NUCLEAR AND RELATED TECHNIQUES IN CONTROL AND EPIDEMIOLOGY OF HAEMOPARASITIC INFECTIONS

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Introduction

Two genera of intra-eryhtrocytic protozoa cause diseases of major importance, *Babesia* causing babesiosis in cattle, sheep, horses and dogs; and *Theileria*, causing theileriosis in cattle and sheep. Babesiosis is one of the most common infection of free living animals world wide and is gaining increasing interest as an emerging zoonosis in humans. These diseases cause high morbidity and mortality rates in livestock production mainly in tropical and subtropical regions of the world. All babesial parasites described to date are transmitted by ixodid ticks to their vertabrate hosts. . They are piriform, round, ameboid, or rod-shaped, depending in part on the genus. The severity of the disease is attributed to erythrocyte destruction and plugging of capillaries with parasitized erythrocytes leading to impaired organ function. They occur in the erytrocytes; some genera occur in the leukocytes or other blood system cells as well. In Turkey, babesiosis is seldom lethal, at least if diagnosed early and cured, but it is always associated with a reduction of the profit from both cattle and sheep breeding.. *Babesia bovis, B.divergens*, and *B.bigemina* are the most important *Babesia* species of bovine babesiosis. Also, *B.ovis*, in sheep, has been found in all climatic regions of Turkey and causes a great economic losses in sheep breeding. Theileriosis is the one of the serious tick-borne diseases in cattle production in the Mediterranean littoral and the Middle East, extending eastward to India. The most important *Theileria* species is *T.annulata*, the causative agent of tropical theileriosis in cattle in Turkey. *Theileria* species occurs commonly in the lymphocytes and erythrocytes of the affected animals. The forms in the erythrocytes are round, oval, rodshaped or comma shaped. Some *Theileria* parasites enter lymphocytes and develop into forms called schizonts. All are transmitted by ticks. The mortality rate of babesiosis and theileriosis is as high as 30-90 % in untreated cases. Medication with anti-babesial and anti-theilerial compounds of affected animals is the main control of these diseases at present. However, in most cases, even if the animals survive, their productivity is severely impaired after recovery. In addition, the agents used for medication cause environmental pollution and the tick forms develop resistance to drugs in time. Vaccination of susceptible animals with antiparasitic vaccines proved to be the method of choice for control of these infections. Since, babesiosis and theileriosis are transmitted by tick, prevention and control depend primarily on tick elimination. For this reason, it is important to determine the areas where the vector ticks are present. Improved serological tests are essential for sensitive and specific diagnosis of the infections to determine the endemic areas. Enzyme-Linked Immunosorbent Assay (ELISA) is one of the serological tests which is simple to perform, higly sensitive and economical. Vaccination of susceptible cattle and sheep with antiparasitic vaccine has considerably reduce the losses caused by babesiosis and theileriosis. The application of safe and effective vaccine will have a beneficial influence on cattle and sheep husbandry whereever babesiosis and theileriosis are endemic.

Serology

Microscopic techniques are still the only appropriate techniques to diagnose acut disease A characteristic feature of this infections is that animals which recover from an acute infection become carriers of the respective haemoparasites. These carrier animals can not be diagnosed by stained films. Thus, in order to identfy infected animals it is necessary to develop serological method to detect specific antibodies and the detection of animals with subclinical infections. Also, in developing control strategies against haemoprotozoan diseases it is essential initally to obtain baseline data before the control programme is introduced, and then to constantly monitor the programme to asses its efficacy.

Immunoassays with their high specificity and widely applicable methodology have been gaining ground at the expense of the chemical or physical analytical methods. One group of such assays employing labelled reagents are at present dominating the immunoassay field.

The label whether it can be an enzyme (enzyme-immunoassay), an isotope (radioimmunoassay) or a flourescent molecule (fluoroimmunoassay), Serodiagnosis of haemoprotozoon diseases is necessary in order to find out the epidemiological status of these diseases. During the past 30 years, seroepidemiological studies using enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and indirect flourescent antibody techniques (IFAT) were used in the world. Also, the following serological tests have been used in the diagnosis of *Babesia spp.* infections: complement fixation (CF), indirect hemagglutination (IHA), gel diffusion, capillary agglutination test. The indirect fluorescent antibody test (IFAT) is widely applied for the diagnosis of human and protozoal diseases (Burridge 1971). The disadvanges of IFA test is its incovenience for the screening of large numbers of sera limit its use in epidemiological surveys. Usually not more than 70-90 samples per day should be exemined and also results are influenced by subjective judgement of the operator which makes standardisation diffucult (Böse et al,1995). In Turkey a number of studies were carried out to determine the epidemiology of theileriosis by using IFA test. (Çakmak et al 1993, Dincer et al,1991). In these studies, high incidence of tropical theileriosis was observed in southern and northern Turkey. The enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Babesia spp.* has been evaluated (Barry et al,1982, Waltishbuhl et al,1987) This technique has been used to detect antibodies against B.divergens, *B.major, B.bovis, B.bigemina* in cattle and *B.ovis* in sheep babesiosis. ELISA was developed to determine antibody levels in cattle infected with *Theileria parva* and *T.annulata,* using antigens prepared from the intra-erythrocytic piroplasms stage of the parasites (Gray et al 1980). The enzme-linked immunosorbent assay technique has been successfully used to detect antibodies against many haemoparasites of medical and veterinary importance. Its sensitivity is greater than that of other serological assays. It does not require expensive equipment and can be fully automated, making it suitable for screening of large numbers of serum samples within a short period. Until now only one large-scale serological survey of bovine babesiosis has been done using ELISA test in Turkey. From the data obtained in this seroepidemiological survey indicate that there is a high incidence of B. bovis infections of some regions of Turkey (Duzgun et al, 1992). Also, an ELISA for the diagnosis of B.ovis infection was developed at Sarayköy, Ankara Nuclear Center for Agriculture and Animal Science, Parasitology Laboratories. İn this study, a crude B.bovis antigen and a synthetic B.bovis-derived antigen were used to screen 1466 sera collected from sheep from 18 regions of Turkey. A high incidence of B.ovis positive reactions was found from all regions (60-80%) in sheep over 1 year old, while from two smaller samples the incidence in young sheep was much less (28 and 52%), (Duzgun et al, 1991). The development of radioimmunoassay (RIA) for the detection of antibody against *B.bovis* enables highly sensitive rapid semi-automated assays to be made. As little as 2-4 ng anti-*Babesia* antibody can be detected using this test (Wrigh,I.G.1984). The review by Wright et al (1988), described that RIA test was somewhat more sensitive than ELISA and it has less application since the radioisotope label ^{125}I has short half-life. It is for this reason that the ELISA is becoming more popular, even if it is somewhat less sensitive. RIA test was developed for the diagnosis of babesiosis at Sarayköy, Ankara Nuclear Center for Agriculture and Animal Science, Parasitology Laboratories But, this test has not been used in seroepidemiological studies in Turkey.

Vaccination

Various vaccination methods have been developed and used under laboratory and field conditions as immunoprophylactic agents against haemoparasitic diseases. Vaccination with attenuated live parasites has been demostrated to be the most effective control measure against babesiosis and theileriosis.

1.Vaccine Against Babesiosis

1.1 Attenuated Babesia Vaccine

The rapid passage technique was developed during 1960s and 1970s. Splenectomised calves were used to produce vaccine against *B.bovis* and *B.bigemina* parasites. It was observed that 20-30 passages were required and low pathogenicity were obtained by rapid passagess in calves. Commercial vaccines became available for both *B.bovis* and *B.bigemina* using the rapid passages technique after it was discovered. (Dalgiest et al 1981). Cell culture vaccines have been developed and used effectively in various countries for the control of these diseases. Principle of producing of culture-derived vaccines against theileriosis and babesiosis are following these criteria Cultivation of *Babesia* and *Theileria* parasites continously in vitro and to obtain avirulent populations of these parasites.

Preparing vaccines from these attenuated parasites and to evaluate the effectiveness of these vaccines. In vitro cultivation of *Babesia spp.* has been a research objective for the production of antigens for serologic test procedures and immunogens against babesiosis. Short- term in vitro cultivation of *B.bovis* was reported by Erp et al (1980). Further progress in the in vitro propagation of *B.bovis* resulted from the development of a microaerophilus stationary phase system (MASP) by Levy and Ristic (1980), Yunker et al (1987), Rodriquez et al (1983). These major advances have made large amount of parasite-derived material available for antigenic and immunogenic studies. In vitro cultivation for prolonged periods may result in the decrease or complete loss of virulance. Culture-derived babesia parasites have been successfully cryopreserved with polyvinylpryrolidone as cryoprotectant (Palmer et al, 1982; Vega et al, 1985).

1.2 Irradiated Vaccines Against Babesiosis

The use of gamma irradiation to induce stable virulent strains of Babesia spp. has been reported in numerous studies and these have been reviewed (Wright,I.G.1984.,Weilgama. et al,1988; Wright et al. 1988). Especially, the irradiation studies of *Babesia* parasites have been concentrated on *B.bovis*. Successful control of babesiosis has been achieved in Australia with a blood vaccine containing attenuated *B.bovis* parasites (Callow 1977). The likely mechanism of irradiation on parasites induced avirulance was conceived. In the first study of the effect of irradiation on *B.bovis* it was shown that with increasing doses of irradiation there was a linear decrease in viable organisms. Also, Wright et al (1983) was observed that *B.bovis* parasites which had either been frehly irradiated or had been reisolated from cattle infected 12 months previously with irradiated organisms were not transmitted transovarially by cattle ticks (*Boophilus microplus*). The parent unirradiated strain of *B.bovis* was readily transmitted in this manner after being in host cattle for 12 months. From the studies on *B.bovis*, it was shown that the irradiation dose of 350 Gy induce an avirulent state in a number of strains. These avirulent strains did not revert to virulance, even after one year, in a carrier animal, however, the parent virulent strains maintained their virulance under the same conditions (Wright et al, 1980). In other study, it was observed that on challenge with virulent parasites the cattle were inoculated with with *B.bovis* parasites irradiated at 300 Gy showed better protection than those that had received parasites irradiated at 350 Gy (Weilgama et al, 1988). A series of experiments were made the effects of irradiation on the infectivity of *B.divergens* and *B.major* in cattle (Purnell et al, 1979). In these experiments, the result of inoculating the calves with blood containing *B.divergens* piroplasms irradiated at doses of 240 to 400 Gy; it was observed that irradiation had a market effect on the pathogenic identity of the parasites. Not only were the prepatent periods extended, but also animals receiving piroplasm irradiated at 240 and 280 Gy had mild rections whereas those receiving piroplasms irradiated at 360 and 400 Gy had no overt reactions. On challenge the animals which had mild reactions were solidly immune and these having no reactions had limited protection. The result of experiment for *B.major*, it was observed that the intact calves can be protected against B.major challenge by the prior inoculation of piroplasms at 240 or 280 Gy doses. Philips (1970), working with rodent *Babesia* species demostrated that irradiation at doses of 400 or 600 Gy prevented intraerythrocytic parasites non-infective but that rats and mice inoculated with them were protected against homologous challenge. Halacheva et al (1977), studied on the effect of ionizing radiation on the virulence and the immunogenic properties of *B.ovis*. In this study, it was observed that irradiation with 300 Gy reduces the virulence of *B.ovis*, parasites irradiated in this dose are capable of producing a relatively permanent immuity.

2. Vaccine Against Theileriosis

The tick vector of Hyalomma spicies, which transmit *T.annulata* and infect cattle while the ticks suck blood. The sporozoites enter the lymphocytes and invade perpheral white blood cells where they develop via trophozoites into multinucleate schizonts, which grow and divide in synchrony and then differentiates into merozoites. The merezoites enter erythrocytes and become piroplasms. Schizonts of *T.annulata* can be cultivated and maintained in vitro. Tsur (1945), first cultivated *T.annulata* schizonts in lymhocytes in small explants of liver or spleen obtained from infected cattle. *T.annulata* schizonts grown in cell culture have replaced schizont-infected blood as the vaccine. Herds are usually protected by vaccination The culture derived vaccine against theileriosis have been developed and used effectively in various countries for the control of this disease (Gill et al,1976;Hasemi-Fesharki et al,1973; Ozkoç et al, 1981). It is important to know the duration of immunity provided by the cell culture vaccine in the vaccinated animals.

Determination of duration of immunity of calves vaccinated with the Theileria annulata schizont cell culture vaccine was studied by Beniwall et al (2000). In this study, it was observed that the calves challenged after 3 months of vaccination were fully immune and protected against Tropical theileriosis. In Turkey, culture-derived *theleria* vaccine is being produced in Pendik Veterinary Research Instutute and this vaccine was found to be partially effective on tropical theilerioisis since the incidence of tropical theileriosis was 1.9 % in vaccinated animals and 10.5 % unvaccinated control group animals in Central Anatolia Region of Turkey.

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