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A BRIEF WORKING PAPER ON MYCOPLASMA DISEASES OF SHEEP AND GOATS IN IRAN (*)

by

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Mycoplasmosis in ruminants and poultry is a serious problem in certain countries, and causes important economic losses to the animal industry. The infection in small ruminants includes two important diseases:

1) Caprine Pleuropneumonia.

2) Contagious Agalactia of sheep and goats (Bridré & Donatien, 1923).

Much work has been done in Iran on Mycoplasmosis in recent years and particularly on Agalactia. The study was undertaken to determine the microbiologic and immunologic properties of Mycoplasma agalactia strains, and for preparation of a vaccine.

1. Caprine Pleuropneumonia

Caprine pleuropneumonia is one of the native diseases of Africa and Asia. The causative agent, Mycoplasma mycoides var. caprine produces in goats and sheep (Longley 1940) a contagious pulmonary disease. It may be confused with other pneumonic diseases, caused by certain viruses (Echthyma, Pulmonary goat pox), bacteria (Pasteurella or Preiz-Nocard) and parasites (Strongylosis).

Longley, 1951, suggests that for the differential diagnosis of the specific Caprine pleuropneumonia the following criteria should be applied:

1) Lobar pneumonia, spreading by oedematous infiltration of the parenchyma with bronchitis.

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2) Transmissibility of the disease by S/C inoculation of infective materials in healthy goats and sheep, and observing a progressive oedema with marked regional vascularity.

3) Observing the agent in the infective materials by dark-field microscopy.

4) Recovery of the organism in pure culture from the pathologic materials on special medium.

5) Absence of other microorganisms.

6) Absence of any untoward reactions in laboratory animals when injected with pleural exudates.

7) The agglutination test, according to the report of Klieneberger could be applied.

In Iran, Caprine Pleuropneumonia was recognized many years ago, and the causative agent was recovered first in 1957 by observers at the Razi Institute. During the last decade many cases of disease have been reported, and the organism was isolated from seven cases in pure culture on Edward's medium. Bacteriological studies were carried out and the close similarity to the Mycoplasma group was shown.

Control: Control where the disease is endemic is based upon vaccination. Va cine is prepared locally with formalinized suspension of lung lesion in saline (Stylianopoulos, 1933 and Hulin, 1943) and with a little modification is used in all parts of the country with satisfactory results.

2. Contagious Agalactia of sheep and goats

The disease caused by Mycoplasma agalactia is known to occur only in parts of Southern Europe, Northern Africa and specially around the Mediterranean coast. It may be acute with the presence of a slight and temporary fever, but is usually chronic, and its clinical features are located in the joints, the eyes, and in the mammary glands of female animals. Abortion occurs in pregnant sheep and goats.

Contagious Agalactia has been prevalent in Iran since long ago, and has been classed among the most infectious diseases of sheep and goats. Three important symptoms: mastitis, keratoconjunctivitis and joint lesions always occur together in infected animals. The mastitis is manifested by the usual symptoms; milk secretion diminishes and in many cases ceases. Sometimes the mammary glands atrophy, but this manifestation may be related to other causative agents. Laboratory examination is desirable and milk samples are the best materials; they have plenty of Mycoplasma organisms which can be grown on culture medium.

For collecting the milk, the Razi Institute provides vials containing serum broth with Thallium acetate at 1/4.000, and sends them to the Veterinary Services in different provinces; here they add 1 or 2 drops of suspected milk and send them to the center laboratory. This procedure stops the spoilage of contaminated samples occurring in the hot season, and especially for areas where rapid shipping of milk samples from the field to the laboratory is difficult.

Control measures. In the past the only practicable method of eradication of the disease in many countries was the slaughter of all infected flocks. But, because of the economic and social conditions of certain underdeveloped countries, adoption of a programme involving slaughter of infected animals is very difficult. On the other hand, animals that have recovered from the Mycoplasmosis are immune for a long period of time. For these reasons the emphasis is placed upon control by vaccination.

Although control by vaccination is much less valuable than the slaughter of infected animals, it seems to prevent the development of disease and to reduce losses to a small amount.

Attempts to immunize animals in various ways have been made by many investigators in different parts of the world. In Italy, Zavagli (1951) described two kinds of vaccines: the first was a killed formalized vaccine prepared from the mixture of infected milk and a serum broth culture of Mycoplasma agalactia and the second was a living attenuated vaccine prepared from mammary gland and brain tissue of experimentally infected lactating ewes, and adsorbed on aluminium hydroxide as an adjuvant.

In Spain, Lopez Y Lopez (1952) applied a killed formalin vaccine from a mixture of serum peptone veal infusion broth culture and of total chick embryo culture.

These vaccines had a prophylactic and curative effect and one or two injections of 5 to 10 ml by the S/C route protected ewes against a lethal dose of virulent culture, but certain authors mentioned that it gave for some time a post vaccinal reaction.

In the U.S.S.R. an attenuated vaccine adsorbed on aluminium hydroxide with saponin protected sheep and goats.

Shamir in Palestine (1954) failed to attenuate a virulent strain by 90 consecutive passages in chicken embryos.

In Iran, during the 5-year period, 1959-63, much work concerning the preparation of a vaccine against contagious agalactia infection was carried out at the Razi Institute with the object of making a vaccine which would give an acceptable immunity after one injection.

Vaccine preparation

The dried stock culture of the chosen strain of Mycoplasma agalactia is sown into peptone broth made with veal at pH 7,4. buffered by phosphate, to which has

been added 20 per cent filtered horse serum with 50 UT/ml of penicillin to prevent combination. After inoculation it is put in an incubator 37° C for 4 days. After growing, the purity tests are made and the organisms killed by adding 1/1000 of formalin, and the culture kept at 37° C for a period of 4 days. Safety and potency tests of vaccine are carried out on sheep and goats in lactation. This product is being used experimentally in some infected areas.

Before a vaccination plan could be applied in field conditions it was essential to carry out a series of studies on the biological properties and the effect of formalin at 37° C on the viability and pathogenicity of the vaccine strains.

An attempt was also made to determine the minimum infecting dose of several strains, estimation of the immunogenicity of the vaccine strains and the sensitivity of fat-tailed sheep and goats to the virulent challenge strains. The results may be summarized as follows:

1) A virulent strain, lyophilised and kept at laboratory temperature lost its pathogenicity after 3½ months. The other virulent strains caused severe reactions and death in a dose of 0.5 ml, which contained 500 infecting doses.

2) Female goats were found to be definitely more sensitive than female sheep.

3) 1 ml. of serum broth culture, 5-6 days old, contains 1,000 infecting doses. Lower dilution did not cause reactions.

4) Some strains were non-pathogenic and when injected into female goats were inoffensive even in very large doses; attenuated strains may set up a severe reaction in certain animals. Vaccine to be used as a single injection should not contain any living organisms.

5) Tests in female goats showed that a virulent strain set up a solid immunity. No immunity was produced by non-pathogenic strains. Equal degrees of immunity followed the use of a killed or an attenuated vaccinal strain. From this result, it is concluded that the vaccinal strain should be killed by the joint action of formalin and heat. Within the limits tested the amount of formalin used has no effect on the antigenicity of the vaccine.

6) There are good immunogenic strains of Mycoplasma agalactia and there are also strains which have no immunogenic value.

7) Immunity of female sheep against at least 100 infecting doses can be produced when a good immunogenic strain is used; female sheep, injected once with a killed vaccine show solid immunity against 100 infecting doses of a virulent strain.

8) Preventive vaccination, using a single dose of vaccine, is valuable in healthy flocks and in flocks at the beginning of an outbreak. (Bory & Entessar, 1962).

Miscellaneous work on serological tests in Mycoplasma Agalactia Infection in sheep and goats

Since the advent of programmes for the control of Mycoplasmosis, the specificity of serologic reactions to this infection has become a factor of major importance. Tube and Plate Agglutination, Complement fixation, Hemagglutination inhibition, and antiglobulin tests have been used.

Baharsefat and Adler, 1965, stated that the Hemagglutination-Inhibition (HI) is usually more specific and sensitive than the agglutination test. The antiglobulin procedure, however, has an advantage over the HI test for detecting antibodies of Mycoplasma gallisepticum in chicken and turkey sera and could have similar advantages in goats or sheep.

During the current year at the Razi Institute Baharsefat and Yamini, 1966 have carried out further work on serological tests for the detection of Mycoplasma agalactia antibodies in sheep and goats sera.

Antigens were prepared from a strain from the milk of a mastitis affected sheep, by the method of Adler **et al.** 1965 for M. gallisepticum. (Details will be provided on request.)

H. I. Test

Goat and chicken red cells gave similar results in the H.I. test the specificity of which is being further studied.

Plate agglutination test with stained Antigen

Tests were made with sheep and goat sera and promising results were obtained; 5 goats out of 10 and 4 sheep out of 107 being negative and the remainder positive to various degrees up to 1/80.

Stained Antigen Agglutination Tests with sera taken from experimentally infected sheep and goats up to 17 days after inoculation showed that peak titers were reached 5 to 7 days after intervenous injection but decreased at 11 to 12 days, whereas after s/c inoculation peak titers were higher and reached at 12 to 15 days, decreasing soon thereafter.

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