# A Serological Survey of Sera from Domestic Animals on Easter Island

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SUMMARY

Animals' sera collected on Easter Island from December 1964 to February 1965 were tested by appropriate methods for the presence of antibodies to various infections. These included, ornithosis, Q-fever, brucellosis, Johne's disease, leptospirosis, toxoplasmosis and vesicular stomatitis viruses. It appeared that the cattle and sheep were exposed to the ornithosis group of agents. The sheep were also exposed to toxoplasmosis. The low-grade reactions observed on the cattle sera with the leptospira and brucella antigens were not sufficient to indicate past infection. All sera tested with Q-fever and Johne's disease antigens gave negative reactions. The results suggested that neither strain of vesicular stomatitis virus had yet been introduced into this restricted animal population.

RESUME

Des sérums d'animaux prélevés à l'Ile de Pâques de décembre 1964 à février 1965, furent éprouvés par des méthodes appropriées pour déceler des anticorps contre diverses infections. Celles-ci comprenaient: l'ornithose, la fièvre-Q, la brucellose, la maladie de Johne, la leptospirose, la toxoplasmose et la stomatite vésiculeuse. Il devint apparent que les bovins et ovins furent exposés à un agent du groupe de l'ornithose. Les moutons furent aussi exposés à la toxoplasmose. Les faibles réactions données par les sérums bovins avec les antigènes de la leptospirose et de la brucellose ne furent pas suffisantes pour indiquer une infection antérieure. Tous les sérums éprouvés avec les antigènes de la fièvre-Q et de la paratuberculose se sont révélés négatifs à ces infections. Les résultats ont suggéré qu'aucun des virus de la stomatite vésiculeuse n'avaient encore été introduits dans cette lointaine population animale.

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## INTRODUCTION

Easter Island is located some 2300 miles west of the coast of Chile, of which country it is a possession. It represents a very isolated spot on the earth's surface. From the 13th of December, 1964, to the 12th of February, 1965, a medical-veterinary survey was carried out by a group of specialists from various parts of the world under the direction of Dr. S. C. Skoryna of McGill University, Montréal.

As part of the veterinary survey, serum samples were collected from cattle, sheep and swine, and the results of the examination of these sera for the presence of antiodies to various disease conditions represent the content of this paper.

Some 38,000 sheep are kept on the Island for wool production. These are of the Chilean Merino breed and are kept on some 14,000 hectares (34,580 acres) of land. There are about 2,500 head of cattle of mixed breeding, most of which are owned by the Islanders and are grazed collectively on a community pasture. Approximately 200 pigs are kept by the Chilean Navy at their farm at Mataveri. Introductions of breeding stock to the Island are infrequent, so that these populations represent fairly stable epidemiological groups.

## MATERIALS AND METHODS

### SERA

Blood samples were taken from 57 cattle by jugular puncture into 10 ml. vacutainer tubes. Additional blood samples were taken from 146 sheep and 13 pigs, at slaughter into sterile 4 oz. bottles. After collection, the blood was allowed to clot in a refrigerator for approximately 12 hours, at which time the serum was removed and stored frozen at –70°C until delivered to the Animal Diseases Research Institute.

At the A.D.R.I. sera were kept at –20°C until tested. They were inactivated in a water-bath at 60°C for 30 minutes before submission to the complement-fixation and serum neutralization tests. They were not heat-inactivated for the agglutination tests.

The cattle sera were tested with ornithosis, Q-fever, brucellosis and Johne's disease antigens using the complement-fixation test. They were also tested for brucellosis using the agglutination test, for leptospirosis using the microscopic-agglutination test (agglutination-lysis) and for vesicular stomatitis using the serum neutralization test.

The sheep sera were tested for ornithosis and toxoplasmosis using the complement-fixation test.

The swine sera were tested for brucellosis using the agglutination test and for vesicular stomatitis using the serum neutralization test.

### COMPLEMENT-FIXATION TESTS

The general procedure described in the Standard Methods of the New York State Department of Health (10) for the titration of complement and amboceptor was followed closely. The test was standardized on the basis of the 50% haemolytic unit of complement. The unit of complement is the amount of fresh, frozen guinea-pig serum necessary to haemolyse 50% of the 2.5% maximally sensitized sheep red blood cells. The density of the non-sensitized red blood cell suspension was adjusted to a 5% concentration by means of a photoelectric colorimeter. The reading of the degree of haemolysis was made with the aid of a color standard prepared with the day's reagents. All dilutions were made in veronal buffered saline containing Mg and Ca ions. Each reagent employed in the test was added in 0.1 ml. amounts, giving in every case a final volume of 0.5 ml. For fixation, the test-serum, the complement and the antigen mixture was incubated at 9°C for 18 hours followed by an additional 30 minutes incubation at 37°C after the addition of the sensitized red blood cell suspension.

*Antigens.* Five different antigens were used in the complement-fixation test. The *ornithosis antigen* was a phenolized, boiled group-reactive antigen prepared from yolk sacs from embryonated eggs inoculated with the virus of enzootic abortion of ewes (2, 5). Normal control antigens were prepared in a similar way from yolk sac membranes of non-inoculated embryonated eggs.

The *Q-fever antigen* (American Strain — Nine Mile) was prepared commercially and bought from Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.

The antigen used in the *brucellosis* complement-fixation test was our stock tube agglutination test antigen. This consists of an 8% suspension of *Brucella abortus* (strain 413) in physiological saline containing 0.5% phenol. This antigen was further diluted 1:200 before incorporation into the test (7).

The complement-fixation antigen for *Johne's disease* consisted of a polysaccharide fraction prepared by phenol extraction of bacilli that had been disintegrated by ultrasonic vibration (1, 8).

The *toxoplasmosis antigen* used in the complement-fixation tests was prepared as described in a previous publication (9) from infected embryonated chicken egg membranes and fluid.

### AGGLUTINATION TESTS FOR BRUCELLOSIS

The sera were tested by both the tube and plate agglutination tests using the official technique employed in Canada.

*Tube agglutination test.* In this test the stock antigen was an 8% suspension of

TABLE I Complement-fixation Test of 57 Cattle Sera Using Ornithosis Group Reactive Antigen and Normal Control Egg Antigen

| Infected ornithosis antigen | Normal control egg antigen | | | | | | |
|---|---|---|---|---|---|---|---|
| | Serum titres | | | | | | |
| Serum titres | — | 1:5 | 1:10 | 1:20 | 1:40 | 1:180 | 1:160 |
| — | 1 | | | | | | |
| 1:5 | 1 | | | | | | |
| 1:10 | 8 | 3 | 3 | | | | |
| 1:20 | 8 | | 7 | 4 | | | |
| 1:40 | 2 | | 2 | 2 | 9 | | |
| 1:80 | | | | | 1 | 3 | |
| 1:160 | 1 | 1 | | | | | 1 |

*Brucella abortus* (strain 413) in physiological saline containing 0.5% phenol. The test antigen was a 1:200 dilution of the stock suspension in phenolized physiological saline which was delivered in 2 ml. amounts in 99 x 11 mm. tubes. Each serum was tested in three dilutions (1:50, 1:100, 1:200) by distributing 0.04 ml., 0.02 ml., and 0.01 ml. to respective tubes containing the 2 ml. of test antigen. The test was incubated for 24 hrs. at 37°C, followed by standing at room temperature for 18 hrs. before reading.

*Plate agglutination test.* The antigen consisted of a 12% *Brucella abortus* (strain 413) suspension in an 8% sodium chloride solution containing 0.5% phenol. The test was set up by mixing 0.04, 0.02 and 0.01 ml. of the serum (1:50, 1:100 and 1:200) with 0.03 ml. of the above antigen on a glass plate. After mixing, the test was incubated in a humidity box at 37°C for 20 minutes, then the reading was recorded.

MICROSCOPIC-AGGLUTINATION TEST FOR LEPTOSPIROSIS

This test, also called agglutination-lysis test, was conducted on the cattle sera using the following leptospiral serotypes: *L. pomona, L. canicola, L. sejroe, L. icterohemorrhagiae, L. autumnalis, L. ballum, L. grippotyphosa, L. bataviae.* The test was conducted as previously described (3, 6). In summary, the sera were screened using 0.1 ml. of 1:10 serum dilution plus 0.1 ml. of antigen consisting of a 7- to 14-day-old motile culture of *Leptospira.* When a reaction was obtained in this dilution, the test was repeated using tenfold dilutions from 1:10 to 1:10,000 inclusive. The tests were incubated for 4 hours at 37°C, and the reactions evaluated in a microscope fitted with a darkfield condenser.

SERUM-NEUTRALIZATION TEST

Fifty-seven bovine, 6 ovine and 10 porcine sera were each tested for the presence of neutralizing antibody to the New Jersey (V.S.N.J.) and Indiana (V.S. Ind.) strains of vesicular stomatitis virus. The technique employed followed closely that previously described (4). Allantoic fluids infected with V.S.N.J. or V.S. Ind. viruses were prepared and held frozen until used. Neutralization

TABLE II Complement-fixation Test of 146 Sheep Sera Using Ornithosis Group Reactive Antigen and Normal Control Egg Antigen

| Infected ornithosis antigen | Normal control egg antigen | | | | | | |
|---|---|---|---|---|---|---|---|
| | Serum titre | | | | | | |
| Serum titres | — | 1:5 | 1:10 | 1:20 | 1:40 | 1:180 | 1:160 |
| — | 1 | | | | | | |
| 1:5 | | 3 | | | | | |
| 1:10 | 5 | 2 | 32 | | | | |
| 1:20 | 1 | | 24 | 46 | | | |
| 1:40 | | | 3 | 6 | 16 | | |
| 1:80 | | | 1 | | 3 | 2 | |
| 1:160 | | | | | | | 1 |

**TABLE III Leptospirosis Test of 57 Cattle Sera**

| No. of sera | Reactions in 1:10 dilution |
|---|---|
| 11 | Negative |
| 10 | *L. autumnalis, L. ballum* |
| 1 | *L. autumnalis, L. ballum, L. bataviae* |
| 2* | *L. autumnalis, L. ballum, L. icterohemorrhagiae* |
| 1 | *L. autumnalis, L. ballum, L. sejroe* |
| 1 | *L. autumnalis, L. grippotyphosa* |
| 28 | *L. ballum* |
| 3 | *L. ballum, L. icterohemorrhagiae* |

*One serum reacted in 1:100 dilution with *L. autumnalis* antigen, the other one reacted 1:100 dilution with *L. autumnalis* and *L. ballum* antigen.

tests were conducted using the same lot of infected egg fluids throughout. For the test, allantoic fluid was diluted in thioglycollate broth in ten-fold dilutions from $10^{-1}$ to $10^{-8}$. All sera were diluted 1:5 in the same diluent and 0.5 ml. amounts added to 0.5 ml. of each virus dilution. Serum-virus mixtures were held on ice for 30 minutes before inoculation into the allantoic sacs of 9-day-old embryonating eggs, one group of four eggs being inoculated with each dilution. Eggs were candled daily and all typical deaths recorded. Known positive and negative control sera and a virus titration were included in each test, in order to provide standards of comparison.

## RESULTS

The results of the various tests were evaluated purely on a serological basis, because it was impossible to correlate them with clinical history of the animals.

*Ornithosis*: The results of the complement-fixation test of the 57 cattle sera using ornithosis group-reactive antigen are given in Table 1. In this Table it can be seen that 1 serum reacted in 1:5, 8 in 1:10, 8 in 1:20, 2 in 1:40 and 1 in 1:160 dilution with the positive ornithosis antigen, and no reaction were given with the normal control egg antigen. Another serum which reacted in 1:160 dilution with the positive antigen also reacted in 1:5 dilution with the normal control egg antigen. The serological reactions given by these 21 sera suggest that these animals had contact with an agent belonging to the ornithosis group. Thirty-six or 63 per cent of the 57 cattle sera tested reacted with the normal control egg antigen. This amount of reaction with normal egg antigen is greater than that observed with Canadian cattle, where approximately 30 per cent gave nonspecific reactions (2).

The reactions given by the 146 sheep sera with ornithosis group antigen are summarised in Table II. Five sera gave a reaction in 1:10 and 1 in 1:20 dilution with the positive ornithosis antigen suggesting contact with this group of agents. However, 139 or 95 per cent gave non-specific reactions with the normal control egg antigen.

*Q-fever*: None of the 57 cattle sera tested reacted in the complement-fixation test with this antigen.

*Brucellosis*: Of the 57 cattle sera tested by the brucellosis agglutination test 26 gave insignificant reactions in the 1:50 dilution in the plate agglutination test. In only 3 instances were similar reactions observed with corresponding sera in the tube agglutination test. None of the 57 sera reacted in the complement-fixation test.

None of the 13 swine sera tested showed evidence of reaction either in the plate or in the tube agglutination tests.

*Johne's disease*: No reactions were given by the 57 cattle sera in the complement-fixation test using Johne's disease antigen.

*Leptospirosis*: Eleven of the 57 cattle sera did not react with any of 8 leptospiral serotypes used in the microscopic agglutination test. The remaining 46 sera gave insignificant, low-grade reactions in the 1:10 serum dilution with the serotypes listed in Table III. These low-grade reactions might be non-specific or represent exposure to leptospirae which took place a long time previous to the test. In countries where vaccination is practised, such reactions are also of common occurrence.

*Toxoplasmosis*: The results of the toxo-

**TABLE IV Complement-fixation Test of 146 Sheep Sera Using Ornithosis Infected and Normal Egg Control Antigens**

| Infected toxoplasmosis antigen | Normal control egg antigen | | | | |
|---|---|---|---|---|---|
| | serum titre | | | | |
| Serum titre | — | 1:5 | 1:10 | 1:20 | 1:40 |
| —..... | 14 | | | | |
| 1:5..... | 23 | 5 | | | |
| 1:10..... | 53 | 23 | 8 | | |
| 1:20..... | 14 | 3 | 1 | | |
| 1:40..... | 2 | | | | |

plasmosis complement-fixation tests on the 140 sheep sera are listed in Table IV. Fourteen sera, 10 per cent, did not react with the infected or normal antigen. Twenty-three reacted in 1:5 dilution, 53 in a 1:10 dilution, 14 in a 1:20 dilution and 2 in 1:40 dilution with infected antigen for a total of 92 (63 per cent). These reactions suggest previous contact with this infection. An additional 40 sera, 27 per cent, gave false reactions with both the infected and the normal control antigens.

*Vesicular stomatitis*: The 57 bovine, 6 ovine and 10 porcine sera tested all proved to be negative for antibody of both the New Jersey and Indiana strains of vesicular stomatitis virus.

## DISCUSSION

Serological tests are only presumptive evidence of infection. Additional weight is given to the tests when paired samples collected at 2 or 3 week intervals show a marked increased in titre. In the absence of paired samples, high titres simply suggest past contact with the infectious agent.

It would appear that the cattle and sheep were exposed to the ornithosis group of agents and the sheep also to toxoplasmosis.

The low-grade reactions observed in the cattle sera with *Leptospira* and *Brucella* antigens were not sufficient to indicate past infection.

All sera tested with Q-fever and Johne's disease antigens gave negative reactions.

Testing for evidence of vesicular stomatitis virus was considered worth-while because animal importation to Easter Island in recent times has been from the Chilean mainland only, a region in which this disease is known to occur. The test of choice seems to be serum neutralization, because of the long-lasting antibody as compared to the shorter persistence of complement-fixing antibody (4). Results presented suggest that neither strain of V.S. has yet been introduced into this restricted animal community.

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