-sorbitol. Both *Y. pestis* and *Y. pseudotuberculosis* were uniformly sorbitol negative by the conventional test in peptone water or the API 50E. With the API 20E, most *Y. pestis* strains gave a sorbitol-positive reaction at 16 h and became negative after 24 h.

Strains of both *Y. pestis* and *Y. pseudotuberculosis* show very little biochemical heterogeneity. The biochemical reactions that both organisms exhibit are listed in Table 2. Biochemical and cultural characteristics useful in separating *Y. pestis* from *Y. pseudotuberculosis* are shown in Table 3. Motility at 28°C, urease activity, fermentation of rhamnose, growth rate, and colony size on nutrient agar remain the best way to differentiate these organisms. Problems related to the choice of suitable media for *Y. pestis* biochemical reactions have been reviewed elsewhere [24]. Differential characters have also been discussed elsewhere [9,24].

[1] Use of trade name is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

In addition to having comparable physiological and biochemical characteristics, the two organisms are very similar in other respects. Eleven of the 18 antigens studied by Lawton, Fukui, and Surgalla are found in strains of both *Y. pestis* and *Y. pseudotuberculosis* [14]. Even the lipid composition in these organisms was shown to be highly related [27].

G+C content averaged 46.5 mol% for *Y. pseudotuberculosis* and 46.0 mol% for *Y. pestis* (Table 4). These G+C percentages confirm previous values already in the literature [12,17]. The 0.5 mol% difference in the G+C values obtained for strains of *Y. pestis* and *Y. pseudotuberculosis* is not meaningful. This kind of minimal difference in G+C percentage is within the technical limits of the optical denaturation method and well within the variability in G+C found among different strains of the same species [3]. Other *Yersinia* species contain slightly more G+C in their DNA [7,13]. The values usually obtained for *Y. enterocolitica* and *Y. ruckeri* are 47–49% G+C.

Hybridization reactions with labeled DNA from *Y. pestis* 1122 and *Y. pseudotuberculosis* strains P62 and P3 and unlabeled DNA from *Yersinia* strains are presented in Table 5. By reacting DNA from a single strain of *Y. pestis* with labeled DNA from *Y. pseudotuberculosis*, two laboratories reported a high degree of relatedness between these organisms [22,25]. Brenner et al. [5] pointed out the intraspecies homogeneity in *Y. pseudotuberculosis* and its close relation to *Y. pestis*. We now report results of DNA relatedness obtained using 13 strains of *Y. pestis* reacted with both labeled *Y. pestis* DNA and two labeled *Y. pseudo-*

Table 2. Biochemical characteristics of *Yersinia pseudotuberculosis* and *Y. pestis*.[a]

Test	*Y. pseudotuberculosis*			*Y. pestis*		
	Reaction	%+	Neotype strain NCTC 10275	Reaction	%+	Reference strain NCTC 5923
Motility (28°C)	+	98	+	−	0	−
Urease	+	100	+	−	0[b]	−
Indole	−	0	−	−	0	−
Simmons' citrate (28°C)	−	1[c]	−	−	0	−
Malonate	V	V[c]	−	−	0	−
NO_3 reduction to NO_2/Type	+	99/B	+/B	V	V[d]	+/B
Tetrathionate reductase	−	0	−	V	15	−
Deoxyribonuclease	V	85	+	+	91	+
Acid production from:						
Glycerol	+	99	+	V	V[d]	−
L-Arabinose	+	100	+	+	98	+
Adonitol	−	5	−	−	0	−
D-Mannose	+	97	+	+	100	+
L-Rhamnose	+	99	+	−	3	−
Maltose	+	100	+	V	84	+
D-Melibiose	+	99	+	V	V[d]	−
D-Raffinose	V	11	−	−	2	−
D-Melizitose	−	0	−	−	2	−
D-Sorbitol	−	2	−	−	0	−
Salicin	V	65	+	V	88	+
Arbutin	V	88	+	+	100	+
Dextrin	−	5	−	V	62	+

[a] All incubations, except where indicated, were done at 28°C. +, 90.0% or more positive within 7 days; V, 10.1–89.9% positive; −, less than 10% positive after 7 days. The following tests gave positive reactions for all strains of *Y. pseudotuberculosis* and *Y. pestis* tested: catalase, methyl red (28°C and 37°C), fermentation in O-F test, β-galactosidase (ONPG; 37°C), β-xylosidase (PNPX; 37°C), polypectate, and acid production from D-glucose, ribose, D-xylose, galactose, D-fructose, D-trehalose, D-mannitol, esculin, *N*-acetylglucosamine, and starch. The following tests gave negative reactions for all strains tested: oxidase, motility (37°C), Voges-Proskauer (28°C and 37°C), Simmons' citrate (37°C), mucate, Christensen's citrate, KCN, gas from D-glucose, H_2S (Kliegler's), phenylalanine deaminase, tryptophan deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, lipase (Tween 80), gelatin (film), and acid production from erythritol, D-arabinose, L-xylose, L-sorbose, dulcitol, D-cellobiose, lactose, sucrose, *i*-inositol, amygdalin, α-methyl-xyloside, α-methyl-D-mannoside, α-methyl-D-glucoside, inulin, amylose, and glycogen.
[b] Some strains give positive reactions when first isolated [20].
[c] Serogroup IV strains of *Y. pseudotuberculosis* are positive.
[d] Reaction varies in different biotypes of *Y. pestis* [19].

Table 3. Differentiation between *Yersinia pestis* and *Y. pseudotuberculosis*.[a]

	Y. pestis	*Y. pseudotuberculosis*
Motility 28°C	−	+
Urease	−[b]	+
L-Rhamnose	−[c]	+
Colony size on nutrient agar in mm	≤1	≥2
Nutritional requirement for amino acids	+	−
Tellurite reduction	−[d]	+
Glycerol (oxidative)[e]	V[f]	+
D-Melibiose[e]	V	+
Adonitol	−	−(5%+)
Maltose	V(84%+)	+

[a] See Table 1 for explanation of +, −, and incubation conditions.
[b] Rare strains are urease positive on primary isolation. They become negative after subculture [20].
[c] Rare strains ferment rhamnose but never in 24 h.
[d] Weak; a few black specks if heavy inoculum is used.
[e] Variable characters used for the classification of *Y. pestis* biogroups [19].
[f] Variable reaction, more than 10% and less than 90% of strains are positive.

Table 4. G+C content in DNA from strains of *Yersinia pestis* and *Y. pseudotuberculosis*.

Source of DNA	G+C (mol%)
Y. pseudotuberculosis P1	46.5
Y. pseudotuberculosis P2	46.5
Y. pseudotuberculosis P3	46.5
Y. pestis P1	46.0
Y. pestis P2	46.0
Y. pestis P3	46.0
Y. pestis P10	46.5
Y. pestis P11	46.0

tuberculosis DNAs. Our results confirm and extend the previous observations. *Y. pestis* strains representing the three described varieties (*orientalis, medievalis,* and *antiqua*) were included in these studies [11]. All varieties were essentially identical by DNA relatedness.

Intra- and interspecies DNA relatedness values for *Y. pestis* and *Y. pseudotuberculosis* are virtually identical. They are 90% or more related in reactions done at 60°C and this percentage does not decrease in 75°C reactions. Percent divergence (%D) is zero to 1% in interspecies reactions. Other *Yersinia* species (*Y. enterocolitica, Y. intermedia, Y. frederiksenii, Y. kristensenii*) are at most 48% related to *Y. pestis* and *Y. pseudotuberculosis*, with %D values greater than 10. Reciprocal hybridization reactions using the different labeled strains did not show any differences in DNA relatedness values. This suggests not only that *Y. pestis* and *Y. pseudotuberculosis* are highly related, but also that their genome size is very similar.

The DNA data support the two following conclusions: First, biochemical distinctions between *Y. pestis* varieties should be considered only as epidemiological markers, not as criteria for subspeciation.

Table 5. DNA relatedness of *Yersinia pseudotuberculosis* and *Y. pestis*.

	Source of labeled DNA								
	Y. pseudotuberculosis P62			*Y. pestis* 1122			*Y. pseudotuberculosis* P3		
Source of unlabeled DNA	60°C RBR[a]	%D[b]	75°C RBR	60°C RBR	%D	75°C RBR	60°C RBR	%D	75°C RBR
Y. pseudotuberculosis P62	100	0	100						
Y. pseudotuberculosis P3	92	4	79				100	0	100
Y. pseudotuberculosis P1							89	1	
Y. pseudotuberculosis P320	92	0	93						
Y. pseudotuberculosis P105	91	0	98						
Y. pestis P1				98	0		96	1	89
Y. pestis P2				97	0		93	0.5	91
Y. pestis P3	87	0	85	97	0	97	94	0	94
Y. pestis P4	87	0		94	0		96	0	98
Y. pestis P5							97	0	95
Y. pestis P6	90	0		99	0	100	95		95
Y. pestis P7	89	0		99	0	99	100	0	97
Y. pestis P8	89	0		99	0	100	98	0.5	95
Y. pestis P9	91	0					100		100
Y. pestis P10	89	0	84				95		99
Y. pestis P11	87	1					98		
Y. pestis P12							97	1	
Y. pestis 1122	86	0		100	0	100			
Y. enterocolitica 497-70	48	12	15						
Y. enterocolitica 3953 (Bottone 48)	44	12							
Y. enterocolitica 867	44	11							
Y. enterocolitica 1474	44	11							
Y. enterocolitica 4052							40		14
Y. enterocolitica 6005 (X2)									
Y. ruckeri 4535-69	33	13	7				44	14	16

[a] RBR, relative binding ratio = % (heterologous DNA bound to HA)/(homologous DNA bound to HA) × 100. RBR is a convenient way to express percent relatedness.

[b] %D, percent divergence. D is calculated on the assumption that a 1°C decrease in thermal stability of a heterologous DNA duplex compared to that of the homologous DNA duplex is caused by each 1% of the bases within the duplex that are unpaired [2]. For example, consider organisms A and B which are 50% related (RBR = 50%). The thermal stability of an A-A duplex is 91°C, and the thermal stability of an A-B duplex is 76°C; the %D of the related DNA is 15. %D is given to the nearest 0.5%.

Second, in addition to being very similar biochemically, antigenically, and structurally, *Y. pestis* and *Y. pseudotuberculosis* genetically belong to the same species. This is certainly true if we agree with the definition of Enterobacteriaceae species cited in a previous paper on *Yersinia* [7].

Epidemiological data and the clinical aspects of infections due to these two organisms are notably different. *Y. pseudotuberculosis* is found frequently, at least in Europe, among *Lagomorpha* and birds, which can be considered its reservoir. In contrast, *Y. pestis* infects and is often epizootic in different genera of rodents (*Citellus, Meriones, Rattus*, etc.). The two organisms grossly share the same ecology in that they are mainly pathogenic for rodents and occasionally for man. The route of human infection and the consequences of infection are, however, quite different.

Humans are usually infected by oral ingestion of *Y. pseudotuberculosis*, which causes a mesenteric lymphadenitis. The bacteria rarely reach the bloodstream to cause septicemia. On the other hand, *Y. pestis* is generally transmitted from animals to humans by a bite from infected fleas. A peripheral adenitis (bubo) develops in the lymph nodes, draining the site where the bacteria entered. Without treatment, bubonic plague will evolve to septicemia or pneumonic plague, which, as a rule, are fatal. Thus, plague must be considered as an epidemic and fatal disease; *Y. pseudotuberculosis*, on the other hand, causes sporadic disease in man and infections are rarely fatal.

We, and most taxonomists, have long believed that no single property, even pathogenicity, is a sufficient basis for designating species. Therefore, on the basis of the data presented and cited here we believe that these two organisms should be treated as a single species, differentiated at the level of subspecies. From a strictly taxonomic point of view, we recommend that *Y. pseudotuberculosis* subsp. *pseudotuberculosis* be the type species of the genus *Yersinia*,

since it was described before *Y. pestis* and therefore has priority. *Y. pestis* would then become *Y. pseudotuberculosis* subsp. *pestis*. It would still be perfectly acceptable for clinical bacteriologists and public health officers to refer to *Y. pseudotuberculosis* and *Y. pestis*. In fact, we strongly recommend this approach. Thus, the taxonomy of these organisms would be consistent with available scientific knowledge and the need of the medical community for practical designations would be met. Strain NCTC 10275 (=ATCC 29833= Thal 14.1) is proposed as the neotype strain for *pseudotuberculosis* subspecies *pseudotuberculosis*. It belongs to serogroup 1. Strain NCTC 5923 (= ATCC 19428) is the type strain for *Y. pseudotuberculosis* subspecies *pestis*. Biochemical reactions for strains NCTC 10275 and ATCC 19428 are given in Table 2.

Literature Cited

1. **Bercovier, H., Brenner, D. J., Ursing, J., Steigerwalt, A. G., Fanning, G. R., Alonso, J. M., Carter, G. P., Mollaret, H. H.** 1980. Characterization of *Yersinia enterocolitica sensu stricto*. Current Microbiology **4**:201–206.
2. **Bonner, T. I., Brenner, D. J., Neufeld, B. R., Britten, R. J.** 1973. Reduction in the rate of DNA reassociation by sequence divergence. Journal of Molecular Biology **81**:123–135.
3. **Brenner, D. J., Fanning, G. R., Johnson, K. E., Citarella, R. V., Falkow, S.** 1969. Polynucleotide sequence relationships among members of the *Enterobacteriaceae*. Journal of Bacteriology **98**:637–650.
4. **Brenner, D. J., Fanning, G. R., Rake, A., Johnson, K. E.** 1969. Batch procedure for thermal elution of DNA from hydroxyapatite. Analytical Biochemistry **28**:447–459.
5. **Brenner, D. J., Steigerwalt, A. G., Falcao, D. P., Weaver, R. E., Fanning, G. R.** 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reactions. International Journal of Systematic Bacteriology **26**:180–194.
6. **Brenner, D. J., Steigerwalt, A. G., Miklos, G. V., Fanning, G. R.** 1973. Deoxyribonucleic acid relatedness among erwiniae and other *Enterobacteriaceae*: The soft-rot organisms (genus *Pectobacterium* Waldee). International Journal of Systematic Bacteriology **23**:205–216.
7. **Brenner, D. J., Ursing, J., Bercovier, H., Steigerwalt, A. G., Fanning, G. R., Alonso, J. M., Mollaret, H. H.** 1980. Deoxyribonucleic acid relatedness in *Yersinia enterocolitica* and *Yersinia enterocolitica*-like organisms. Current Microbiology **4**:195–200.
8. **Brzin, B.** 1968. Tellurite reduction in *Yersinia*. Experientia **24**:405.
9. **Brubaker, R. R.** 1972. The genus *Yersinia*. Biochemistry and genetics of virulence. Current Topics in Microbiology and Immunology **57**:111–158.
10. **Cherm, B. K., Hallick, R. B.** 1976. Changes in the expression of the chloroplast genome of *Euglena gracilis* during chloroplast development. Biochemistry **15**:593–599.
11. **Devignat, R.** 1951. Variétés de l'espèce *Pasteurella pestis*. Nouvelle hypothèse. Bulletin de l'Organisation Mondiale de la Santé **4**:247–263.
12. **Domaradskij, V., Marchenkou, V., Shimanjuk, N.** 1973. New data obtained in comparative studies of plague agent and related organisms. Contributions to Microbiology and Immunology **2**:2–3.
13. **Ewing, W. H., Ross, A. J., Brenner, D. J., Fanning, G. R.** 1978. *Yersinia ruckeri* sp. nov., the redmouth (RM) bacterium. International Journal of Systematic Bacteriology **28**:37–44.
14. **Lawton, W. D., Fukui, G. M., Surgalla, M. J.** 1960. Studies on the antigens of *Pasteurella pestis* and *Pasteurella pseudotuberculosis*. Journal of Immunology **84**:475–479.
15. **Malassez, L., Vignal, W.** 1883. Tuberculose zoogléique (forme ou espèce de tuberculose sans bacilles). Archives de Physiologie Normale et Pathologique, 3rd series, **2**:369–412.
16. **Mandel, M., Marmur, J.** 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods in Enzymology **12B**:195–206.
17. **Marmur, J., Doty, P.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. Journal of Molecular Biology **5**:109–118.
18. **Mollaret, H. H.** 1965. Sur la nomenclature et la taxonomie du bacille de Malassez et Vignal. International Bulletin of Bacteriological Nomenclature and Taxonomy **15**:97.
19. **Mollaret, H. H., Mollaret, C.** 1965. La fermentation du mélibiose dans le genre *Yersinia* et son intérêt pour le diagnostic des variétés de *Y. pestis*. Bulletin de la Société de Pathologie Exotique et de Ses Filiales **58**:154–156.
20. **Mollaret, H. H., Nguyen, V. B., Vandekerkove, M., Karimi, Y., Eftekhari, M.** 1964. Sur l'uréase du bacille de Yersin. Annales de l'Institut Pasteur **107**:424–429.
21. **Mollaret, H. H., Thal, E.** 1974. Genus XI. *Yersinia* Van Loghem 1944, pp. 330–332. In: Buchanan, R. E., Gibbons, N. E. (eds.), Bergey's manual of determinative bacteriology, 8th ed. Baltimore: Williams & Wilkins.
22. **Moore, R. L., Brubaker, R. R.** 1975. Hybridization of deoxyribonucleotide sequences of *Yersinia enterocolitica* and other selected members of *Enterobacteriaceae*. International Journal of Systematic Bacteriology **25**:336–339.
23. **Nonoyama, M., Pagano, J. S.** 1973. Homology between Epstein-Barr virus DNA and viral DNA from Burkitts lymphoma and nasopharyngeal carcinoma determined by DNA-DNA reassociation kinetics. Nature **242**:44–47.
24. **Pollitzer, R.** 1954. Plague. Geneva: World Health Organization.
25. **Ritter, D. B., Gerloff, R. K.** 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. Journal of Bacteriology **92**:1838–1839.
26. **Steigerwalt, A. G., Fanning, G. R., Fife-Asbury, M. A., Brenner, D. J.** 1976. DNA relatedness among species of *Enterobacter* and *Serratia*. Canadian Journal of Microbiology **22**:121–137.
27. **Tornabene, T. G.** 1973. Lipid composition of selected strains of *Yersinia pestis* and *Yersinia pseudotuberculosis*. Biochimica et Biophysica Acta **306**:173–185.
28. **Van Loghem, J. J.** 1944. The classification of plague-bacillus. Antonie van Leeuwenhoek Journal of Microbiology and Serology **10**:15–16.
29. **Yersin, A.** 1894. La peste bubonique a Hong-Kong. Annales de l'Institut Pasteur **8**:662–667.