

Carrion's Disease. IV. Natural Sources of Bartonella in the Endemic Zone.

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This paper presents the results of a somewhat limited study of the possible reservoirs of the virus of Carrion's disease (Oroya fever and *verruca peruana*). The investigations were, for the most part, carried on in Peruvian villages in the zone of endemicity of the disease. The methods consisted chiefly of the examination of blood films and the making of blood cultures in leptospira-medium in an effort to detect *Bartonella bacilliformis* in human and animal inhabitants of the verruga zone. At Callahuanca, a village in the endemic district, blood cultures and films were obtained from a random group of 53 residents. None of this group gave any sign or symptom of Carrion's disease. Positive blood cultures of *Bartonella bacilliformis* were obtained, however, from 5 of these persons. In none of the 5 could bartonellæ be demonstrated in blood films. Three cases had no past history of Carrion's disease; the other 2 gave a history of *verruca peruana* 6 months previously.

These asymptomatic cases are not hospitalized and continue to reside in the verruga zone where they live in close proximity to the presumptive vector, and inhabit dwellings which render them easily accessible to hematophagous arthropods. It is, therefore, possible that they may constitute an important reservoir of virus for Carrion's disease.

The only animals from the verruga zone available in sufficient numbers to permit of study were guinea pigs and dogs. One guinea pig coming from San Bartolomé, a village within the region where Carrion's disease is endemic, was found to be spontaneously infected with an organism of the bartonella group. Further investigation showed that the laboratory stock of guinea pigs (all of Peruvian origin) was likewise infected. No indubitable spontaneous bartonella infection has been previously reported in splenectomized guinea pigs studied in various countries.^{1, 2, 3} We have not found such infection in guinea pigs obtained in Boston.

¹ Schwetz, J., *Bull. Soc. Path. Exot.*, 1934, **27**, 515.

² Schin, C., *J. Chosen Med. Assn.*, 1930, **20**, 1073.

³ Noguchi, H., *J. Exp. Med.*, 1928, **47**, 235.

Four animals, or about 17%, of a series of 23 splenectomized Peruvian guinea pigs were found to be infected. The first animal (origin unknown) had been inoculated with human Oroya fever blood one day prior to splenectomy. The other 3 animals were not inoculated; one came from San Bartolomé, while the other 2 were of unknown origin. The parasite was observed only after removal of the spleen. The period of incubation after splenectomy varied from 5 to 9 days. The parasites were observed in or on red cells, of which about 0.1% were infected. A single erythrocyte presented from 8 to 15 microorganisms, staining an intense, slightly violet red with Giemsa's solution. The parasites were rod-shaped, and frequently took an accentuated bipolar stain. Their average measurements were 2μ in length by $\frac{1}{2}\mu$ in width, with many longer and shorter forms.

An attempt was made to cultivate this guinea pig bartonella. Several tubes of leptospira medium were inoculated with the blood of the naturally infected guinea pig from San Bartolomé. In these tubes, kept at 28°C ., structures resembling clumps of *Bartonella bacilliformis* had developed by the tenth day. These cultures were injected, 2 months later, into 5 bartonella-free splenectomized guinea pigs obtained in Boston. After 2 weeks, 4 of these inoculated animals presented in their blood films the same organism seen in the naturally infected animals in Peru. This guinea pig bartonella has not yet been fully identified, but evidence at present available indicates that it is not *Bartonella bacilliformis*.

Fifteen non-splenectomized Peruvian guinea pigs inoculated with either infected blood, verruga tissue, or cultures of *Bartonella bacilliformis* did not develop any symptoms of Carrion's disease, nor were bartonellæ found in the blood films.

Verruga peruana is reported to occur in dogs. We observed neither cutaneous lesions nor parasites in the blood, and obtained no positive blood cultures from 10 dogs, of which 4 came from Puente Verrugas, the other 6 from Callahuanca. The dog bartonella, *B. canis*, of wide geographic distribution, has been found in Peru.⁴

Monkeys, which are receptive to the disease, are not reported to occur in the verruga zone. They, therefore, cannot play any important rôle as a natural reservoir of virus.

Summary. Healthy inhabitants of a village in the verruga zone were discovered to be asymptomatic carriers of *Bartonella bacilliformis*. Five individuals of a group of 53 investigated yielded positive

⁴ Weiss, P., and Muzzo, J. P., *Actualidad Med. Per.*, 1936, **2**, 330.

blood cultures. Three of these had no past history of Carrion's disease; 2 had previously suffered from *verruca peruana*. It is possible that asymptomatic cases may constitute a reservoir of virus which is important in the natural transmission of the disease.

Peruvian guinea pigs were found to be spontaneously infected with an unidentified bartonella. This infection has not been found in guinea pigs from other regions than Peru, but was transmitted to splenectomized previously uninfected stock guinea pigs obtained in Boston. This guinea pig bartonella is being further studied, in an attempt to identify it more accurately.

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Carrion's Disease. V. Studies on *Phlebotomus* as the Possible Vector.

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Shannon¹ collected *Phlebotomus* sandflies in the verruga zone of the Rimac Valley, Peru, and shipped them under refrigeration to New York where they were injected into monkeys.² Four of 10 batches, including chiefly *Phlebotomus noguchii* and *P. verrucarum* produced inapparent infection with *Bartonella bacilliformis* as evidenced by blood culture. Microscopic examination of these sandflies was not reported. These experiments demonstrated the harboring but not the actual transmission, of bartonella by *Phlebotomus*, since no living sandflies were used.

During March to June, 1937, the writer collected sandflies in Verrugas Canyon where much of Shannon's field work was done. Houses yielded almost entirely *P. verrucarum*; material from excavations included both *P. noguchii* and *P. verrucarum*. They were collected and transported in 10x75 mm. cotton-stoppered test tubes, inverted in moist pots. Moist plaster-lined pots were used for rearing.³ Larvae were fed on moist guinea pig's feces. A second method was devised, permitting more ready examination of larvae

¹ Shannon, R. C., *Am. J. Hyg.*, 1929, **10**, 78.

² Noguchi, H., Shannon, R. C., Tilden, E. B., and Tyler, J. P., *J. Exp. Med.*, 1929, **49**, 993.

³ Young, C. W., and Hertig, M., *Proc. Soc. Exp. Biol. and Med.*, 1926, **23**, 611.