The effects of oxytetracycline on *Theileria parva in vitro*

P. R. SPOONER

International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya

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SUMMARY

When bovine peripheral blood leucocytes were infected with *Theileria parva* sporozoites, immediate treatment with oxytetracycline (OTC) inhibited the development of sporozoites to mature schizonts. The extent of inhibition was dependent on drug concentration and duration of treatment. Concentrations of 5 μg/ml OTC, or higher, for 8 days completely inhibited the establishment of schizonts and their ability to transform host cells. A cytostatic effect on schizont-infected cell lines was found with three tetracyclines and was also demonstrated on uninfected lymphoblasts. The parasites were found to be sensitive to OTC during development to schizonts, but when mature and established within host cells, schizonts were not demonstrably affected. The infectivity of sporozoites and the binding of sporozoites to lymphocytes were not directly inhibited by OTC. The results may explain the action of tetracyclines when used prophylactically during immunization against East Coast fever, and also the reasons for the ineffectiveness of these drugs when used therapeutically during patent disease.

Key words: *Theileria parva*, East Coast fever, *in vitro*, oxytetracycline, immunization.

INTRODUCTION

East Coast fever (ECF) is an important disease of cattle caused by the protozoan parasite *Theileria parva* (Theiler, 1904). The disease affects cattle in a large part of East and Central Africa and is transmitted between cattle by the brown ear tick *Rhipicephalus appendiculatus*. Neitz (1953, 1957) reported that chlortetracycline and oxytetracycline (OTC) suppressed development of the disease following experimental tick-induced *T. parva* infections in cattle. Tetracyclines were administered at 10 mg/kg body weight, 8–12 times during 20 days from the day following tick application. Treated cattle recovered and were immune to homologous challenge. This prophylactic effect of tetracyclines has been exploited in the development of an 'infection and treatment' method of immunization (reviewed by Purnell (1977) and Radley (1981)). Tetracyclines appeared to be more effective when given during the incubation period of the disease, since they were found to have little therapeutic activity (Wilde, 1967; Mugera, Mworia & Munyua, 1973). Effective therapeutic drugs, parvaquone, buparvaquone and halofuginone, have been developed (Schein & Voigt, 1979; McHardy, Hudson & Rae, 1980; Dolan, 1981; McHardy & Wekesa, 1985) and buparvaquone has been shown to suppress disease when given at the same time as infection with *T. parva* sporozoites (McHardy & Wekesa, 1985; Mutugi *et al.* 1988). While buparvaquone shows considerable promise, tetracyclines, particularly OTC, are currently the drugs of choice for use in immunization. The mode of action of tetracyclines against *Theileria* is not understood. Neitz (1957) suggested that tetracyclines were schizonticidal, thereby causing a reduction in the numbers of schizont-infected lymphocytes which are detected following immunization. Wilde, Hulliger & Brown (1966) considered that the infectivity of the sporozoite stage of *T. parva* was inhibited by tetracyclines but Radley (1978) showed that this was unlikely, since sporozoites were infective for cattle following exposure to 160 μg/ml OTC for 1 h *in vitro*. However, the sporozoite preparations in this latter study were not quantified and therefore it was not possible to determine whether there was a reduction in the number of viable sporozoites. More recently, Dolan (1981) considered that tetracyclines act by inhibiting the growth of the rapidly multiplying infected cell thus slowing the development of the macroschizont. This was supported by the *in vitro* studies of McHardy, Haigh & Dolan (1976) who reported that the growth of a *T. parva*-infected cell line was inhibited by exposure to 100 μg/ml OTC.

The purpose of this study was to examine the effects of tetracyclines, particularly OTC, *in vitro*, which might be relevant to their action in cattle during immunization against ECF. The effects of these drugs on infected and uninfected bovine lymphocytes, on sporozoites, and on the infection and transformation of lymphocytes by sporozoites were studied.

MATERIALS AND METHODS

Cell lines and drugs

The lymphoblastoid cell line, IR.TpM.1 infected

with *T. parva* (Muguga) (Brocklesby, Barnett & Scott, 1961), was isolated from the peripheral blood of an infected bovine (Kurtti, Buscher & Hirumi, 1978; Kurtti *et al.* 1981). A cell line, H12, cloned by limiting dilution from the IR.TpM.1 line, was used. The *T. parva* (Marikebuni) cell line was an uncloned line originally isolated from an animal at the Kenya coast (Irvin *et al.* 1983). The uninfected T cell clone, A2, was derived following stimulation of bovine peripheral blood mononuclear cells (PBM) with concanavalin A (con A), and grown in the presence of T cell growth factor (TCGF) without continued addition of con A (Brown & Grab, 1985). The same clone was also studied after infection with *T. parva* (Muguga) (Brown & Logan, 1986). B cells were isolated from bovine PBM by staining with a monoclonal antibody that recognizes bovine Class II major histocompatibility complex antigens and selecting the positive cells using a fluorescence activated cell sorter (Lalor *et al.* 1986). These cells were infected with *T. parva* (Muguga) and then cloned by limiting dilution.

Oxytetracycline hydrochloride was supplied by Pfizer Ltd, Kent, UK, and tetracycline and chlortetracycline as hydrochlorides were purchased commercially (Sigma Ltd, St Louis, USA). Drugs were dissolved in growth medium, filtered through a 0·22 μm membrane filter (Millipore Corporation, California, USA) and then diluted in medium to the appropriate concentration before use.

Cell culture

Liebovitz's L15 medium (Flow Laboratories, Ayrshire, Scotland) containing 20% (v/v) foetal bovine serum (Flow Laboratories) was used for the culture of all *T. parva*-infected cells utilizing culture methods similar to those reported previously (Brown, 1979). RPMI 1640 with 20 mM HEPES and 20% foetal bovine serum (Flow Laboratories), supplemented with T cell growth factor (Brown & Grab 1985), was used for the uninfected T cell line. Cell lines for growth rate studies were seeded at 1×10^5 or $1{\cdot}5 \times 10^5$ cells in 10 ml of medium/25 cm^2 flask (Falcon Products, Becton Dickinson, Oxford, UK). Flasks were set up in duplicate for each drug concentration and for controls. Aliquots were removed daily for viable cell counts following staining with 1% trypan blue. Cell numbers were expressed as log viable cells/ml and were the mean counts of the duplicate samples. To change the medium, the contents of each flask were centrifuged at 200 *g* for 5 min, the cell pellet was resuspended in 10 ml of fresh growth medium with or without drug and returned to the original flask. Adjustments in cell concentrations could be made at this time. Two *Bos taurus* Friesian cattle were used as blood donors. PBM were separated from whole blood by a method similar to that of Lalor *et al.* (1986). Sporozoites were prepared from salivary glands dissected from adult *R. appendiculatus* infected with *T. parva* (Muguga). For different batches of ticks the mean number of infected acini ranged between 50 and 100/tick. Glands were suspended in medium at 50 pairs of glands/ml, homogenized as described by Kurtti *et al.* (1981) and sporozoite suspensions clarified by centrifugation at 50 *g* for 5 min.

In vitro *infections*

Sporozoite binding studies were carried out following the addition of 250 μl of undiluted sporozoite suspension (approximately 625–1250 infected acini) to a pellet of 10^7 PBM in the presence or absence of 20 μg/ml OTC. Samples of the sporozoite/PBM mixture were taken after 2 h at 37 °C and resuspended in 10 ml of PBS. Following centrifugation at 200 *g* for 5 min the cells were counted and 10^6 PBM in 1 ml of PBS were added to 1 ml of 4% paraformaldehyde and the cells held at 4 °C for 15 min. Then 8 ml of 50 mM ammonium chloride in PBS were added and mixed for 10 min. The fixed cells were centrifuged at 200 *g* for 5 min and resuspended in 100 μl of 0·2% gelatin in PBS. Drops of this suspension were placed in the wells of multispot slides (Wellcome Diagnostics, Dartford, UK), and immediately removed leaving a residual film of evenly distributed cells which rapidly dried (for staining procedure, see below).

The infectivity of sporozoite preparations was determined following log dilution steps in growth medium. Diluted suspensions of sporozoites in 100 μl volumes were added to 6×10^5 pelleted PBM. After incubation for 1 h at 37 °C, each cell pellet was washed once, resuspended in medium and distributed into 10 wells of a 96-well cluster plate (Costar Products, Massachusetts, USA). Culture wells used for these studies contained a 3–4 day monolayer of an adherent cell line derived from a foetal bovine thymus (reference BT6-isolated at ILRAD) as a feeder layer. Cells were cultured with or without drug in a total volume of 200 μl.

Studies of the effect of OTC on the early events after infection were carried out similarly except that 1·5 ml volumes in 24-well plates (Costar Products) were used. PBM concentrations were adjusted to 5×10^5 cells/well for studies with feeder layers, or 2×10^6 cells/well when feeder layers were not used. This procedure permitted repeated sampling of the same wells during the early course of infection (days 1–8). Parasites were identified as schizonts when several distinct nuclei could be detected, or as immature forms (pre-schizont stages) when vacuolated intracytoplasmic bodies were observed, often with peripheral chromatin but without distinct schizont nuclei.

The end-points of the infectivity titrations were calculated from the percentage of wells, for each

dilution, containing schizont-infected lymphocytes at 4 weeks. The end-point was expressed as that logarithmic dilution of sporozoites which transformed PBM in 50% of the wells (Karber, 1931).

Staining procedures

For observations by light microscopy, cell suspensions were centrifuged onto slides using a cytocentrifuge (Shandon Southern Products, Cheshire, UK). These samples were air-dried, fixed with methanol and stained with 5% Giemsa's stain for 30 min. For studies of the mean schizont nuclear number (MSN) (Brown, 1979; Irvin, Ocama & Spooner, 1982), fixed infected cells on slides were subjected to hydrolysis with 1 M hydrochloric acid before staining (Kurtti *et al.* 1981). Results were compared using Student's *t*-test.

Sporozoites binding to lymphocytes were detected by an indirect fluorescent antibody (IFA) test (Dobbelaere *et al.* 1985) using an anti-sporozoite monoclonal antibody (Dobbelaere *et al.* 1984). Schizont antigen in infected lymphocytes was detected by an IFA test, following acetone fixation of cytocentrifuge preparations. The primary antibody was monoclonal antibody No. 4 (Minami *et al.* 1983), raised against *T. parva* (Muguga) macroschizonts (Pinder & Hewett, 1980). This antibody was used at a dilution of 1:800 and, following washing with PBS, a rabbit anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate (Nordic Immunological Laboratories, Tilburg, Holland) was added at a dilution of 1:400.

RESULTS

The action of tetracyclines was initially assessed using a *T. parva*-infected cell line. The results depicted in Fig. 1 demonstrate the inhibition by OTC of the growth of a *T. parva* (Muguga) line. The effect was dose dependent and was detected at 2·5 μg/ml OTC, the lowest drug concentration studied. Growth inhibition of the cultures was found within 24 h at concentrations of 40 μg/ml OTC and above, and growth was completely inhibited within 3 days by these concentrations. The inhibitory effect was reversible; on drug removal, the rate of recovery of infected cell growth depended on drug concentration and duration of treatment. When the cultures had been treated with 40 μl/ml OTC or less, the recovery following treatment for 4 days was rapid, and by day 9 growth rates were similar to those of control cultures. While the growth rate was also inhibited by 10 μg/ml, the percentage parasitosis of the *T. parva* (Muguga) line was not affected by this concentration administered for 3 days. Control cultures had an infection rate of 96·5% and treated cultures 97·0%. Samples from these cultures stained

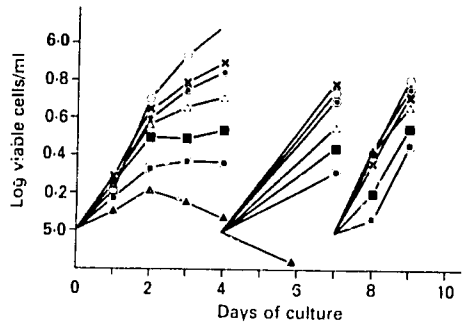

Fig. 1. The effect of oxytetracycline on the growth rate of a *Theileria parva* (Muguga)-infected cell line. Drug concentrations in μg/ml as follows: (▲) 80; (▣) 40; (■) 20; (△) 10; (◉) 5; (✖) 2·5; (○) control. Culture medium was changed with OTC on day 2. On day 4, drug was removed by changing the medium and cell numbers reduced to 10^5/ml. Culture medium was changed without drug on day 7, and cell numbers were again reduced to 10^5/ml, except for 80 μg/ml OTC which did not survive the treatment. Each point is the mean of viable cell counts from duplicate flasks.

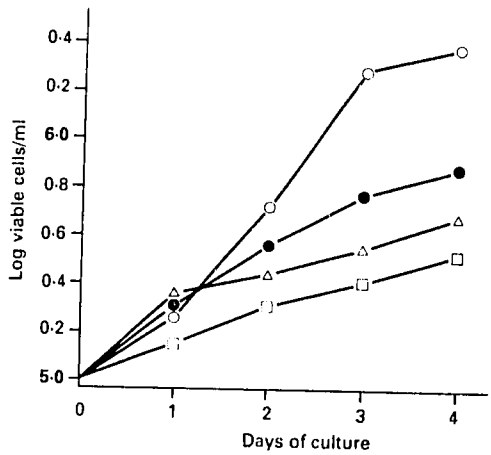

Fig. 2. Comparison of the effect of 3 tetracyclines on the growth rate of a *Theileria parva* (Marikebuni)-infected cell line. Each drug at 10 μg/ml, symbols as follows: (●) chlortetracycline; (△) oxytetracycline; (□) tetracycline; (○) control. Culture medium was not changed during the experiment.

with Giemsa's stain showed no differences in schizont morphology between control and drug-treated cells. The mean schizont nuclear counts (MSN) for cells treated with 10 μg/ml OTC for 3 days was 12·15 (±6·53) which was not significantly different from the MSN of 12·11 (±6·86) for control cells.

The growth rate of the *T. parva* (Marikebuni)-infected cell line was also inhibited by 10 μg OTC and similar inhibitory effects were detected following treatment with chlortetracycline and tetracycline (Fig. 2). An uninfected bovine T cell line (clone A2),

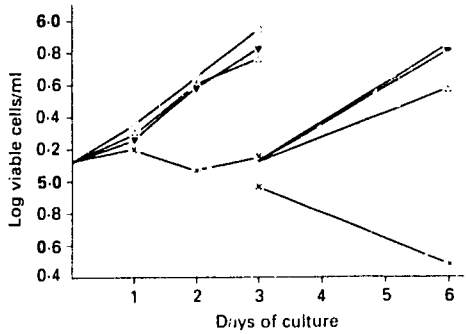

Fig. 3. The effect of oxytetracycline on the growth rate of an uninfected T cell line (clone A2). Drug concentrations in μg/ml as follows: (⊗) 100; (△) 10; ▼) 1; (○) control. Culture medium was changed with drug on day 3 and cell numbers reduced as indicated.

Table 1. The infectivity of *Theileria parva* sporozoites for bovine PBM was assessed following incubation of sporozoites with 20 μg/ml OTC at 5 or 37 °C for the times indicated

(Results of infectivity titres are end-points expressed as the log dilution of sporozoites which infected and transformed PBM in 50 % of the culture wells at 4 weeks.)

Treatment	Temperature of incubation (°C)	Incubation time (h) 0	3	24	48
Control	5	2·8	2·9	1·3	0·3
OTC 20 μg/ml	5	2·7	2·9	1·2	0·2
Control	37	3·1	3·2	N.D.	N.D.
OTC 20 μg/ml	37	3·1	3·1	N.D.	N.D.

N.D., No transformation detected.

Table 2. The effect of different concentrations of OTC on the transformation of a fixed number of bovine PBM by log dilutions of *Theileria parva* sporozoites

(Drug was added immediately after the 1 h infection period and removed on day 8 of culture. Culture wells were examined for the presence of schizont-infected transformed lymphocytes at 4 weeks.)

	Concentration of OTC (μg/ml) 20	10	5	2·5	1·25	0·625	0
Sporozoite dilution (log) giving transformation in 50 % of wells	N.D.	N.D.	N.D.	0·88	1·38	1·25	1·25

N.D., No transformation detected.

grown in the presence of TCGF, was similarly inhibited (Fig. 3). This same cell line when infected with *T. parva* (Muguga), and a bovine B cell line (RI⁺ clone 1), also infected with *T. parva* (Muguga), showed similar sensitivities to the drug (details not shown).

The infectivity of *T. parva* sporozoites, held at 5 or 37 °C, for bovine lymphocytes was not influenced by the presence of 20 μg/ml OTC (Table 1). The sporozoite infectivity remained stable for 3 h at both temperatures, but subsequently there was a more rapid decline at 37 °C, with no infectivity detected at 24 h in either control or treated preparations. At 5 °C, significant infectivity was detected after incubation for 24 h, and residual infectivity found at 48 h.

The number of sporozoites binding to bovine PBM was not altered following incubation of sporozoite/PBM mixtures with 20 μg/ml OTC for 2 h. In control preparations, 20–25 % of PBM were found to have bound sporozoites, while the numbers of PBM binding sporozoites in drug-treated cultures were within ±1 % of these values.

Table 3. Detection of schizont antigen in lymphocytes following incubation with sporozoites and treatment 1 h later with 20 μg/ml OTC

(Schizont antigen was detected by IFA with an anti-schizont monoclonal antibody. Results are expressed as the percentage of cells with fluorescence.)

	Days after infection 1	2	3	4	6
Control culture	9·5	22·6	23·2	33·2	38·6
OTC-treated culture	10·5	21·0	22·2	22·8	12·0

When bovine lymphocytes were infected with sporozoites, treatment for 8 days with concentrations of 5 μg/ml OTC, or higher, completely inhibited the establishment of the schizont stage of the parasite within the host cell (Table 2). While complete inhibition was not achieved with 2·5 μg/ml, the extent of cell transformation was reduced. Schizont antigen could be detected, by the IFA test, within

Table 4. Schizont development in lymphocytes following incubation with sporozoites and treatment 1 h later with 20 μg/ml OTC, as assessed after staining of cell preparations with Giemsa's stain

(Results are expressed as the percentage parasitosis (including both immature and mature stages) based on examination of 1000 cells, and the percentage of schizonts (in parentheses) assessed from day 3 by examining 100 infected cells. Feeder layers were not used in this experiment.)

	Days after infection					
	1	2	3	4	6	8
Control culture	11·0	12·0	15·0 (12)	26·0 (28)	29·3 (89)	35·4 (96)
OTC-treated culture	11·0	10·2	12·4 (8)	19·5 (1)	10·2 (5)	5·6 (4)

20 h of infection, and both control and treated (20 μg/ml OTC) preparations showed a similar increase in the percentage of cells showing fluorescence of schizonts to day 3 (Table 3). While the percentage of control cells with detectable schizont antigen increased to day 6, the percentage of treated cells which contained schizont antigen was at a maximum on day 4 and declined to 31% of the control value by day 6. The fluorescent schizonts were smaller in cells treated with OTC than those in control cells.

By examination of Giemsa-stained samples, immature schizonts could be detected within 24 h of infection in both treated and untreated preparations (Table 4). The percentage parasitosis increased steadily in control cultures and this was accompanied by a maturation of the schizonts. By day 8, 35·4% of cells were infected and 96% of these infected cells contained mature schizonts. In treated cells, the percentage parasitosis increased to 19·5% on day 4 and then rapidly declined to 5·6% on day 8. This decline was accompanied by a failure of the majority of parasites to mature. Most remained as immature, vacuolated forms with peripheral chromatin whereas control cells contained mature schizonts with several distinct nuclei.

At day 3, the MSN in the low percentage of cells in which schizont nuclei could be detected, following treatment with 20 μg/ml OTC, was 2·44 (±0·99) and in control infected cells was 5·7 (±2·38). By day 8, the control MSN had risen to 8·0 (±3·78), but insufficient numbers of cells containing schizonts were found in treated cells to permit a calculation of the MSN.

DISCUSSION

The effect of OTC on parasite development after infection was studied by quantification of host cell transformation, detection of schizont antigen immunofluorescence, and examination of schizont morphology. Tetracyclines were found to inhibit the growth rate of infected and uninfected lymphoblasts. This effect was not cell type-specific as the growth of B cells was also inhibited. Following infection of bovine lymphocytes with *T. parva* sporozoites, drug-induced growth inhibition resulted in a significant effect on the developing parasite. The addition of OTC immediately after infection was found to reduce the number of cells containing schizonts and to inhibit the development of those schizonts that were detected. The majority of parasites remained at an immature stage, resembling those described previously in the early stages of untreated cultures (Stagg *et al.* 1981). A reduction in schizont size (as assessed by MSN) was observed in the few treated lymphocytes in which parasites developed past the immature stage.

This inhibitory effect on schizont development resulted in the failure of parasites to establish within host cells, as determined by host cell transformation assessed at 4 weeks. The parasite appeared to be more sensitive to OTC during the early stages of schizont development, since treatment resulted in an irreversible effect on the parasite. Once established as schizont-infected cell lines, treatment with similar concentrations of drug resulted in a reversible inhibition of infected cell growth, without affecting the percentage parasitosis or the morphology of the schizonts.

The effects of OTC against *T. parva* appeared to be confined to the events following sporozoite infection of lymphocytes. The drug did not directly affect sporozoites or sporozoite binding to target cells.

Inhibitory effects of OTC on schizont development were detected *in vitro* at 2·5 μg/ml, the lowest concentration studied. This concentration can be found in the serum of cattle for 24–30 h following a single dose of 20 mg/kg OTC (Terramycin LA, Pfizer), as used during immunization, and concentrations above 0·5 μg/ml have been found for over 80 h (Davey, Ferber & Kaye, 1985). While drug concentrations *in vitro* were maintained for a longer period, it seems likely that the inhibition of parasite maturation observed in this study also occurs *in vivo*. Treatment of cattle with OTC, during immunization, suppresses the disease, caus-

ing a prolonged pre-patent period, with schizonts detected in low numbers (Neitz, 1957; Radley, 1978). This limitation of the rate and extent of parasite development in the bovine host by OTC, may allow time for the development of an immune response which controls the lower level of infection that may develop following immunization.

A similar inhibition of schizont maturation, caused by tetracyclines, has been reported with the related protozoan, *Eimeria tenella* (Ball & Warren, 1966; Ryley & Wilson, 1976). This effect was detected following treatment of experimental infections in chickens and was considered to be coccidiostatic rather than coccidiocidal since relapses occurred after drug removal.

A schizonticidal activity of tetracyclines suggested by Neitz (1953, 1957) seems unlikely, since stasis rather than a killing effect has been demonstrated in this study. In contrast, a schizonticidal effect on a *T. parva*-infected lymphoblastoid cell line has been demonstrated with the anti-theilerial drugs parvaquone, halofuginone and buparvaquone, at concentrations of 0·006, 0·006 and 0·0003 μg/ml respectively (Morgan & McHardy, 1982; McHardy *et al.* 1985). It has also been possible to 'cure' cells of schizont infection with parvaquone (Pinder *et al.* 1981). A similar schizonticidal effect was not demonstrated with concentrations of 10–100 μg/ml OTC (McHardy *et al.* 1976; Spooner, 1987).

During clinical disease, tetracyclines may act to control the multiplication of schizont-infected lymphocytes (Dolan, 1981); however, these drugs have been shown to have little therapeutic value (Wilde, 1967; Mugera *et al.* 1973; Brown *et al.* 1977; Dolan, 1981). The present study indicates that tetracyclines may not be effective therapeutically against *T. parva*, because these drugs have a reversible cytostatic action and schizonts when established within host cells are not eliminated by this treatment. However, the inhibition of the multiplication of infected cells is unlikely to be the major action of tetracyclines during immunization, since the results presented here indicate that these drugs most probably inhibit schizont development before the infected cell has been stimulated to divide. The mechanism(s) by which OTC causes the effects demonstrated in this paper is unknown. However, tetracyclines have been reported to inhibit mitochondrial protein synthesis in rapidly growing mammalian tumour cells (Van den Bogert *et al.* 1981; Van den Bogert, Dontje & Kroon, 1983). Such an effect could result in decreased energy levels available to the developing parasite. The role of these mechanisms in the inhibition of development of *T. parva* is currently under investigation.

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REFERENCES

BALL, S. J. & WARREN, E. W. (1966). Effects of chlortetracycline, oxytetracycline and spiramycin on *Eimeria tenella* in chicks. *Journal of Comparative Pathology* **76**, 255–9.

BROCKLESBY, D. W., BARNETT, S. F. & SCOTT, G. R. (1961). Morbidity and mortality rates in East Coast fever (*Theileria parva* infection) and their application to drug screening procedures. *British Veterinary Journal* **117**, 529–31.

BROWN, C. G. D. (1979). Propagation of *Theileria*. In *Practical Tissue Culture Applications* (ed. Maramorosch, K. & Hirumi, H.), pp. 223–254. New York: Academic Press.

BROWN, C. G. D., RADLEY, D. E., CUNNINGHAM, M. P., KIRIMI, I. M., MORZARIA, S. P. & MUSCKE, A. J. (1977). Immunization against East Coast fever (*Theileria parva* infections of cattle) by infection and treatment: chemoprophylaxis with *N*-pyrrolidinomethyl tetracycline. *Tropenmedizin und Parasitologie* **28**, 342–6.

BROWN, W. C. & GRAB, D. J. (1985). Biological and biochemical characterization of bovine interleukin 2. Studies with cloned bovine T cells. *Journal of Immunology* **133**, 3184–90.

BROWN, W. C. & LOGAN, K. S. (1986). Bovine T-cell clones infected with *Theileria parva* produce a factor with IL 2-like activity. *Parasite Immunology* **8**, 189–92.

DAVEY, L. A., FERBER, M. T. & KAYE, B. (1985). Comparison of the serum pharmacokinetics of a long-acting and a conventional oxytetracycline injection. *Veterinary Record* **117**, 426–9.

DOBBELAERE, D. A. E., SPOONER, P. R., BARRY, W. C. & IRVIN, A. D. (1984). Monoclonal antibody neutralizes the sporozoite stage of different *T. parva* stocks. *Parasite Immunology* **6**, 361–70.

DOBBELAERE, D. A. E., WEBSTER, P., LEITCH, B. L., VOIGT, W. P. & IRVIN, A. D. (1985). *Theileria parva*: studies on expression of a sporozoite surface coat antigen. *Experimental Parasitology* **60**, 90–100.

DOLAN, T. T. (1981). Progress in the chemotherapy of theileriosis. In *Advances in the Control of Theileriosis* (ed. Irvin, A. D., Cunningham, M. P. & Young, A. S.), pp. 186–208. The Hague: Martinus Nijhoff.

IRVIN, A. D., OCAMA, J. G. R. & SPOONER, P. R. (1982). Cycle of bovine lymphoblastoid cells parasitised by *Theileria parva*. *Research in Veterinary Science* **33**, 298–304.

IRVIN, A. D., DOBBELAERE, D. A. E., MWAMACHI, D. M., MINAMI, T., SPOONER, P. R. & OCAMA, J. G. R. (1983). Immunization against East Coast fever: correlation between monoclonal antibody profiles of *Theileria parva* stocks and cross immunity *in vivo*. *Research in Veterinary Science* **35**, 341–6.

KARBER, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Archiv für Experimentelle Pathologie und Pharmakologie* **162**, 480–7.

KURTTI, T. J., BUSCHER, G. & HIRUMI, H. (1978).

Establishment and characterization of bovine lymphoblastoid cell lines infected with the intracellular protozoan *Theileria parva*. *In Vitro* **14**, 389.

KURTTI, T. J., MUNDERLOH, U. G., IRVIN, A. D. & BUSCHER, G. (1981). *Theileria parva*: early events in the development of bovine lymphoblastoid cell lines persistently infected with macroschizonts. *Experimental Parasitology* **52**, 280–90.

LALOR, P. A., MORRISON, W. I., GODDEERIS, B. M., JACK, R. M. & BLACK, S. J. (1986). Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system. *Veterinary Immunology and Immunopathology* **13**, 121–40.

McHARDY, N., HAIGH, A. J. B. & DOLAN, T. T. (1976). Chemotherapy of *Theileria parva* infection. *Nature, London* **261**, 698–9.

McHARDY, N., HUDSON, A. T. & RAE, D. G. (1980). Therapy of *Theileria parva*: correlation of results in culture and in cattle. In *The in Vitro Cultivation of the Pathogens of Tropical Diseases* (ed. ILRAD), pp. 149–152. Basel: Schwabe and Co.

McHARDY, N. & WEKESA, L. S. (1985). Buparvaquone (BW 720C): a new anti-theilerial naphthoquinone: its role in the therapy and prophylaxis of theileriosis. In *Immunization against Theileriosis in Africa* (ed. Irvin, A. D.), p. 88. Nairobi: ILRAD.

McHARDY, N., WEKESA, L. S., HUDSON, A. T. & RANDALL, A. W. (1985). Antitheilerial activity of BW 720C (buparvaquone): a comparison with parvaquone. *Research in Veterinary Science* **39**, 29–33.

MINAMI, T., SPOONER, P. R., IRVIN, A. D., OCAMA, J. G. R., DOBBELAERE, D. A. E. & FUJINAGA, T. (1983). Characterization of stocks of *Theileria parva* by monoclonal antibody profiles. *Research in Veterinary Science* **35**, 334–40.

MORGAN, D. W. T. & McHARDY, N. (1982). Comparison of the antitheilerial effect of Wellcome 993C and halofuginone. *Research in Veterinary Science* **32**, 84–8.

MUGERA, G. M., MWORIA, G. M. & MUNYUA, W. K. (1973). Treatment of East Coast fever with Aureomycin. *Bulletin of Epizootic Diseases of Africa* **21**, 501–5.

MUTUGI, J. J., YOUNG, A. S., MARITIM, A. C., LINYONYI, A., MBOGO, S. K. & LEITCH, B. L. (1988). Immunization of cattle using varying infective doses of *Theileria parva lawrencei* sporozoites derived from an African buffalo (*Syncerus caffer*) and treatment with buparvaquone. *Parasitology* **96**, 391–402.

NEITZ, W. O. (1953). Aureomycin in *Theileria parva* infection. *Nature, London* **171**, 34–5.

NEITZ, W. O. (1957). Theileriosis, gonderiosis and cytauxzoonoses: A review. *Onderstepoort Journal of Veterinary Research* **27**, 275–430.

PINDER, M. & HEWETT, R. S. (1980). Monoclonal antibodies detect antigenic diversity in *Theileria parva* parasites. *Journal of Immunology* **124**, 1000–1.

PINDER, M., KAR, S., WITHEY, K. S., LUNDIN, L. B. & ROELANTS, G. E. (1981). Proliferation and lymphocyte stimulatory capacity of *Theileria*-infected lymphoblastoid cells before and after elimination of parasites. *Immunology* **44**, 51–60.

PURNELL, R. E. (1977). East Coast Fever: some recent research in East Africa. *Advances in Parasitology* **15**, 83–132.

RADLEY, D. E. (1978). Immunization against East Coast Fever by chemoprophylaxis. *FAO Technical Report* 1. AG: DP/RAF/67/077, Rome.

RADLEY, D. E. (1981). Infection and treatment method of immunization against theileriosis. In *Advances in the Control of Theileriosis* (ed. Irvin, A. D., Cunningham, M. P. & Young, A. S.), pp. 227–237. The Hague: Martinus Nijhoff.

RYLEY, J. F. & WILSON, R. G. (1976). Laboratory studies with some older anticoccidials. *Parasitology* **73**, 287–309.

SCHEIN, E. & VOIGT, W. P. (1979). Chemotherapy of bovine theileriosis with halofuginone. *Acta Tropica* **36**, 391–4.

SPOONER, P. R. (1987). Oxytetracycline and *Theileria parva*: the effects of the drug and its mechanisms of action with respect to the 'infection and treatment' method of immunizing cattle against East Coast fever. Ph.D. thesis, Brunel University, Uxbridge, UK.

STAGG, D. A., DOLAN, T. T., LEITCH, B. L. & YOUNG, A. S. (1981). The initial stages of infection of cattle cells with *Theileria parva* sporozoites *in vitro*. *Parasitology* **83**, 191–7.

THEILER, A. (1904). East Coast Fever. *Transvaal Agricultural Journal* **2**, 421–38.

VAN DEN BOGERT, C., DONTJE, B. H. J., WYBENGA, J. J. & KROON, A. M. (1981). Arrest of *in vivo* proliferation of Zajdela tumor cells by inhibition of mitochondrial protein synthesis. *Cancer Research* **41**, 1943–7.

VAN DEN BOGERT, C., DONTJE, B. H. J. & KROON, A. M. (1983). Arrest of *in vivo* growth of a solid Leydig cell tumor by prolonged inhibition of mitochondrial protein synthesis. *Cancer Research* **43**, 2247–51.

WILDE, J. K. H. (1967). East Coast Fever. *Advances in Veterinary Science* **11**, 207–59.

WILDE, J. K. H., HULLIGER, L. & BROWN, C. G. D. (1966). Some recent East Coast fever research. *Bulletin of Epizootic Diseases of Africa* **14**, 29–35.