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FIRST REPORT OF JOHNE'S DISEASE IN IRAN (*)

by

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One of the most important diseases causing economic loss in many countries in the world is Johne's disease.

This disease has been described as a chronic infectious enteritis affecting cattle, sheep, goats and some other species of animals.

The causal agent, *Mycobacterium johnei* was found for the first time from an infected cow by JOHNE and FROTHINGHAM in Germany (1).

Other authors have since reported the disease in sheep, goats and buffalo in Europe, America and Asia, but until now the disease has not been definitely confirmed in any susceptible animal in Iran.

Since 1957, the Razi institute has been receiving reports from the southern region of Iran of a contagious chronic enteritis among the foreign breeds of cows (2).

Johne's disease was suspected, and research was carried out on the isolation of the organism until 1962.

Mycobacterium johnei was isolated for the first time from a specimen sent by the Razi institute's branch laboratory in Ahwaz, collected from cows of a herd belonging to the National Oil Company in Abadan.

Severe outbreaks of the disease were frequently seen from 1957 until 1962 in this area and in spite of the application of sanitary measures and the use of usual types of astringents and sulphonamides for sick cows, there was no favourable response to treatment; and more than 12 cows of the Sindhi and Jersey breeds died from the disease.

However, we have not yet observed or obtained any confirmation of the existence of the disease among the indigenous breeds of cattle in any part of the

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country, although we have tested more than 300 specimens collected at the Teheran slaughterhouse from cows of local breeds.

These cows were brought each day from different parts of Iran for slaughter for meat.

The specimens tested were collected from the cattle which were showing symptoms of weakness and diarrhoea.

In spite of attempts to isolate *Mycobacterium johnei* by the same techniques as those for the Abadan positive sample, we failed to recover the organism from any of the 300 specimens.

MATERIALS AND METHODS

Macroscopic examination : In a specimen of small pieces of affected intestine and mesenteric lymph nodes taken from a dead Jersey cow affected with chronic diarrhoea for several months and showing emaciation and weakness, specific lesions of Johne's disease were found, consisting of intestinal ulceration with congestion, wrinkled mucous membrane covered by whitish mucus and slightly raised with gelatinous liquid, and swelling of lymph nodes which contained serous infiltration.

Bacteriological examination: Direct microscopic examination of smears from the intestinal mucous membrane of the affected cow, stained by the Ziehl-Neelsen method, revealed many clumps of short acid-fast rods, 0.5 micron wide, 1-2 micron long and morphologically indistinguishable from *Mycobacterium johnei.*

Isolation of Mycobacteria on artificial media: The mucous membrane of the specimen was collected, treated with 5 percent oxalic acid in a grinder for suppression of growth of other organisms and concentrated by centrifugation for 20 minutes at 2 000 r.p.m. (3).

The sediment was inoculated onto 20 screw cap bottles of SMITH (4), and DUBOS (5) solid media (6) containing alcoholic extract of *Mycobacterium phlei* (7), chloramphenicol, penicillin and actidion.

The bottles were incubated at 38° C and examined at weekly intervals for evidence of growth.

After 8 weeks, small greyish colonies of *Mycobacterium johnei* were observed on the surface of some of the bottles, and following this, rapid growth of the organisms was attempted by several subcultures, using solid media slopes.

For growing the organism on liquid media, the mycobactin tween serum and mycobactin tween albumin Dubos media were used.

After 6 weeks, the first pellicles were seen on the surface of the liquid media, then by several subcultures, it was possible to obtain culture sufficient in amount to make johnin.

Animal inoculation: Cultures of *Mycobacterium johnei* from two bottles of solid media were suspended in 4 ml saline for injection into the following laboratory animals :

Young rabbit No 7 received 0.5 ml intravenously;

Young rabbit No 9 received one ml. intravenously;

Two guineapigs Nos 42 and 43, each received 0.25 ml. intraperitoneally;

Two guineapigs, Nos 47 and 48, each received 0.25 ml. subcutaneously.

After 5 months, rabbit No 9 died from severe chronic diarrhoea and emacination.

On post-mortem examination many clumps of acid-fast *Mycobacteria* were found in smears from the mucous membrane of the intestine.

A piece of the mucous membrane was treated with oxalic acid and inoculated onto synthetic solid slop medium containing *Mycobacterium phlei* extract, and after 6 weeks incubation, the first colonies of *Mycobacterium johnei* were seen on the surface of the medium.

The possibility of tuberculosis was excluded as neither of the injected guineapigs showed any tuberculosis lesions.

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SUMMARY

It is recognized that the occurrence of Johne's disease is established in most countries in the world among all species of ruminant.

The first case in this country was found in the foreign breed of cattle (Jersey) which showed the clinical disease, and it was confirmed by macroscopical and bacteriological examination.

Mycobacterium johnei was isolated on synthetic special media and by inoculation of laboratory animals.

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RESUME

Il est admis que la paratuberculose existe dans la plupart des pays du monde chez toutes les espèces de ruminants.

Le premier cas qui fut constaté en Iran a été suspecté chez des bovins de race étrangère (Jersey) et confirmé par des examens nécropsiques et bactériologiques.

L'isolement de *Mycobacterium johnei* a été réalisé sur des milieux spéciaux synthétiques et par inoculation aux animaux de laboratoire.

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RESUMEN

Esta admitido que la paratuberculosis existe en la mayor parte de los paises del mundo en todas las especies de rumiantes.

El primer caso, comprobado en Iran, se sospecho primeramente en bovinos de raza extranjera (Jersey), quedando confirmado despues en los examenes necropiscos y bacteriologicos.

Se realizo el aislamiento de *Mycobacterium johnei* en medios especiales sintéticos e inoculando a los animales de laboratorio.

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