REPORT OF THE

EXPERT CONSULTATION ON RESEARCH ON TRYPANOSOMIASES

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

APPENDIX I

Report on Dourine Epidemiological Surveillance 1980 and Research in Italy

by

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1. INTRODUCTION

Dourine has reappeared in Italy in 1975 after 25 years from the last reported case.

Its presence has fostered field and laboratory work finalized to diagnosis, control and research on epidemiology and immunological mechanisms.

2. EPIDEMIOLOGICAL SURVEILLANCE

2.1 Distribution of the disease

The first clinical case of the disease has been diagnosed in August 1975 in the Marsica mountainous zone of the Abruzzi Region (Central Italy). The veterinary service of the whole nation was, therefore, alerted and further evidence of disease presence was found in the Marsica as well as in various other Italian Regions.

An extensive sero-epidemiological survey, was consequently carried out by Regional and private veterinarians. This survey was carried out by clinical and/or serological examinations of solipeds (horses, donkeys and mules) resident in the zones where the disease was reported. The serological test employed was the micro-complement fixation test (Caporale et al., 1976).

The disease, in addition to the outbreaks in the Abruzzi was reported in the Lazio, Umbria, Marche, Romagna, and Sicily Regions. On epidemiological evidence it was concluded that in the latter Regions, the outbreaks were secondary to the primary outbreaks in the Abruzzi. The Italian Veterinary Authority enforced a slaughter policy of horses clinically and/or serologically positive, to face the epidemic. The stamping-out was successful and the national territory was considered free of the disease by the end of 1976 but for the Marsica in the Abruzzi Region, where the disease became endemic. A special epidemiological study was carried out in the solipeds population of the latter territory to: (1) identify the geographic limits of the endemic zone; (2) ascertain the prevalence of the infection; (3) study the disease dynamics.

2.1.1 Epidemiological survey and surveillance in the Marsica (Abruzzi)

The survey was carried out, before the beginning of the 1976 mating season. Each

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sampled of the Marsica zone bled three times, at approximately 20 days interval, and the sera were tested by complement-fixation. For each animal relevant epidemiological informations were collected and recorded on special forms. An Olivetti P6060 computer and a specifically designed software (SOMOCO) were used for data storage and analysis. A total of 2,841 horses, 1,255 donkeys and 200 mules were examined. The average prevalence rates were 7.4% and 1.3% in the horse and in the donkey populations respectively. The prevalence rate in horse stallions was 16.1%; in horse mares 6.8%; in geldings 7.6%; in sexually immature males 4.5%; in sexually immature females 5.4%. The infection was not evenly distributed on the whole territory. A statistically significant clustering was evident in certain zones, while other appeared to be free. Furthermore on the basis of the analysis of the data it has been concluded that: (1) the infection was introduced in the population at least one breeding season prior to the one when the survey was carried out; and it went unnoticed because of the low incidence rate, the paucity of clinical symptoms, and poor animal health surveillance; (2) the spread of the infection began among horses kept on high range pastures, where they lived in small groups, each with its own stallion. Subsequently the infection passed to the foothill and spread by the males of the studs; (3) although in very few cases non-sexual transmission cannot be excluded the coital route appeared the only epidemiologically relevant. The infected male being the main vector of the spread of the epidemic.

Therefore existing Italian veterinary regulation that no stallion be kept unless authorized for stud, and periodically subjected to veterinary control would be, if correctly enforced, sufficient to eradicate the disease also in the endemic zone, provided that only serologically negative mares are brought to service.

3. DIAGNOSTIC TEST DEVELOPMENT

Four serological techniques have been compared with micro-complement fixation (CF); immunoelectrosmophoresis (IEO), indirect immunofluorescence (IF), solid phase radioimmuno assay (RIA), and solid phase enzime linked immuno-sorbent assay (ELISA).

For comparison purpose 115 sera from 2 experimentally infected horses, and 2 horses naturally affected by Dourine, 94 sera from field cases either positive to CF test or with clinical symptoms of the disease, and 64 sera from horses of areas known to be free of the disease, were used. The results of the comparative tests indicated that specificity was similar for all tests. As for sensitivity, the number of sera considered positive by CF was similar to those considered positive by ELISA, but titers with the latter were higher (4 to 7 fold). The titers obtained by RIA were also higher than CF titers, but a fairly high number of sera considered positive by CF and ELISA resulted to be negative (46/183). This discrepancy was probably due to the type of antigen used for RIA, and further trials are in progress to test this hypothesis. Results with IEO confirmed earlier work (Caporale et al. 1976).

4. TRYPANOSOMA EQUIPERDUM ANTIGENS

Proteic antigens of Trypanosoma equiperdum have been isolated and are being characterized. 'In vitro' cultivation assays of T. equiperdum have been unsuccessful; therefore, for parasite production, albino rats were used. Parasites, purified from rats blood by low speed centrifugation and DEAE-chromatography, were frozen and thawed three times and sonicated. The sonicated material was centrifuged at high speed and Sephadex chromatography, and rocket immunoelectrophoresis were carried out. With the latter technique 3 lines of precipitation were observed. By column chromatography, 6 soluble protein fractions were obtained. Before chromatography treatment with urea 2 and 6 M, ether, and lypase had no influence upon the precipitating antigens, while urea 8 M, trypsin hydrolisis, chymotrypsin, pepsin, papain and lysozime resulted in loss of activity, as determined by rocket immunoelectrophoresis. Antigenic activity was evidenciated by ELISA test in 3 of the 6 fractions isolated by sephadex chromatography. Glycoprotein were present in different

concentration, in all of the three fractions; in one of the three also lypids were present.

At present, further biochemical and antigenic characterization of the various fractions is under investigation. Lymphocytes transformation assays with the different isolates are also in progress. It is hoped to identify the antigenic fractions responsible for humoral and cell-mediated immune response, respectively. If these attempts will be successful immunization assays with inbred strains of experimental animals and transfer of immunocompetent cells from immunized donors to susceptible recipients will be carried out. This to clarify the role in protection against T. equiperdum infection, of humoral and cell-mediated immune responses, respectively.

5. IMMUNOLOGICAL ASPECTS OF TRYPANOSOMA EQUIPERDUM INFECTION IN THE HORSE

The immunological response of two horses with clinical history of Dourine (EMCM) and of two horses infected experimentally with Trypanosoma equiperdum (EIS-Try) has been studied.

After infection a statistically significant increase of B-lymphocytes was observed in the EIS-Try, while no increase was observed for T-lymphocytes. Antibodies against T. equiperdum appeared 6-days post-inoculation in both animals and persisted until death, 50 and 68 days post-inoculation respectively. In both EIS-Try animals B-lymphocytes and antibody curves showed oscillations, which were more marked in one of the two. Trypanosoma was observed in the peripheral blood from the 8th day post-inoculation. While it persisted constantly, until death in one animal, in the other disappeared cyclically from the peripheral blood. Circulating monocytes behaved similarly. Although monocytosis was present in both horses, in one animal monocytes levels were constantly above 15%, while, in the other, values under 10% were observed (normal range in the horse being 4-8%).

Also in the two naturally infected horses (EMCM) mean B-lymphocytes values were above normal; T-lymphocytes, on the contrary, remained within normal range. Circulating antibodies were constantly present in both horses, and a marked increase of CF titers was observed in the oestral period. In the same period in one of the horses a recrudescence of the clinical symptoms was also observed. Monocytes in both horses were always within normal range and Trypanosoma was never observed in the peripheral blood of either animal.

In the two EIS-Try circulating antibodies, B-lymphocytes, and monocytes increase, either preceded or closely followed parasites level increase in the blood. Although B-lymphocytes functionality has to be tested further, specific immunosuppression does not seem to be present. Trials with heterologous antigens and mitogen transformation assay are under investigation at present. Also T-lymphocytes activity has to be tested further. Although some evidence of '*in vivo*' specific delayed hypersensitivity response is already available, on the basis of some preliminary results it seems that, at least in one chronic infection case, mitogen transformation of T-lymphocytes is depressed.

In conclusion, it is hypothesized that in the acute phase of infection disappearance of circulating parasites probably correlates with monocytes activity. Efficiency of monocytes is increased by specific antibody activity, which at present is not known whether complement dependent or not. Antibody formation, however, is probably not necessarily dependent on parasite presence in the peripheral blood.

All research on Trypanosomiasis, whose summary has been presented in this paper was carried out with grants of the Direzione Generale dei Servizi Veterinary - Ministero della Sanità - Roma (Italy).