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A STUDY OF TWO STRAINS OF SAPROPHYTIC LEPTOSPIRAS ISOLATED IN THE IRANIAN REGION OF THE CASPIAN SEA.

by

B. Babudieri (*)

In September 1968 we have drawn a certain number of water specimens in various zones of Northern and Central Iran.

These specimens have been seeded in dishes of Zuelzer's culture medium and the cultures have been checked with frequent observations, for the period of one month.

In two dishes we have observed the development of leptospiras. These spirochaetas have been isolated in pure culture by filtering the washing-liquid of the dishes of Zuelzer's medium through a Millipore filter with a porosity of 0,2m μ and by seeding the filtered matter in Korthof-Babudieri's medium. In order to ascertain whether the two isolated strains are really pure and not constituted by mixtures of leptospiras of different nature, we have adopted the technical proceeding already described in another paper (B. Babudieri).

One of the strains isolated in this manner has been drawn from a slowly-flowing fresh water canal, in tea plantation, in the neighbourhood of the village of Khoshamian, in the region of the Caspian Sea. Suspected cases of leptospirosis had previously been signalled in this zone. This strain has been called "Khoshamian".

The second strain, instead, has been isolated from the waters of the Caspian Sea, drawn in the vicinity of the beach, by the small town of Shahsavar. This strain has been named "Caspian Sea".

It must be stressed that the Caspian Sea has a salinity of 14‰, which is sufficient to prevent a prolonged survival of pathogenic leptospiras and of saprophytic leptospiras living in fresh water.

The two strains are growing very vigorously in Korthof-Babudieri's medium; they are able to resist the action of copper sulphate at 1/100000, they grow in a medium added with 200γ/ml of 8-azoguanine and in a medium with 0,1% sodium bicarbonate according to Mazzonelli. For these characteristics and for their capacity to develop in Zuelzer's medium, the two strains are to be included among saprophytic leptospiras.

(*) Professor B. Babudieri, Istituto Superiore di Sanita, Laboratori di Microbiologia. Roma WHO/FAO Leptospira Reference Laboratory.

The two strains have been tested with 6 human sera containing antibodies for pathogenic leptospiras and able to agglutinate at a rather high titer the saprophytic leptospiras of the Semaranga and Andamana groups (Addamiano and Babudieri, Petelin and Cinco). These sera have been unable to agglutinate the strain "Caspian Sea"; the strain "Khoshamian" has been agglutinated in a single case and at the not very significant titer of 1:100.

The two strains have survived for some days in the media added with NaCl at 12‰., not in those with NaCl at 25‰. In this respect they have behaved very much like control strains of saprophytic leptospiras of our collection.

The systematic study of the two strains has been executed by preparing, with them, from the rabbit, the relative immune sera and by testing them with reference strains of all the water-leptospira serogroups we have known so far. On the other hand the immune sera prepared with these strains have been tested with the two strains under study.

To the reference strains of the various serogroups we have added some more strains representing serotypes of greater interest, as well as three strains (8m, 11d, 11m) isolated by Castelli and Cinco from the waters of the Adriatic, in the neighbourhood of the mouth of the Timavo, and not classified yet.

With the following strains there has not been any cross-agglutination in any of the two directions: "Veldrat S173", "Sâo Paulo", "CH 11", "Monte Valerio", "Cau", "Ausrisina", "Percedol 1", "8m", "11m", "11d".

The serum anti-"Caspian Sea" (titer 1/50.000) has agglutinated at 1:5000 the strain "Basovizza", at 1:20.000 the strain "San Giusto", at 1:500 the strains "Bovedo" and "Farneti".

Reciprocally, the strain "Caspian Sea" has been agglutinated only by the serum anti-"Basovizza" titer 1:50,000) at a titer of 1:50,000 and by the serum anti-"San Giusto" (titer 1:1000.000) at 1:100.000.

The only weak cross-agglutination obtained by the serum anti-Khoshamian (titer 1:100.000) has been that for the strain "Patoc 1", at a titer of only 1:100. The strain "Khoshamian" has not been agglutinated by any of the sera used in tests.

On the ground of the results of these experiences the strain "Khoshamian" is to be considered as the representative of a new serotype and serogroup which we have both named "Khoshamian".

The strain "Caspian Sea", instead, has shown a clear affinity with the serogroup "Basovizza" and is to be attributed to it. This group comprises two serotypes: "Basovizza" and "San Giusto", each of them being represented by the strain bearing the same name (Cinco and Petelin).

The cross-absorbtion tests of the agglutinins effected between these strains have given the following results:

SERUM SAN GIUSTO

	non absorbed	absorbed with "Caspian Sea"
San Giusto	1:5000	1:10
Caspian Sea	1:5.000	1:10
Basovizza	1:500	<1:10

SERUM BASOVIZZA

	non absorbed	absorbed with "Caspian Sea"
Basovizza	1:500	< 1:10
Caspian Sea	1:5000	1:10
San Giusto	1:1000	< 1:10

SERUM CASPIAN SEA

	non absorbed	absorbed with:	
		San Giusto	Basovizza
Caspian Sea	1:5000	< 1:10	1:5000
San Giusto	1:5000	1:10	1:2000
Basovizza	1:5000	< 1:10	< 1:10

These results prove that a substantial antigenic identity is existing between the two strains "Caspian Sea" and "San Giusto" they both posses, on the contrary, an antigen or a group of antigens which cannot be found in "Basovizza." "Caspian Sea" is consequently to be attributed to the "San Giusto" serotype, which is a component of the "Basovizza" serogroup.

The isolation and identification of this strain appears particularly interesting, be it because it has been isolated from sea water, though at a not very high salinity, be it because it has the same antigenic pattern of a strain isolated at a distance of about km. 4000, at Trieste, from fresh water. This antigenic identity and its biologic characteristics, particularly its being unable to grow in a medium containing a high concentration of sodium chloride, lead us to the opinion that the strain "Caspian Sea" should not be considered a genuine marine microorganism. It is likely to be a fresh water leptospira, possibly conveyed to the sea by the waters of some brook, able to survive for some time in salt water.

We still have to explain how it is possible for water leptospira strains of the same antigenic type to be found in regions so far away from each other, without obvious connections.

This fact is explainable in the case of pathogenic leptospiras which are very often the usual guests of small rodents or domestic animals, capable to emigrate actively or passively, but it can hardly be explained when saprophytic leptospiras are concerned, as they are not carried by any animal, are not spore-forming, they do not resist to drying, and are extremely sensitive to any bactericide environmental factor.

SUMMARY.

We examine two strains of saprophytic leptospiras isolated in the Caspian region of Iran. The first strain, "Khoshamian", has been isolated from a fresh water canal and is to be considered as the representative of a new serogroup and serotype of leptospiras, both called "Khoshamian." The second strain, called

"Caspian Sea", has been isolated from the waters of the Caspian Sea and has the antigenic characteristics of the serotype "San Giusto," belonging to the serogroup "Basovizza."

LITERATURE.

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