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# NON-SPECIFIC IMMUNIZATION AGAINST BOVINE TROPICAL THEILERIOSIS (*THEILERIA ANNULATA*) USING KILLED *CORYNEBACTERIUM PARVUM*

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## ABSTRACT

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Killed *Corynebacterium parvum* was used as an adjuvant for the production of non-specific resistance against *Theileria annulata* in cattle.

Groups of cross-bred (*Bos indicus* × *Bos taurus*) calves were administered *C. parvum* adjuvant subcutaneously and were then challenged with *T. annulata*-infected ticks on 45, 60 or 90 days later. The challenge caused mild reactions in the protected calves. None of the 10 immunized calves died due to theileriosis, whereas all three pairs of susceptible control calves died due to theileriosis.

It appears from this pilot study that cattle can be protected non-specifically with *C. parvum* adjuvant against *T. annulata*.

## INTRODUCTION

*Corynebacterium parvum*, a potent immunopotentiator, has been used as an adjuvant either alone or with live or killed vaccines of *Plasmodium* spp. and *Babesia* spp. (Clark et al., 1977), *Toxoplasma gondii* (Swartzberg et al., 1975) and *Trypanosoma cruzi* (Bomford and McHardy, 1979) to enhance resistance of animals against these parasites. This paper reports an attempt to use *C. parvum* to produce non-specific resistance against *Theileria annulata* infection in cattle.

## MATERIALS AND METHODS

### *Experimental animals*

Sixteen 8—12-week-old male calves of mixed breed (*Bos indicus* × *Bos taurus*) were used. The calves were procured from a farm where strict acari-

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cidal regimes are practised, and were maintained under tick-free conditions. Prior to the start of experiment, the *Theileria*-free status of the calves was ascertained by blood smear examination and by the complement-fixation test as described by Dhar and Gautam (1977).

*Parasites*

A virulent strain of *Theileria annulata* isolated locally and maintained cyclically in the tick *Hyalomma anatolicum anatolicum* and cattle, was used for challenge purposes.

The seed culture of *C. parvum* (*Propionibacterium acnes*-11829) was procured from the American Type Culture Collection, MD, U.S.A. The organisms were grown under anaerobic conditions, and killed *C. parvum* adjuvant was prepared following the method of Fisher et al. (1970) and finally freeze-dried in 5-ml amounts.

*Experimental design*

The calves were divided into 4 groups. Group I consisted of 10 calves, and Groups II, III and IV of 2 calves each. Each of the 10 calves from Group I was inoculated subcutaneously (s/c) with 200 mg of *C. parvum* reconstituted in 2.5 ml of distilled water. The inoculated calves were subdivided into Groups I(A), I(B) and I(C) consisting of 3, 4 and 3 calves and were challenged on Days 45, 60 and 90, respectively, post-inoculation by allowing 30 *H. a. anatolicum* adults (from a *T. annulata*-infected batch) to feed on their ears. Two susceptible control calves (Groups II, III and IV) were infected with each subgroup.

*Parameters studied*

The response of the host was evaluated by recording the rectal temperature daily and examining Giemsa's stained biopsy material aspirated from the right parotid lymph node from the day of its enlargement. Blood smears stained with Giemsa were also examined from the day when schizonts were first detected in lymph node biopsy smears. Haematology (haemoglobin (Hb) content, packed cell volume (PCV), differential leucocyte count) was done on each calf twice a week. The reactions were classified as mild when the maximum number of schizont-infected leucocytes detected was less than 1%, and severe when over 1% was detected. When an animal died, a post-mortem examination was conducted.

RESULTS

The results are summarised in Tables I and II. No significant differences in the reactions (prepatent period, day to onset of fever, maximum thermal

TABLE I

Reaction (mean values) of calves to challenge with *T. annulata*-infected ticks

| Particulars | Group | | | | | |
|---|---|---|---|---|---|---|
| | I(A) | II | I(B) | III | I(C) | IV |
| Day of challenge | 45 | — | 60 | — | 90 | — |
| Total number of calves | 3 | 2 | 4 | 2 | 3 | 2 |
| Mean prepatent period (days) | 14.7 | 11.9 | 15.3 | 11.0 | 15.3 | 11.3 |
| Mean time to onset of fever in days | 11.3 | 8.2 | 10.8 | 7.9 | 11.0 | 8.0 |
| Mean maximum febrile response (°C) | 40.3 | 41.6 | 40.2 | 41.4 | 40.0 | 41.6 |
| Mean time to onset of lymph node enlargement in days | 10.7 | 5.0 | 9.8 | 5.5 | 8.7 | 5.5 |
| Mean time to detection of schizonts in days | 12.3 | 7.6 | 11.8 | 7.8 | 12.7 | 7.9 |
| Mean maximum percent schizonts | 0.5 | 56.4 | 0.6 | 64.8 | 0.6 | 60.0 |
| Mean maximum percent parasitaemia | 0.3 | 60.4 | 3.5 | 59.8 | 0.6 | 68.5 |
| Mean maximum percent fall in Hb | 11.9 | 24.4 | 20.0 | 34.7 | 15.0 | 26.3 |
| Mean maximum percent fall in PCV | 26.5 | 38.2 | 28.1 | 45.3 | 33.2 | 41.5 |
| No. died | — | 2 | 1 | 2 | — | 2 |

reaction, day to detection of schizonts, percent schizonts and parasitaemia and percent fall in Hb and PCV) of animals in the three subgroups (Groups I(A), I(B) and I(C) were observed on challenge). One calf of Subgroup I(B) which died on Day 28 post-challenge showed severe congestion of lungs at the post-mortem examination. Nothing abnormal was detected in the other organs. On the other hand, all the control calves of Groups II, III and IV showed all the typical symptoms of severe theileriosis (Table I) and died within 21 days post-challenge due to acute theileriosis. Impression smears from lymph nodes, spleen, kidneys and abomasal ulcers showed the presence of schizonts.

## DISCUSSION

All the 10 calves inoculated with *C. parvum* were protected against a lethal *T. annulata* challenge which killed all the 6 control calves. One calf of subgroup I(B) which died on the 28th day of challenge, showed severe pneumonic changes and lesions were not suggestive of theileriosis. Thus, the non-specific resistance which developed as a result of inoculation of *C. parvum* alone was stronger and more durable than that observed against *Plasmodium* spp. (Nussenzweig, 1967; Clark et al., 1977); *Trypanosoma* spp. (Kierszenbaum, 1975; Murray and Morrison, 1979), *Babesia* spp. (Clark et al., 1977) and several types of tumours (Halpern et al., 1966; Smith and Scott, 1972; Milas and Scott, 1978; Avent et al., 1979) in rodents.

A fall in neutrophil count and a corresponding increase in monocytes following immunization and again after tick challenge is difficult to explain,

TABLE II

Mean differential leucocyte count of calves immunized with *C. parvum* and challenged with *T. annulata*-infected ticks

| Cell type (%) | Group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1A | | | 1B | | | 1C | | |
| | Pre-immunization | Post-immunization | Post-tick challenge | Pre-immunization | Post-immunization | Post-tick challenge | Pre-immunization | Post-immunization | Post-tick challenge |
| Neutrophil | 28.6 | 20.0 | 16.0 | 29.2 | 21.0 | 15.5 | 28.2 | 21.4 | 15.1 |
| Lymphocyte | 65.8 | 70.8 | 69.4 | 64.6 | 67.2 | 69.0 | 65.3 | 69.5 | 69.1 |
| Monocyte | 3.4 | 7.4 | 13.0 | 3.4 | 8.9 | 13.3 | 3.5 | 7.1 | 14.0 |
| Eosinophil | 2.2 | 1.8 | 1.6 | 2.8 | 3.0 | 2.3 | 3.0 | 2.0 | 1.8 |

though a transient neutropaenia has been reported in the peripheral blood of mice after immunization with *C. parvum* (Woodruff and Dunbar, 1973). An increased percentage of monocytes (macrophages) probably suggests that protection is mediated through them, either by releasing a soluble non-antibody mediator of immunity which destroys the invading parasites as observed by Clark et al. (1977) in *Babesia* and *Plasmodium* infection of mice, or by cytotoxic destruction of parasites by activated macrophages as observed by Milas and Scott (1978) in antitumour activity or due to cytotoxic means by arresting DNA synthesis as observed by Cottrell et al. (1977) in murine malaria. It is, therefore, not clear which of the above mechanisms operated in stimulating resistance in the present study against *T. annulata* challenge, and this needs further investigation.

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