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Development of an Experimental Tissue Culture Vaccine Against Mediterranean Theileriosis in Spain

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With 2 figures

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Summary

Vaccines against Mediterranean theileriosis have been developed in several countries where the disease is an economic problem. Tissue culture vaccines have been widely and successfully used to immunize cattle. Although Mediterranean theileriosis represents a constraint to dairy cattle production in Spain, no vaccines against this disease have been developed previously. The successful development of a tissue culture vaccine consisting of attenuated *Theileria annulata* schizont infected cells from an enzootic area of Spain and its efficacy under experimental conditions is reported. Vaccinated calves were resistant to homologous challenge showing no signs of theileriosis while non-vaccinated calves showed typical signs of disease.

Introduction

Mediterranean or tropical theileriosis is a disease of cattle caused by the protozoan parasite *Theileria annulata*, which can potentially affect 250 million cattle (TAIT and HALL, 1990) from countries of the Mediterranean basin, the southern regions of the former USSR, the Middle East and areas of Asia such as China and India (PURNELL, 1978; DOLAN, 1989).

Traditionally, in many countries where Mediterranean theileriosis represents an economic problem, vaccines have been developed against the disease. With the development of the *Theileria* infected lymphoblast cultures (reviewed by BROWN, 1987), these cells have been widely used to produce vaccines. Such tissue culture vaccines are presently available and applied in Israel (PIPANO, 1995); in Iran, where a 14 year vaccination campaign has been carried out (HASHEMI-FESHARKI, 1988); and in India (SINGH et al., 1993), each using strains isolated in their own country. Vaccines derived from a local isolate have always been more successful in protecting against disease caused by an homologous strain (PIPANO, 1977; OZKOC and PIPANO, 1981).

In Spain, many cases of Mediterranean theileriosis have been reported since the first third of this century (SALVANS-BONET, 1928), and some areas are theileriosis-enzootic (BRANDAU et al., 1989; VISERAS, 1994; GARCÍA-FERNÁNDEZ et al., 1996). It is very important that a vaccine should be found to protect against a disease which is a constraint to livestock improvement in these areas. Recently, very high prevalences of Mediterranean theileriosis in dairy cows (BRANDAU et al., 1989) and in fighting bulls (GARCÍA-FERNÁNDEZ et al., 1996) have been reported in Spain. For these reasons, a protective vaccine could be very beneficial to the livestock industry.

This paper describes the development of the first experimental vaccine consisting of *T. annulata* infected lymphocytes from an enzootic area of Spain.

Material and Methods

Calves

All calves used in the experiments were from a non-enzootic area and were kept in tick proof stalls. These calves were tested by Giemsa-stained blood smears for the presence of piroplasms of *T. annulata* into erythrocytes, and the immunofluorescence antibody test (IFAT) for the presence of specific antibodies to determine if animals had previous exposure to *T. annulata*.

Source of T. annulata parasites

The *T. annulata* infected lymphoblastoid cell line 28E used was previously isolated from a Friesian cow in an enzootic area of Andalucía (southern Spain) which was acutely ill and died 3 days later due to Mediterranean theileriosis (VISERAS et al., 1997).

Culture for attenuation of the virulence

Once the *in vitro* culture was established, infected cells were grown in a monolayer cell culture in RPMI-1640 (Sigma Chemical Co., St. Louis, MO., USA) +20% (v/v) heat-inactivated fetal bovine serum (Sigma), at 37°C and 5% CO₂ (BROWN, 1987; PIPANO, 1989). These cells were subcultured every 3–4 days in order to get the attenuation of the virulence of *T. annulata* schizonts (BROWN, 1987; PIPANO, 1989). Cells were cryopreserved every 10 passages of culture in order to avoid the accidental loss of the cell line.

Considering that the number of passages needed to produce attenuation can be between 30 and 300 depending on the strain (PIPANO, 1989), it was decided to assess the degree of attenuation after 35 passages.

Checking the degree of attenuation

To assess the degree of attenuation, two *T. annulata*-free calves were inoculated subcutaneously with a cell suspension containing 5×10^6 infected cells in RPMI-1640 medium (without serum) from a 35th passage culture. The cells were harvested 1 h before the inoculation and kept in darkness at 4°C until inoculation into the calf.

Assessing safety and efficacy of the vaccine

The vaccine consists of a suspension of 5×10^6 non-virulent *T. annulata* infected lymphoblastoid cells inoculated subcutaneously into each host according to SINGH et al. (1993). Vaccinated animals were monitored for body temperature in the morning, anti-*T. annulata* antibodies by IFAT, piroplasms in Giemsa-stained blood smears, packed cell volume (PCV) and enlargement of lymph nodes, approximately weekly for 4.5 months. Afterwards, the calves were monitored monthly until challenged.

Challenge

In order to test the efficacy of the vaccine, two 8 month old post-vaccination calves, and two non-vaccinated, *T. annulata*-free control calves were inoculated with an homologous stabilate of 4×10^7 virulent schizont infected cells from a 15 passage culture. Afterwards, the inoculated calves were monitored twice a week for 2.5 months, in the same manner as described above.

Results

Attenuation of virulence

Total attenuation of virulence of the 28E cell line was achieved by continuous passage up to 35 (two or three per week) in *in vitro* culture, following the criteria of PIPANO (1989).

Rectal temperature, antibody titre and PCV of the two inoculated calves are depicted in

Fig. 1. There were no parasites detected in blood smears or in lymph node biopsies. There was no detectable increase in body temperature (Fig. 1a). The PCV values (Fig. 1b) were the same as those measured prior to inoculation. No lymph node enlargement or any other clinical signs of Mediterranean theileriosis (OUHELLI, 1985; NAVARRETE et al., 1992) were observed in these calves. As is shown in Fig. 1c, both animals produced specific antibodies against *T. annulata*, which reached the highest titres about 40 days post-inoculation.

Testing the efficacy of the vaccine

After challenge, both the vaccinated calves and the non-vaccinated calves (susceptible controls) were examined clinically and the responses recorded. Following the classification of IRVIN et al. (1983), responses were 'none' in the case of the vaccinated calves, whereas for the

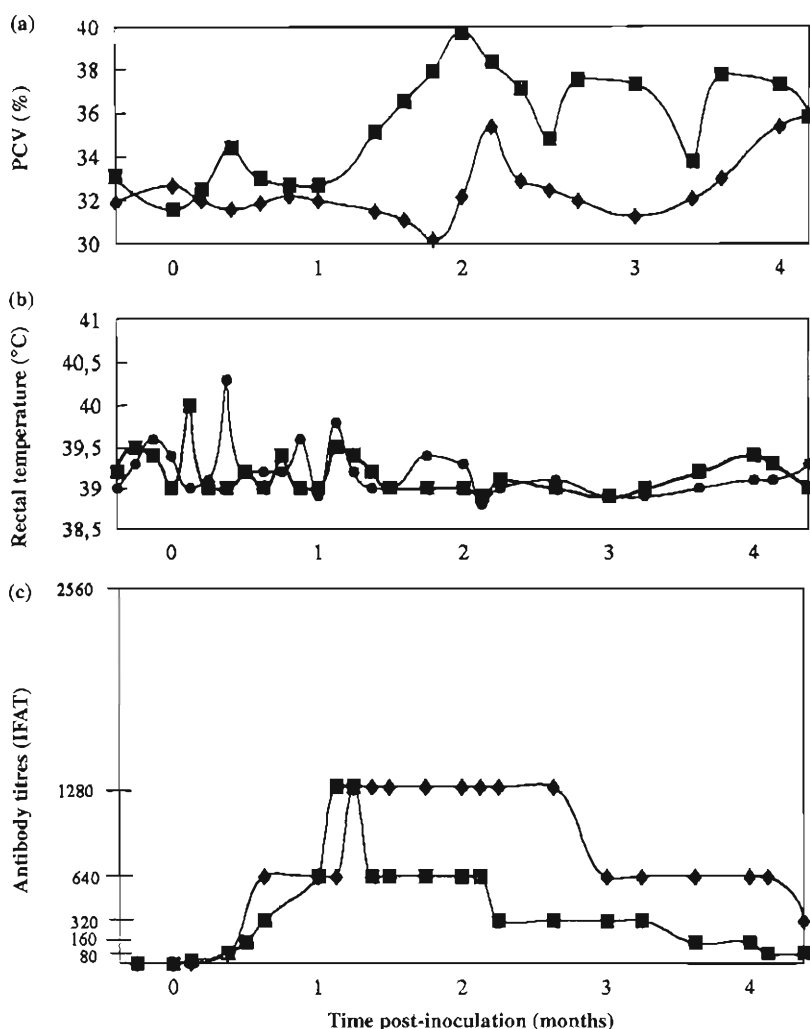


Fig. 1. (a) Percentage of packed cell volume (PCV), (b) rectal temperature (°C), and (c) titre of specific antibodies against *Theileria annulata*, measured by IFAT, of two calves inoculated with 5×10^6 *T. annulata* infected cells from a 35 passages culture, throughout time post-inoculation

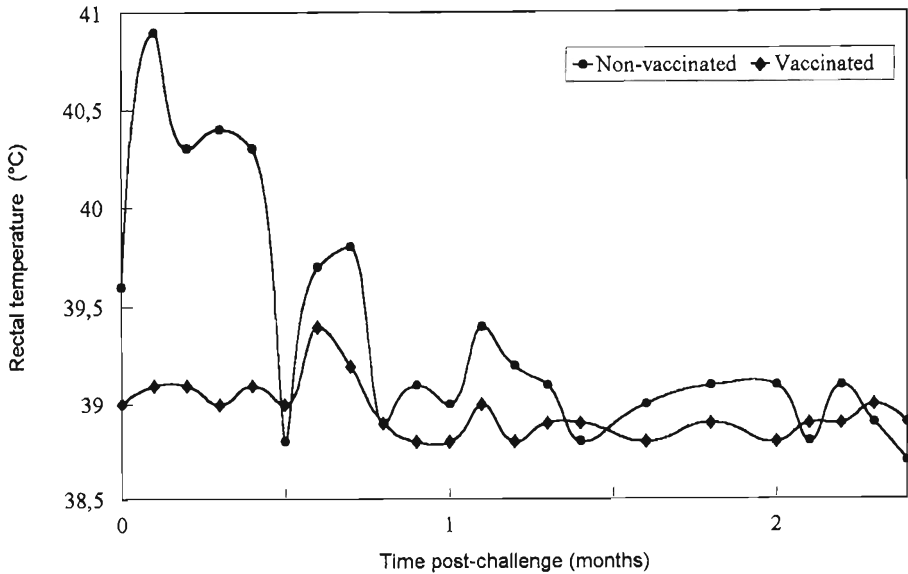


Fig. 2. Rectal temperature (°C) of the calves challenged with 4×10^7 *Theileria annulata* infected cells from a virulent culture, throughout time post-inoculation. Data depicted are representative of the two calves studied in each case

non-vaccinated calves the responses were 'moderate' with an absence of changes in the PCV values.

The host response in the non-vaccinated calves consisted of: 1. Increase in rectal temperature (Fig. 2); 2. Enlargement of lymph nodes close to the inoculation site, which was detectable for more than 2 months; 3. Intraerythrocytic piroplasms, detected for the first time on the 25th day post-inoculation; 4. Schizonts in enlarged lymph nodes which were detectable even 2 months post-inoculation; 5. Tearful, petechiae in eyes, and intense nasal mucus production over several days. These symptoms started 1 week after the challenge.

No harmful effects resulted from the challenge in the vaccinated cattle.

Discussion

As has been reported before (SINGH et al., 1993), prevention of tropical or Mediterranean theileriosis by control of the vector is not feasible and practical under field conditions. The chosen method is vaccination of cattle with vaccines produced from isolates common to the area in question. Such vaccines have proved to be only partially or non-effective in other areas with different strains of *T. annulata* (PIPANO, 1989). Hence OZKOC and PIPANO (1981) consider that it is preferable to use local strains, instead of strains isolated in remote areas. In Spain, although there are theileriosis-enzootic areas and many cases of the disease every year, no vaccine was available to prevent this problem of such sizeable economic importance until now.

PIPANO (1989) considered that total attenuation was achieved when inoculating the cells to the calves, these showed no increase in body temperature and no parasites were detected in lymph nodes or blood, and sufficient production of antibodies against *T. annulata* could be detected by serological methods (e.g. IFAT). Such totally attenuated cells could then be used as an attenuated live vaccine.

To check the degree of attenuation, 5×10^6 infected cells from a culture of 35 passages were inoculated. Following PIPANO (1979), it was considered that total attenuation was achieved because after inoculation there were no modifications of rectal temperature and PCV (Fig. 1a,b), or other symptoms of the disease. Schizonts and piroplasms were not detected. The conclusion

was that the virulence of the schizonts was attenuated at 35 passages, which was not much higher than the minimum (30 passages) necessary for this parasite (PIPANO, 1989).

To document the response of calves to the immunization process, the development of the antibody titres of animals after vaccination has been followed (Fig. 1c), which is considered a suitable method by many authors (PIPANO et al., 1969; PIPANO, 1971; MORZARIA et al., 1987; SUBRAMANIAN et al., 1989). An increase in titre of specific antibodies has been observed, with a maximum at 1–2 months post-inoculation, similar to results obtained by other authors (PIPANO and CAHANA, 1968; PIPANO, 1971; PIPANO et al., 1977).

Bearing in mind that the schizonts maintained in the cell culture of a low number of passages, inoculated into susceptible cattle, cause clinical theileriosis (PIPANO, 1979) and that SAMISH et al. (1984), in trials of experimental transmission of *T. annulata*, successfully infected several calves by inoculation with schizonts from a cell culture maintained in the laboratory, cells of the line 28E with a low number of passages in culture were used to produce an homologous challenge. After this, the different responses observed between the vaccinated calves and the non-vaccinated calves were evident. The vaccinated calves did not show any sign of the disease, whereas fever with temperatures between 40 and 41°C over several days was observed in the non-vaccinated calves (Fig. 2). Other typical signs of the disease were observed, such as enlargement of lymph nodes close to the inoculation area that was detectable for more than 2 months, and other signs noted earlier indicating protection occurred by the vaccination.

With these results, this study concludes that cell line 28E of bovine lymphocytes infected with *T. annulata* schizonts was completely attenuated after 35 passages in culture, and, inoculated in a dose of 5×10^6 from fresh cultures, it can be used successfully for vaccination of cattle against Mediterranean theileriosis. Trials of field immunization with this experimental vaccine are in progress to assess its efficacy under field conditions.

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