REVIEW ARTICLE

Immunomodulatory effect of various anti-parasitics: a review

M. S. SAJID*, Z. IQBAL, G. MUHAMMAD *and* M. U. IQBAL

Department of Veterinary Parasitology, Veterinary Clinical Medicine and Surgery, University of Agriculture, Faisalabad – 38040, Pakistan

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SUMMARY

This paper reviews the immunomodulatory effects (immunosuppression or immunoactivation) of various anthelmintics including levamisole, fenvalerate, dieldrin, carbofuran, aminocarb, thiabendazole, fenbendazole, oxfendazole and ivermectin. The induced modulation of immune function may occur via direct and/or indirect mechanisms. The immunomodulatory effects of these anti-parasitics have been studied in a variety of bacterial (e.g. brucellosis, salmonellosis, paratuberculosis, mastitis), viral (e.g. infectious bovine rhinotracheitis, Herpes, foot and mouth disease), parasitic (e.g. onchocerciasis, coccidiosis, ascariasis, schistosomiasis) and neoplastic diseases. Some antiparasitics have also been used to boost immunity in a number of human diseases including leprosy, Hodgkin's disease, rheumatoid arthritis, and in adjuvanted therapy of colorectal cancer. The ability to stimulate the immune response of animals offers a new means of disease intervention. Future research on immunomodulatory effects of anti-parasitics, for humans and domestic farm animals, will provide additional methods of treating immunosuppressed subjects. The immunopotentiating or immunosuppressing activity of anti-parasitics will dictate whether co-administration of vaccines and anthelmintics or administration of vaccines during the window of immunoactivation is justified or not.

Key words: immunomodulation, antiparasitics, levamisole, fenvalerate, dieldrin, carbofuran, aminocarb, thiabendazole, fenbendazole, oxfendazole, ivermectin.

INTRODUCTION

Immunomodulators are the substances that have the capability to either augment or suppress an immune response. In addition to an altered immune response, modulation of haematopoiesis, including increased RBC and WBC counts, an increased PCV and enhanced macrophage activation, have also been reported (Cox, 1988). Certain anti-parasitics have also been reported to have an immunomodulatory activity. Examples include levamisole (Fernie, Ripley and Walker, 1983; Giambrone and Klesius, 1985; Blecha, 1988; Rehman, Fatima and Jagannath, 1989; Mondal, Sinha and Tiwary, 1993; Naylor and Hadden, 2003; Mojzisova *et al*. 2004), thiabendazole (Blecha, 1988), fenvalerate (Singh and Jha, 1996; Singh, Singhal and Chauhan, 2001), dieldrin (Fournier *et al*. 1988; Flipo *et al*. 1992), oxfendazole (Stankiewicz *et al*. 1994), fenbendazole (Cabaj *et al*. 1994; Parish *et al*. 1996; Dvoroznakova *et al*. 1998) niridazole, metronidazole (Hewlett *et al*. 1981) carbofuran, malathion (Flipo *et al*. 1992) and ivermectin (Rao, Chandrashekar and Subrahmanyam, 1987; Blakley and Rousseaux, 1991; Savanur *et al*. 1995, 1996; Sajid, 2004). Immunostimulatory activity of oxfendazole has also been documented in poultry (Razzaq, 2000). Variable degrees of immunosuppression associated with stress, infectious disease, or nutritional deficiencies may respond favourably to treatment with such immunomodulating agents (Flesh, Harel and Nelken, 1982; Blecha, 1988).

* Corresponding author: Tel: +92 41 9201106. E-mail: drsohailuaf@hotmail.com

MECHANISMS OF ACTION

Chemical structures and modes of action of various immunomodulators for use in domestic farm animals have been proposed by Fenichel and Chirigos (1984), Kende, Gainer and Chirigos (1984) and Mulcahy and Quinn (1986). The induced modulation of immune function may occur via direct and/or indirect mechanisms. The direct mechanisms of immunomodulation involve interaction of an immunomodulator and/or its metabolite with a component of the immune cell itself. Stimulation of the immune cell-associated component induces alterations in immune cell function directly. The indirect mechanisms of immune modulation involve interaction of the immunomodulator and/or its

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metabolite with a component of a non-immune cell. Interaction with a non-immune cell stimulates or inhibits the release of a biological messenger possessing immunomodulatory activity. A direct mechanism of immunomodulation is measurable both *in vivo* and *in vitro*, whereas an indirect mechanism is measurable only *in vivo* but not *in vitro* (Sanders *et al*. 1991). Immunomodulation can also be induced by the excretory or secretory (ES) products of parasites, e.g. by the filarial parasite (Harnett and Harnett, 1999; Harnett *et al*. 1993). ES products are mainly glycoproteins, a number of which are covalently modified with phosphorylcholine (PC) groups (Harnett and Parkhouse, 1995; Harnett and Harnett, 2001). The presence of PC on these proteins supports their possible immunomodulatory role since PC has previously been shown to have immunomodulatory capabilities (Mitchell and Lewers, 1976; Sloan, Docg and Joyce 1991; Bordmann, Rudin and Favre, 1998; Gunter, Roger and Rudolf, 2000).

Studies have shown PC antigens in the internal membranes of lung larvae of *A. suum* (Gutman and Mitchell, 1977) and also in the gut (Harnett *et al*. 1989); certain internal structures, such as egg, uterine and intestinal membranes, but not on the cuticle (Gualzata, Weiss and Heusser, 1986; Gualzata *et al*. 1988). These PC antigens have also been reported from *B. malayi* adult male and female worms, as well as microfilariae (Wenger, Forsyth and Kazura, 1988), *Trichinella spiralis* (Dea-Ayuela and Bolas-Fernandez, 1999), *W. bancrofti* (Day *et al*. 1991), *Dictyocaulus viviparus* (Gilleard, Duncan and Tait, 1995), and L3 infective larvae, adult worms and eggs of *Nippostrongylus brasiliensis* (Pery *et al*. 1979). The density-dependent alterations in immune response were reported by Wenger *et al*. (1988) who correlated the quantification of serum PC epitope levels with parasite burdens in humans with lymphatic filariasis. However, the complexity of immune responses of host against parasites makes it difficult to explore whether the immunity observed was due to control of drug over the parasites or it has some immunopotentiating ability (Stankiewicz *et al*. 1995).

Mechanistic specificity of various anti-parasitics

The immunorestorative or immunopotentiating effects of various anti-parasitics may be occur via the various mechanisms illustrated in Table 1.

ANTI-PARASITICS AND THEIR IMMUNOMODULATORY ACTIONS AND USES

Levamisole

Levamisole (6-phenyl 2,3,5,6-tetrahydroimidazo (2,1-b) thiazole hydrochloride), a levo-isomer of tetramisole and member of thioimidazoles, was initially developed as an anthelmintic (Thienpont *et al*. 1966). It has been used for many years for the treatment of gastrointestinal nematodes and lungworms. Later, the drug received considerable attention as an immunomodulator (Renoux and Renoux, 1974; Jansen, 1976; Symoens and Rosenthal, 1977; Symoens, Cree and Van Bever, 1979; Brunner and Muscoplat, 1980; Lowe, 1980; Webster, 1985). It was the first chemically defined agent shown to have an immunomodulatory effect (Renoux, 1986).

Levamisole modulated the immune function at a dose of 2–5 mg kg^{-1} body weight (Rojas *et al*. 1976; Brunner and Muscoplat, 1980; Babiuk and Misra, 1981) or a total dose of 150 mg day^{-1} for a week in mice (Renoux and Renoux, 1977). Intermittent treatment was reported to be more effective than continuous treatment in restoring the immune response of bovines (Lejan and Asso, 1981). *In vitro* studies have demonstrated that various concentrations of levamisole increased the blastogenic activity of bovine lymphocytes stimulated with pokeweed mitogen (PWM) (Babiuk and Misra, 1981). Levamisole has got a beneficial effect due to an immunopotentiating activity in cows during the dry period by reducing the incidence of mastitis from 9·6% to 3·7% (Flesh *et al*. 1982). According to Alex, Alikutty and Mathew (1995), levamisole failed to control mastitis in cows when administered during the dry period. Levamisole, when administered in combination with canine parvovirus (CPV) vaccine, enhanced antibody production against parvo-virus, increased phagocytic activity and stimulated the proliferation activity of lymphocytes (Mojzisova *et al*. 2004). It was also shown that, *in vitro*, levamisole potentiated *Brucella abortus*-induced blastogenesis in lymphocytes from sterile cattle (Kaneene *et al*. 1981). Antibody titres in heifers given levamisole and *Brucella abortus* strain 19 vaccine were moderately higher than those given vaccine only (Chukwu, 1985). Research on cattle and swine shows that levamisole can influence both the primary and secondary immune responses and the ability of lymphocytes to respond to mitogen (Kehrli and Roth, 1990; Quinn, 1990). In turkeys, levamisole has been reported to be an immunorestorative agent under immunosuppressive conditions caused by antibiotics or X-irradiation (Panigraphy *et al*. 1979).

There are contradictory reports concerning the effect of levamisole on serum immunoglobulin levels of chickens, some indicating decreased antibody titres after using levamisole with Newcastle disease virus (NDV) vaccination, while others reporting no effects on antibody production (Guerrero, 1977). Six-week-old birds subjected to levamisole hydrochloride (I/M) at the dose of 8 mg kg^{-1} for 3 successive days before NDV (LaSota strain intraocular)

Table 1. Mechanistic specificity of various anti-parasitics

Sample no.	Mechanistic specificity	Anti-parasitics	References
Immunopotentiation			
1	Restoration of normal functions of the effector cells of cell mediated immune response	Levamisole	Lejan and Asso, 1981
2	Enhancement of interleukin-1 and other cytokine production	Levamisole, Thiabendazole, Ivermectin, Dieldrin	Lundy and Lovett, 1978; Kimball *et al*. 1991; Soboslay *et al*. 1994; Sceto *et al*. 2000; Pelletier *et al*. 2001
3	Activation of production of superoxide anion (O_2) and phagocytic activity of peritoneal macrophages	Thiabendazole, Fenbendazole, Ivermectin, Dieldrin	Hersey *et al*. 1981; Dvoronznakova *et al*. 1998; Pelletier *et al*. 2001
4	Reduction of suppressor T-cell function	Levamisole	Hersey *et al*. 1981
5	Increased production of natural killer (NK) cells	Levamisole	Holcombe, 1998
6	Induction of maturation of granulocytes and functioning of T-lymphocytes	Levamisole	Lejan and Asso, 1981
7	Increased number of lymphocytes, total serum proteins and immunoglobulins	Thiabendazole, Ivermectin	Mailboroda and Shevchenko, 1978; Savanur *et al*. 1995
8	Augmented ability of lymphocytes to respond to mitogens; enhanced blastogenic activity of the lymphocytes, mixed lymphocyte reactivity (MLR) and increased antibody production	Levamisole, Ivermectin	Chukwu, 1985; Kaneene *et al*. 1981; Kehrli and Roth, 1990; Quinn, 1990; Soboslay *et al*. 1992; Sajid, 2004
9	Increase in the number of cells of lymph glands, spleen, bone marrow and thymus	Levamisole, Thiabendazole	Guerrero, 1977; Maiboroda and Shevchenko, 1978; Donskaya *et al*. 1982
10	Enhanced thymic hormone-like factors affecting activity and maturation of thymus-dependent lymphocytes	Levamisole	Hadden, 1994
11	Enhanced delayed type of hypersensitivity (DTH) response	Levamisole	Hadden, 1994
12	Disruption of interaction of ferritin with T-lymphocytes in conditions of ferritin excess	Levamisole	Wigginton, 1995
Immunosuppression			
1	Decreased phagocytic activity of macrophages	Dieldrin	Jolicoeur *et al*. 1988; Krzystyniak *et al*. 1989
2	Injuries to macrophage functional activities at antigen processing stage leading to suppressed humoral response	Dieldrin	Krzystyniak *et al*. 1989
3	Decreased lymphocyte blastogenesis	Oxfendazole	Vercruysse *et al*. 1987; Stankiewicz *et al*. 1994
4	Inhibition of mixed lymphocyte reactivity	Dieldrin	Hugo *et al*. 1988*a*
5	Reduction in total leucocyte count, absolute lymphocyte count and DTH reaction	Dieldrin, Fenvalerate, Oxfendazole	Fournier *et al*. 1988; Khurana *et al*. 1999; Singh *et al*. 2001
6	Lowered specific antibody production	Fenbendazole, Oxfendazole, Dieldrin, Aminocarb	Bernier *et al*. 1987, 1988; Cabaj *et al*. 1994; Khurana and Chauhan, 2003

vaccination showed a significant increase in the haemagglutination inhibition antibody titres after 1 week of vaccination (Hassan *et al*. 1989). The immunomodulatory effect of levamisole hydrochloride in *Mycobacterium paratuberculosis*-infected rabbits was demonstrated through leukocyte migration following levamisole treatment (Mondal *et al*. 1993). Giambrone and Klesius (1985) reported the effect of levamisole on the response of commercial broilers to coccidiosis vaccination and levamisole was found to improve the development of immunity to coccidiosis. It has been observed that immunity against *Eimeria tenella* developed in chickens treated with levamisole prior to infection within 2 weeks post-infection, reached its peak within 3–4 weeks and showed a gradual decline from the fifth week onwards (Rehman *et al*. 1989).

Levamisole is able to restore normal functions of effector cells of the cell-mediated immune response. Evidence is available that the maturation of granulocytes and functioning of T lymphocytes is induced *in vivo* but not *in vitro*. The potential effects of levamisole in combination with vaccination or the increase in resistance to viral challenge are rather variable depending on the method used for the evaluation of the trial (Lejan and Asso, 1981). In cattle experiments involving levamisole as immunomodulator with infectious bovine rhinotracheitis, Herpes, foot and mouth disease virus and brucellosis, favourable results have been reported (Schimied and Rosenbusch, 1973; Kaneene *et al*. 1981) and its use in cattle during the last stage of pregnancy resulted in the prevention or reduction of the disease conditions (Espinasse, 1980).

The effect of levamisole on cell-mediated immunity is mainly on anergic or depressed thymus-dependent lymphocytes, macrophages and polymorphonuclear leukocytes (Guerrero, 1977). Levamisole enhances macrophage and T-lymphocyte function and reduces suppressor T-cell function (Hersey, Ho and Werkmeister, 1981). Modulations of T-cell maturation as well as cytokine production are potential mechanisms. Both enhancement of the production of IL-1 in a macrophage cell line (Kimball *et al*. 1991) and a shift to Th1 cytokine production are the clues to its mode of action (Sceto, Gillespie and Mathieson, 2000).

Levamisole is a drug extensively used to boost immunity in a number of human diseases as well, including leprosy, some cancers (Sceto *et al*. 2000), Hodgkin's disease, rheumatoid arthritis, and in adjuvanted therapy of colorectal cancer (Chirigos, 1992; Kumran, 1993). Moreover, it has also been found to speed the recovery of malnourished children suffering from various infections (Prakash, Rao and Reddy, 1998). It also appears to possess immunomodulatory properties and be capable of disrupting the interaction of ferritin with T lymphocytes and, therefore, is therapeutically useful in conditions of ferritin excess, such as progressive human immunodeficiency virus (HIV) infection and its associated opportunistic complications (Wigginton, 1995).

Agencies worldwide, with the hopes of demonstrating anticancer activity, have sponsored numerous pre-clinical evaluations and clinical trials with levamisole in the cancer arena. Trials in advanced breast cancer, lung cancer, colorectal cancer, melanoma, and lymphoproliferative diseases have generally been negative or inconclusive (Stevenson *et al*. 1991). However, adjuvanted use of levamisole has shown to enhance delayed-type hypersensitivity (DTH) (Hadden, 1994); for example, it has a synergistic effect in conjunction with 5-fluorouracil (5-FU) in the post-surgical treatment of adenocarcinoma of the colon (Moertel, Fleming and Macdonald, 1990, 1995; Holcombe *et al*. 2001; Tall, Van Tinteren and Zoetmulder, 2001). Levamisole has been shown to increase natural killer (NK) cells and activated T-cells in this adjuvanted treatment (Holcombe, Li and Stewart, 1998). Later, the studies of Gwilt *et al*. (2000) concluded that there is no evidence that the pharmacokinetics of levamisole are altered by 5-FU administered immediately prior to levamisole administration and levamisole has been approved for use in patients with colon cancer.

Levamisole treatment has also been found to enhance protective antibody response to hepatitis B vaccination in haemodialysis patients (Kayatas, 2002). Usually, it is believed that levamisole does not affect B-lymphocytes directly, but can still influence humoral responses indirectly by affecting macrophages and T-lymphocytes (Pelletier, Willoughby and Giroud, 1978) and this immunopotentiation is pronounced in immunologically compromised hosts. *In vivo* studies on humans show that imidothiazoles might enhance the serum levels of thymic hormone-like factor (Hadden, 1994). However, *in vitro* studies on the immunological effects of levamisole showed that it is not a potent modulator of immune parameters including effects on monocyte and lymphocyte cytotoxicity, activation, proliferation, induction of cytokine-induced proteins, and the expression of tumor-associated antigens (Schiller *et al*. 1991).

Not all workers have been able to demonstrate that levamisole has an immunostimulatory effect. For example, Irwin *et al*. (1976) found that levamisole depressed the primary humoral response of calves vaccinated with infectious bovine rhinotracheitis virus. Further, Van Der Maaten *et al*. (1983) found that levamisole did not affect the virological and serological responses of bovine leukaemia virus-infected cattle and sheep. In contrast to the previously mentioned work of Chukwu (1985), Saini *et al*. (1991) found that levamisole treatment resulted in a significant decrease in

agglutination antibody titre against *Brucella abortus* strain 19. Levamisole had no consistent enhancing effect on the blastogenic responses to Con A or PWM, in one-way mixed lymphocyte reaction or the antibody-dependent cellular cytotoxicity of lymphocytes from dexamethasone-treated cattle (Roth, Kaeberle and Hubbard, 1984).

Fenvalerate

Fenvalerate is a synthetic pyrethroid insecticide extensively used for the control of insect pests in agriculture and ectoparasites in veterinary practice. Oral administration of fenvalerate at a dose of 15 mg kg^{-1} body weight daily for 270 days and vaccination of goats with *Brucella abortis* strain 19 after 90 days of dosing, resulted in the reduction of both the humoral and cell-mediated immune response as assessed by standard tube agglutination test and the delayed hypersensitivity test, respectively (Singh and Jha, 1996). Oral administration to sheep of fenvalerate at 1·25 ppm, along with other pesticides including lindane at 1·25 ppm, monocrotophos at 0·025 ppm and carbofuran at 2·5 ppm, resulted in suppression of cell-mediated immune response as indicated by significant reduction in the DTH reaction to dinitrofluorobenzene (DNFB) (Khurana, Mahipal and Chauhan, 1999). Immunosuppressive effects of the drug at 1·25 mg kg^{-1} body weight were also observed against *Brucella* antigen in lambs (Khurana and Chauhan, 2000). Studies on the fenvalerate-induced cell mediated alterations in chicken showed that the birds fed with 20 ppm fenvalerate in feed daily for a period of 6 months resulted in significant reduction in total leukocyte count, absolute lymphocyte count and DTH reaction. This confirmed the suppression of CMI responses in fenvalerate-treated birds (Singh *et al*. 2001). A significant reduction of haemagglutination titres against sheep red blood cells (SRBC) and skin thickness in DTH response of fenvalerate-treated calves demonstrated that fenvalerate causes immunosuppression following a single administration (0·6% dermal spray) in buffalo calves (Singh *et al*. 2003). Fenvalerate at the dose rate of 1·25 mg kg^{-1} body weight in lambs showed significant suppression in serum globulins, gammaglobulins and specific *Brucella melitensis* Rev. 1 antibodies in comparison to controls (Khurana and Chauhan, 2003).

Dieldrin, carbofuran and aminocarb

Dieldrin, a non-aromatic organo-chlorinated insecticide has been shown to be a potent immunomodulator. It was observed that cell-mediated immunity can be a potential target for adverse effects of this pesticide. Studies concerning the effects of agricultural pesticides on the immune system of northern leopard frog (*Rana pipiens*) and its resistance to parasitic infection concluded that these pesticides could alter the immune response of frogs and affect their ability to deal with parasitic infection (Christin *et al*. 2003). The effect of single, sublethal intraperitoneal (i.p.) injection of dieldrin on the primary response to T-cell-dependent (SRBC) and T-cell-independent (lipopolysaccharides, LPS) antigens, investigated in inbred C571B1/6 mice showed significant suppression of the anti-SRBC IgM, IgG and anti-LPS IgM response at 7–24 days and at 4–14 days, respectively after exposure to 0.6 LD50 dieldrin (Bernier *et al*. 1987). A decrease in the anti-mouse hepatitis virus 3 (MHV3) IgM serum antibody titre by aminocarb was found to be comparatively less marked than in the dieldrin group (Bernier *et al*. 1988).

Carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) is a carbamate pesticide. The primary IgM antibody response to SRBC antigen and macrophage phagocytosis returned to control levels indicating a lack of any synergistic or additive effect of dieldrin in combination with a carbofuran (Flipo *et al*. 1992). Suppression of *in vitro* phagocytosis and killing of bacteria by peritoneal exudate cells and significant lowering of antibody response to *Salmonella typhimurium in vivo* after sublethal i.p. administration of dieldrin, confirms that dieldrin inhibits the natural resistance of mice to bacterial infection (Jolicoeur, Fournier and Krzystyniak, 1988). A marked decrease in the phagocytosis of fluorescein-labelled microspheres and *Salmonella typhimurium* has also been documented (Krzystyniak *et al*. 1989) and a transient inhibition of the mixed lymphocyte reactivity (MLR) was noted at 7 days after i.p. exposure to 0.6 LD50 dieldrin (Hugo *et al*. 1988*a*). Similar results were demonstrated following assessment of lymphoid cells from mice injected i.p. 7 days earlier with 36 mg kg^{-1} b.w. (0.6 LD50) dieldrin for their ability to recognize a foreign antigen and to proliferate in a MLR at 4, 7, and 24 days post-treatment (Hugo *et al*. 1988*b*). Dieldrin-induced immunosuppression of the cellular immune response has been seen in mice infected with the MHV3 virus (Fournier *et al*. 1988).

Aminocarb (4-dimethylamino-3-methylphenyl N-methylcarbamate 4-dimethylamino-*m*-tolyl N-methylcarbamate) is another carbamate pesticide. Comparative immunotoxic studies of sublethal exposure to aminocarb and dieldrin in mice revealed that virus-induced cytopathic effects in peritoneal macrophages were augmented to a lesser extent in the aminocarb as compared to dieldrin group (Bernier *et al*. 1988). In addition, macrophage antigen processing of a single protein, avidin, was significantly suppressed in dieldrin-treated animals (Bernier *et al*. 1988; Krzystyniak *et al*. 1989), while the avidin processing in macrophages was unaffected by aminocarb (Bernier *et al*. 1988). Dieldrin

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markedly affected the presentation of avidin on the macrophage surface and release of processed antigen. Thus, antigen processing could be a sensitive target for dieldrin-related injury of macrophage functional activities which, in consequence, could produce suppression of the humoral immune response (Krzystyniak *et al*. 1989). *In vitro* studies on the effect of dieldrin along with other environmental contaminants showed adverse effects on the immune function and reproductive physiology in mice (Wade *et al*. 2002). Dieldrin, which has pro-inflammatory properties *in vivo*, could not alter the ability of human neutrophils to phagocytose opsonized SRBC at non-necrotic concentrations (0·1, 1, 10, and 50 μM) but did increase human neutrophil superoxide production, RNA synthesis, and the pro-inflammatory cytokine (interleukin-8) production (Pelletier *et al*. 2001).

Thiabendazole

Thiabendazole (TBZ), a relatively non-toxic thiazole derivative, appears to be an immunorestorative agent, demonstrating maximum immunopotentiation in immunosuppressed hosts (Lundy and Lovett, 1978). Almost complete restoration of the delayed hypersensitivity responses (90% of control) in animals, immunosuppressed by sublethal exposure of radiation (450 Rads) has been observed when the irradiated animals were treated with TBZ. The immunosuppressive effects of adriamycin could also be reversed in a similar fashion (Lundy and Lovett, 1976). Initial *in vivo* and *in vitro* immune studies indicated that the drug was most effective when given 24 h prior to, or at the time as, administration of antigen. Single doses are more effective than multiple daily doses. One cell population potentiated by TBZ is the macrophage, either by direct activation or secondary to increased lymphokine production (Lundy and Lovett, 1978). However, oral administration of TBZ (16 mg kg^{-1} day^{-1} for 6 days and 20 mg kg^{-1} day^{-1} for 5 days) beginning 24 h prior to antigen and dexamethasone administration failed to prevent the dexamethasone-induced suppression of lymphocyte blastogenic or antibody responses and was associated with a significantly lowered antibody response to *Brucella abortus* (Roth *et al*. 1984). TBZ may have a role as an adjunct in cancer therapy (Lundy and Lovett, 1976). The use of TBZ as an immunorestorative drug in the peri-operative period resulted in an improved cytotoxic response and a significant decrease in pulmonary metastases of tumor growth. Peri-operative immunotherapy can be an effective adjunct to surgery in preventing the growth of micrometastatic foci (Lundy and Lovett, 1979). TBZ has significant effects on the development and differentiation of both lymphoid and haemopoietic cells. *In vivo* administration of TBZ along with the thymus-dependent neoantigen, dinitrofluorobenzene (DNFB) caused changes in expansion of the T-lymphocyte compartment and an increase in extramedullary haemopoiesis of lymph nodes and/or spleens, while the effect on B-cells appeared to be antigen independent (Donskaya *et al*. 1982). Smaller increases in the numbers of plasmacytes in the spleen, lymph glands, thymus and bone marrow were observed in hamsters treated with TBZ (10 mg/100 g body weight) either before or after infection with *Ascaris suum*, than in untreated, infected animals (Maiboroda and Shevchenko, 1978). Studies on the effect of TBZ on lung granuloma formation around *Schistosoma mansoni* eggs and delayed footpad oedema in response to *Schistosoma* egg antigen showed that the drug caused significant reduction in these responses in unsensitized animals when given as a single dose and in sensitized animals using a multiple dose regimen. These findings support the hypothesis that some of the clinical activities of TBZ may be mediated by interference with host response to antigenic stimuli (Hewlett *et al*. 1981). Contrary to what has been seen with fenbendazole (22·2% Panacur TM granules at 0·02 g/kg), TBZ (0·1 g/kg) has been found to raise the immune status in sheep having natural infection of nematodes (Movsesyan *et al*. 1988).

Fenbendazole

Fenbendazole is a member of benzimidazole group of anthelmintics. Repeated use of anthelmintics of the benzimidazole group may interfere with the immune responsiveness in young sheep (Parish *et al*. 1996). The studies in mice as judged by Reiss, Herrman and Hopkins (1987) concluded that this type of anthelmintic treatment did not interfere with immune responses in mice as examined by induction of allospecific cytolytic T-lymphocytes (CTLs) *in vitro*, influenza-specific memory T-cells *in vivo*, influenza-specific antibody secretion *in vivo*, or influenza-specific helper T-cells and CTLs *in vitro*. Reductions in the primary and secondary humoral responses to bovine viral diarrhoea vaccination were documented in both parasitized and parasite-naïve fenbendazole-drenched (5 mg kg^{-1} b.w.) lambs as measured by serum neutralization titre (Parish *et al*. 1996). Antibody responses were similar in the fenbendazole-drenched and control lambs against human erythrocytes and ovalbumin antigens. However, after the second injection, there was a significant reduction in antibody response to human erythrocytes in the fenbendazole-treated lambs (Cabaj *et al*. 1994). Administration of fenbendazole to healthy mice stimulated the proliferative response of T- and B-cells to non-specific polyclonal activators, but partially inhibited the percentage of CD4+ and CD8+T-lymphocytes

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and the production of superoxide anion (O^{-2}) increased insignificantly. The treatment of infected mice considerably stimulated the proliferative response of B-cells in comparison with T-cells. The percentage of CD4+ cells in spleen was moderately reduced after treatment while that of CD8+ increased significantly. A considerable activation of the production of peritoneal macrophage superoxide anion (O_2) was also observed by day 28 after the last administration of fenbendazole (Dvoroznakova *et al*. 1998).

Oxfendazole

Oxfendazole, another benzimidazole, has since long been used in the treatment of gastrointestinal nematode infections (Campbell, 1990; Sangster *et al*. 1991; Ali and Chick, 1992). It has been shown that benzimidazole derivatives affect rat bone marrow cells by inducing a delay in division at the metaphase stage (Lapteva, 1988). Decreased lymphocyte blastogenesis, especially in the secondary response to human erythrocyte and ovalbumin antigen, confirmed that the immune response to nematode parasites is impaired by the use of oxfendazole (Vercruysse *et al*. 1987; Stankiewicz *et al*. 1994). However, high levels of acquired immunity to reinfection under natural and experimental conditions were achieved when animals were treated with oxfendazole (Jacobs *et al*. 1987; Borgsteede, deLeeuw and van de Burg, 1988; Downey, 1988; Eysker and Boersema, 1989; Eysker, Boersema and Kooyman, 1990). Oxfendazole is metabolized in the rumen to fenbendazole and both of these compounds are metabolized by the liver to fenbendazole sulphone (Marriner and Bogan, 1981; Gottschall, Theodorides and Wang, 1990). It is, therefore, not clear which of these compounds is responsible for the immunomodulatory and other effects seen as a consequence of drenching lambs with oxfendazole (Stankiewicz *et al*. 1994).

Ivermectin

Ivermectin (22,23-dihydroavermectin B_1a), a macrocyclic lactone derivative is a semi-synthetic analogue of avermectin B_1a (abamectin) originally isolated from the fermentation of *Streptomyces avermitilis* (Miller *et al*. 1979). Ivermectin paralyses and ultimately kills parasitic nematodes, arachinids and insects and is now extensively used to control and treat a wide variety of parasitic nematodes (roundworms) and arthropods (insects, ticks and mites) that plague livestock and other domestic animals. However, there is growing evidence that the activity of ivermectin may not be limited strictly to neurophysiology of parasites, but may also influence the immune system of the laboratory animals and humans (Rao *et al*. 1987).

The immunomodulatory effects of ivermectin reported in the literature can be described as variable at best. Ivermectin has also been found to have immunomodulatory properties that are associated with altered function of T-lymphocytes (T-helper lymphocytes in particular). Antibody production against SRBC, a T-lymphocyte and macrophage-dependent response, was enhanced by ivermectin treatment in mice (Blakley and Rousseaux, 1991). At a therapeutic dose, ivermectin altered the lymphocyte count without affecting the total serum immunoglobulins, total serum proteins or phagocytic index of animals (Savanur *et al*. 1996). However, an increase in total immunoglobulins against the specific antigen and total serum proteins has been observed in ivermectin-treated rabbits (Savanur *et al*. 1995). Similar antibody responses were seen for the treated and control groups in response to intravenous injections of human erythrocytes and subcutaneous administration of ovalbumin antigens except that after the second injection, there was a significant reduction in antibody response to ovalbumin in ivermectin-treated lambs. There were no differences in serum complement or serum nitric oxide levels between the two groups at any stage, but insulin-like growth factors were significantly reduced in the ivermectin-treated groups 4 days after the treatment (Stankiewicz *et al*. 1995). In lambs, ivermectin has been found to decrease the blastogenic activity (Stankiewicz *et al*. 1995). In rabbits, the drug has been found to have a dose-dependent immunopotentiating response against *Pasturella multocida* type II (capsular polysaccharide extracted protective) antigen and SRBC, i.e. increase in the dose caused increase in immunopotentiating response and *vice versa*. The cell-mediated immunity (determined by macrophage phagocytic activity and dinitrochlorobenzene test) and the humoral immunity (determined by indirect haemagglutination assay and Jerne haemolytic plaque formation assay) were both found to be significant in the ivermectin-treated groups (Sajid, 2004).

Human patients with onchocerciasis are usually expected to receive parasitological and clinical relief from ivermectin (at 150 μg kg^{-1} body weight) for at least 10 months after their initial treatment; suppression of pruritis usually occurs for at least 12 months (Brieger *et al*. 1998). The *in vitro* cellular reactivity of *O. volvulus*-derived antigen (Ov Ag) was reduced in ivermectin-untreated patients as compared with controls, and the lymphocyte blastogenic response improved up to 14 months after treatment (Soboslay *et al*. 1992). After ivermectin treatment, the Ov Ag-induced production of IL-1 beta and TNF-alpha increased significantly ($P<0{\cdot}05$) at 1 and 14 months, and PHA-induced production of IL-2 and IL-4 increased 1 month after treatment. The results suggested that

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onchocerciasis-mediated immunosuppression is reversible following ivermectin-mediated permanent clearance of microfilariae from the skin. The parasite-specific cellular immunity and consistent production of IL-2 and IFN-gamma contribute to control the re-infection (Soboslay *et al*. 1994). In younger children (5–9 years), repeated treatment of ivermectin resulted in some enhancement of *Onchocerca*-specific responses measured 6 months after administration of the drug (Luty *et al*. 1992). Ivermectin has been reported to be central to the control of onchocerciasis through self-sustainable community-based treatment (Ali *et al*. 2002). Comparative study of the effects of diethylcarbamazine (DEC) and ivermectin on filarial antigen-specific immune responses concluded no differences between the two anti-parasitic drugs in terms of humoral and cellular reactivity to filarial antigen (Lammie *et al*. 1992). Although, ivermectin and DEC are believed to exert their anti-parasitic activity via different mechanisms, the same pattern of serological changes was observed in patients treated with either drug for the control of microfilaraemia (Zheng *et al*. 1991). However, ivermectin seemed to synergize host immune factors against infection (Rao *et al*. 1987).

Not all workers, however, have been able to demonstrate that ivermectin has an immunostimulatory effect. For example, Uhlir and Volf (1992) administered ivermectin subcutaneously to observe its influence on the specific immune response in rabbits infested with mites (*Psoroptes cuniculi*) and in rats infested with lice (*Polyplax spinulosa*). A pronounced specific antibody activity and a change in immunoblotting pattern were observed in rabbits after the ivermectin treatment. However, in rats the antibody activity decreased and the profile of specific antibodies, tested by immunoblotting remained the same as before the treatment. These investigators concluded that the enhanced immune response in the mite-infested rabbits was caused by the massive release of antigens associated with the synchronous death of the mites. Stankiewicz *et al*. (1995) reported that the effect of ivermectin on immunity in lambs seems to be limited to its depressive effect on lymphocytes and the secondary antibody response to ovalbumin.

CONCLUSIONS

Based on the information reviewed above, it can be concluded that apart from anti-parasitic activity, levamisole, TBZ, oxfendazole, febendazole and ivermectin have immunopotentiating or immunorestorative activity while fenvalerate and dieldrin have immunosuppressive effects. As far as ascertained, no report is available on the immunomodulatory effect of anti-parasitics along with bystander infection. Restoration of normal immune function may increase resistance to infectious disease, reduce severity of disease, or shorten the recovery period. The immunomodulatory activities vary from one chemical/product to the other. In general, however, they are dose dependent and also relate with the parasite species, their density and biology with particular reference to male and female populations and fecundity. The doses of anti-parasitics used in the field for the control of parasites, therefore, depend upon the (i) nature and extent of parasite infection/infestation, (ii) general condition of the animal, (iii) therapeutic index of the drug and (iv) objectives of the treatment.

Targeting the therapeutic treatment and control of ecto- or endo-parasites with anti-parasitics, one may induce a state of immunoactivation or immunosuppression. If administration of anti-parasitics results in a period of immunosuppression then one should avoid co-administration of vaccines during this window. Contrarily, if administration of anti-parasitics induces immunoactivation then co-administration of vaccines and anti-parasitics or administration of vaccines during the window of immunoactivation is justified and should be incorporated into ecto- and endoparasite control and infectious disease control programmes. In the future, immunomodulating anti-parasitics may provide a useful therapeutic adjunct for treatment of certain disease states in humans and domestic animals.

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REFERENCES

Alex, P. C., Alikutty, K. M. and Mathew, S. (1995). Effect of levamisole administration on the incidence of bovine mastitis. *Journal of Veterinary and Animal Science* **26**, 134–135.

Ali, D. N. and Chick, B. F. (1992). Effect of feed type on the pharmacokinetic disposition of oxfendazole in sheep. *Research in Veterinary Science* **52**, 382–383.

Ali, M. M. M., Mukhtar, M. M., Baraka, O. Z., Homeida, M. M. A., Kheir, M. M. and Mackenzie, C. D. (2002). Immunocompetence may be important in the effectiveness of Mectizan® (ivermectin) in the treatment of human onchocerciasis. *Acta Tropica* **84**, 49–53.

Babiuk, L. A. and Misra, V. (1981). Levamisole and bovine immunity: in vitro and in vivo effects on immune responses to herpes virus immunization. *Canadian Journal of Microbiology* **27**, 1312–1319.

Bernier, J., Fournier, M., Blais, Y., Lombardi, P. and Chevalier, G. (1988). Immunotoxicity of aminocarb. I. Comparative studies of sublethal exposure to aminocarb and dieldrin in mice. *Pesticide Biochemistry and Physiology* **30**, 138–250.

Bernier, J., Hugo, P., Krzystyniak, K. and Fournier, M. (1987). Suppression of humoral immunity in

inbred mice by dieldrin. *Toxicological Letters* **35**, 231–240.

Blakley, B. R. and Rousseaux, C. G. (1991). Effect of ivermectin on the immune response in mice. *American Journal of Veterinary Research* **52**, 593–595.

Blecha, F. (1988). Immunomodulation: a means of diseased prevention in stressed livestock. *Journal of Animal Science* **66**, 2084–2090.

Bordmann, G., Rudin, W. and Favre, N. (1998). Immunisation of mice with phosphatidylcholine drastically reduces the parasitaemia of subsequent *Plasmodium chajandi chajandi* bleed-stage infections. *Immunology* **94**, 35–40.

Borgsteede, F. H. M., deLeeuw, W. A. and van de Burg, W. P. J. (1988). A comparison of efficacy of four different long acting boluses to prevent infections with *Dictyocaulus viviparous* in calves. *Veterinary Quarterly* **10**, 177–186.

Brieger, W. R., Awedoba, A. K. C., Eneanya, I., Hagan, M., Obguagu, K. F., Okello, D. O., Ososanya, O. O., Ovuga, E. B., Noma, M., Kale, O. O., Burnham, G. M. and Remme, J. H. (1998). The effects of ivermectin on Onchocercal Skin Disease and severe itching: results of a multicenter trial. *Tropical Medical Institute of Health* **3**, 951–961.

Brunner, C. J. and Muscoplat, C. C. (1980). Immunomodulatory effect of levamisole. *Journal of American Veterinary Medical Association* **176**, 1159–1162.

Cabaj, W., Stankiewicz, M., Jones, W. E. and Moore, L. G. (1994). Fenbendazole and its effect on the immune system of the sheep. *New Zealand Veterinary Journal* **42**, 216–220.

Campbell, W. C. (1990). Benzimidazoles: veterinary uses. *Parasitology Today* **6**, 130–133.

Chirigos, M. A. (1992). Immunomodulators: current and future development and application. *Thymus-Research* **19**, 517–520.

Christin, M. S., Gendron, A. D., Brousseau, P., Menard, L., Macrogliese, D. J., Cyr, D., Ruby, S. and Fournier, M. (2003). Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. *Environmental Toxicology and Chemistry* **22**, 1127–1133.

Chukwu, C. C. (1985). Serological response of cattle following *Brucella abortus* strain 19 vaccination and simultaneous administration of levamisole. *International Journal of Zoonoses* **12**, 196–202.

Cox, W. I. (1988). Examining the immunologic and haematopoietic properties of an immunostimulant. *Veterinary Medicine* **6**, 424–428.

Day, K. P., Spark, R., Garner, P., Raiko, A., Wenger, J. D., Weiss, N., Mitchell, G. F., Alpers, M. P. and Kazura, J. W. (1991). Serological evaluation of the macrofilaricidal effects of diethylcarbamazine treatment in bancroftian filariasis. *American Journal of Tropical Medicine and Hygiene* **44**, 528–535.

Dea-Ayuela, M. A. and Bolas-Fernandez, F. (1999). Trichinella antigens: a review. *Veterinary Research* **30**, 559–571.

Donskaya, E., Lundy, J., Simon, R. and Goldschneider, I. (1982). Thiabendazole (TBZ), an immunomodulator. I. Effects on lymph node and spleen. *International Journal of Immunopharmacology* **4**, 487–496.

Downey, N. E. (1988). Effect of treatment of first season calves with an OPRB on their immunity to lungworm in the second season. *Veterinary Record* **123**, 571–572.

Dvoroznakova, E., Boroskova, Z., Dubinsky, P., Velebny, S., Tomasovicova, O. and Machicka, B. (1998). Changes in cellular immunity in mice treated for larval toxocariosis with fenbendazole. *Helminthologia* **35**, 4, 189–195.

Espinasse, J. (1980). Immunostimulation par le levamisole en clinique veterinaire. *Cah. Med. Vet.* **49**, 5–13.

Eysker, M. and Boersema, J. H. (1989). Delay in timing of the oxfendazole pulse release bolus in calves in the Netherlands. *Veterinary Quarterly* **11**, 210–215.

Eysker, M., Boersema, J. H. and Kooyman, F. N. J. (1990). Immunity of calves treated with an oxfendazole pulse release bolus to challenge with *Dictyocaulus viviparus*. *Research in Veterinary Science* **48**, 301–305.

Fenichel, R. L. and Chirigos, M. A. (1984). *Immune Modulation Agents and their Mechanisms*. Marcell Dekker, Inc, New York.

Fernie, D. S., Ripley, P. H. and Walker, P. D. (1983). Swine dysentery protection against experimental challenge following single-dose parentral immunization with inactivated *Treponema hyodysenteriae*. *Research in Veterinary Science* **35**, 217–221.

Flesh, J., Harel, W. and Nelken, D. (1982). Immunopotentiating effect of levamisole in the prevention of bovine mastitis, fetal death and endometritis. *The Veterinary Record* **111**, 56–57.

Flipo, D., Bernier, J., Girard, D., Krzystyniak, K. and Fournier, M. (1992). Combined effect of selected insecticide on humoral immune response in mice. *International Journal of Immunopharmacology* **14**, 747–752.

Fournier, M., Chevalier, G., Nadeau, D., Trottier, B. and Krzystyniak, K. (1988). Virus-pesticide interactions with murine cellular immunity after sublethal exposure to dieldrin and aminocarb. *Journal of Toxicology and Environmental Health* **25**, 103–118.

Giambrone, J. J. and Klesius, P. H. (1985). Effect of levamisole on the response of broilers to coccidiosis vaccination. *Poultry Science* **54**, 1033.

Gilleard, J. S., Duncan, J. L. and Tait, A. (1995). *Dictyocaulus viviparus*: surface antigens of the L3 cuticle and sheath. *Experimental Parasitology* **80**, 441–453.

Gottschall, D. W., Theodorides, V. J. and Wang, R. (1990). The metabolism of benzimidazole anthelmintics. *Parasitology Today* **6**, 115–124.

Gualzata, M. D., Rudin, W., Weiss, N. and Heusser, C. H. (1988). The cross-reactive immune response between infective larvae and adult worms of *Acanthocheilonema* (*Dipetalonema*) *viteae* is dominated by phosphorylcholine. *Parasite Immunology* **10**, 481–491.

Gualzata, M., Weiss, N. and Heusser, C. H. (1986). *Dipetalonema viteae*: phosphorylcholine and non-phosphorylcholine antigenic determinants in infective larvae and adult worms. *Experimental Parasitology* **61**, 95–102.

Guerrero, J. (1977). Immunological modulating influence of levamisole. *Proceedings of the Heartworm Symposium*

Atlanta, Georgia, 18–20 March 1977. American Heartworm Society, Veterinary Medicine Publishing Co., Bonner Springs, Kansas, USA, 1978, pp. 104–108.

Gunter, L., Roger, D. D. and Rudolf, G. (2000). Phosphorylcholine substituents in nematodes: structures, occurrence and biological implications. *Biological Chemistry* **381**, 839–847.

Gutman, G. A. and Mitchell, G. F. (1977). *Ascaris suum*: location of phosphorylcholine in lung larvae. *Experimental Parasitology* **43**, 161–168.

Gwilt, P., Tempero, M., Kremer, A., Connolly, M. and Ding, C. (2000). Pharmacokinetics of levamisole in cancer patients treated with 5-fluorouracil. *Cancer Chemotherapy and Pharmacology* **45**, 247–251.

Hadden, J. W. (1994). T–cell adjuvants. *International Journal of Immunopharmacology* **16**, 703–710.

Harnett, W. and Harnett, M. (1993). Inhibition of murine B cell proliferation and down-regulation of protein kinase C levels by a phosphorylcholine-containing filarial excretory-secretory product. *Journal of Immunology* **151**, 4829–4837.

Harnett, W. and Harnett, M. M. (1999). Phosphorylcholine: friend or foe of the immune system? *Immunology Today* **20**, 125–129.

Harnett, W. and Harnett, M. M. (2001). Modulation of the host immune system by phosphorylcholine-containing glycoproteins secreted by parasitic filarial nematodes. *Biochimica et Biophysica Acta* **1539**, 7–15.

Harnett, W. and Parkhouse, R. M. E. (1995). Nature and function of parasitic nematode surface and excretory-secretory antigens. In *Perspectives in Nematode Physiology and Biochemistry* (ed. Sood, M. L. and Kapur, J.), pp. 207–242. Narendra Publishing House, Delhi.

Harnett, W., Worms, M. J., Kapil, A., Grainger, M. and Parkhouse, R. M. E. (1989). Origin, kinetics of circulation and fate *in vivo* of the major excretory-secretory product of *Acanthocheilonema viteae*. *Parasitology* **99**, 229–239.

Hassan, A. B., Atta, A. H., Soliman, R. T. and Afifi, N. A. (1989). Effect of levamisole hydrochloride on the immune response to Newcastle disease virus vaccine in chicken. *Indian Veterinary Journal* **66**, 389–394.

Hersey, P., Ho, K. and Werkmeister, J. (1981). Inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures of T and B cells by levamisole *in vitro* and *in vivo*. *Clinical Experimental Immunology* **46**, 340–349.

Hewlett, E. L., Hamid, O. Y., Ruffier, J. and Mahmoud, A. A. (1981). *In vivo* supression of delayed hypersensitivity by thiabendazole and diethylcarbamazine. *Immunopharmcology* **3**, 325–332.

Holcombe, R. F., Li, A. and Stewart, R. M. (1998). Levamisole and interleukin-2 for advanced malignancy. *Biotherapy* **11**, 255–258.

Holcombe, R. F., Milovanovic, T., Stewart, R. M. and Brodhag, T. M. (2001). Investigation in the role of immunomodulation for colon cancer prevention: results of an *in vivo* dose escalation trial of levamisole with immunologic endpoints. *Cancer Detection and Prevention* **25**, 183–191.

Hugo, P., Bernier, J., Krzystyniak, K., Potworowski, E. F. and Fournier, M. (1988*a*). Abrogation of graft-versus-host reaction by dieldrin in mice. *Toxicology Letters* **41**, 11–22.

Hugo, P., Bernier, J., Krzystyniak, K. and Fournier, M. (1988*b*). Transient inhibition of mixed lymphocyte reactivity by dieldrin in mice. *Toxicology Letters* **41**, 1–9.

Irwin, M. R., Holmberg, C. A., Knight, H. D. and Hjerpe, C. A. (1976). Effects of vaccination against infectious bovine rhinotracheitis and simultaneous administration of levamisole on primary humoral responses in calves. *American Journal of Veterinary Research* **37**, 223–226.

Jacobs, D. E., Pitt, S. R., Foster, J. and Fox, M. T. (1987). Interaction between chemoprophylaxis and immunity to bovine parasitic gastroenteritis and bronchitis pilot studies using an oxfendazole pulse release bolus. *Research in Veterinary Science* **43**, 273–275.

Jansen, P. A. J. (1976). The levamisole story. *Progress in Biophysiology and Moleclular Biology* **20**, 347–383.

Jolicoeur, P., Fournier, M. and Krzystyniak, K. (1988). Suppression of microbiocidal activity of peritoneal exudate by sublethal dieldrin exposure of outbred and inbred mice. *Pesticide Biochemistry and Physiology* **31**, 203–212.

Kaneene, J. M. B., Okino, F. C., Anderson, R. K., Muscoplat, C. C. and Johnson, D. W. (1981). Levamisole potentiation of antigen specific lymphocyte blastogenic response to *Brucella abortus* exposed but non-responsive cattle. *Veterinary Immunology and Immunopathology* **2**, 75–85.

Kayatas, M. (2002). Levamisole treatment enhances protective antibody response to hepatitis B vaccination in haemodialysis patients. *Artificial Organs* **26**, 492–496.

Kehrli, M. E. Jr and Roth, J. A. (1990). Chemically induced immunomodulation in domestic food animals. *Advances in Veterinary Science and Compararative Medicine* **35**, 103–119.

Kende, M. M., Gainer, J. and Chirigos, M. (1984). *Chemical Regulation of Immunity in Veterinary Medicine*. Alan R. Liss, Inc., New York.

Khurana, R. and Chauhan, R. S. (2000). Immunopathological effects of fenvalerate on cell mediated immune response in sheep. *Journal of Immunology and Immunopathology* **2**, 56–59.

Khurana, R. and Chauhan, R. S. (2003). Effect of fenvalerate on humoral immunity in sheep. *Journal of Immunology and Immunopathology* **5**, 44–46.

Khurana, R., Mahipal, S. K. and Chauhan, R. S. (1999). Effect of pesticide on delayed type hypersensitivity reaction in sheep. *Indian Journal of Animal Science* **69**, 880–881.

Kimball, E. S, Clark, M. C., Scheinder, C. R. and Persico, F. J. (1991). Enhancement of *in vitro* lipopolysaccharide-stimulated interleukin-1 production by levamisole. *Clinical Immunology and Immunopathology* **58**, 385–398.

Krzystyniak, K., Flipo, D., Mansour, S. and Fournier, M. (1989). Suppression of avidin processing and presentation by mouse macrophages after sublethal exposure to dieldrin. *Immunopharmacology* **18**, 157–166.

Kumran, M. R. (1993). Recent clinical trials with levamisole. *Annals of the New York Academy of Sciences* **685**, 269–277.

Lammie, P. J., Hightower, A. W., Richards, Fo Jr., Bryan, R. T., Spencer, H. C., McNeeley, D. F., McNeeley, M. B. and Eberhard, M. L. (1992).

https://doi.org/10.1017/S0031182005009108 Published online by Cambridge University Press

Alterations in filarial antigen specific immunologic reactivity following treatment with ivermectin and diethylcarbamazine. *American Journal Tropical Medicine and Hygiene* **46**, 292–295.

Lapteva, L. A. (1988). Study of the mitotic activity of the somatic cell populations in the bone marrow of rats under the influence of benzimidazole derivatives. *Bulleten 'Vsesoyuznogo Instituta Gel'mintolgii im. K. I. Skryabina* **49**, 32–34.

Lejan, C. and Asso, J. (1981). Induction of immunity in calves. In *Current Therapy in Theriogenology* (ed. Morrow, D. A.), pp. 224–226. W. B. Saunders Company, Philadelphia.

Lowe, R. J. (1980). Levamisole as an immunomodulant. *Veterinary Record* **106**, 390.

Lundy, J. and Lovett, E. J. 3rd. (1978). Immunomodulation with thiabendazole: a review of immunologic properties and efficacy in combined modality cancer therapy. *Cancer Treatment Reports* **62**, 1955–1962.

Lundy, J., Lovett, E. J. 3rd., Conran, P. and Goldblatt, P. J. (1976). Thiabendazole: a potential adjuvant in cancer therapy. *Surgery* **80**, 636–640.

Lundy, J., Lovett, E. J. 3rd., Wolinsky, S. M. and Conran, P. (1979). Immune impairment and metastatic tumor growth: the need for an immunorestorative drug as an adjuvant to surgery. *Cancer* **43**, 945–951.

Luty, A. J., Downham, M. D., Whilworth, J. A., Morgan, D. and Taylor, D. W. (1992). Immunolgical studies on onchocerciasis in Sierra Leone. 2. Cell mediated immune response after repeated treatment with ivermectin. *Tropical Medicine and Parasitology* **43**, 54–58.

Maiboroda, L. A. and Shevchenko, L. L. (1978). Dynamic of the immunobiological reaction in hamsters infected with *Ascaris* and treated with thiabendazole. *I Vsesoyuznyi s'' ezd parazitotsenologov* (*Poltava, Sentyabr' 1978 g*). *Tezisy dokladov. Chast' 3*. ''*Naukova Dumka*'', *Kiev, USSR*, pp. 88–90.

Marriner, S. E. and Bogan, J. A. (1981). Pharmacokinetics of oxfendazole in sheep. *American Journal of Veterinary Research* **42**, 1143–1145.

Meroni, P. L. and Barcellini, W. (1985). *In vitro* and *in vivo* effects of a new immunomodulating agent (Biostim) on human lymphocytes. *International Journal of Immunopharmacology* **7**, 47.

Miller, T. W., Chaiet, L., Cole, D. J., Cole, L. J., Flor, J. E., Goegelman, R. T., Gullo, V. P., Joshua, H., Kemph, A. J., Krellwitz, W. R., Monaghan, R. L., Ormond, R. E., Wilson, R. E., Albers-Schonberg, G. and Putter, I. (1979). Avermectins, new family of potent anthelmintic agents: Isolation and chromatographic Properties. *Antimicrobial Agents and Chemotherapy* **15**, 368–371.

Mitchell, G. F. and Lewers, H. M. (1976). Studies on immune responses to parasite antigens in mice. IV. Inhibition of an anti-DNP antibody response with antigen, DNP-Ficoll containing phosphorylcholine. *International Archives of Allergy and Applied Immunology* **52**, 235–240.

Moertel, C. G., Fleming, T. R. and MacDonald, J. S. (1990). Levamisole and Fluorouracil for adjuvant therapy of resected colon carcinoma. *New England Journal of Medicine* **322**, 352–358.

Moertel, C. G., Fleming, T. R. and MacDonald, J. S. (1995). Fluorouracil and levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Annals of Internal Medicine* **122**, 321–326.

Mojzisova, J., Hromada, R., Paulik, S., Ondrasovic, M. and Bajova, V. (2004). Immune response and immunomodulatory effect of levamisole in immunosuppressed dogs vaccinated against Parvo virus. *Bulletin of Veterinary Institute in Pulawy* **48**, 93–97.

Mondal, D., Sinha, R. P. and Tiwary, B. K. (1993). Levamisole-induced immunomodulation in *Mycobacterium paratuberculosis* infections. *Indian Veterinary Journal* **70**, 207–209.

Movsesyan, S. O., Agadzhanyan, A. M., Balayan, K. S. and Galstyan, S. Kh. (1988). The effect of thiabendazole and Panacur on the immune status of sheep with gastrointestinal nematodes. *Biologicheskii Zhurnal Armenii* **41**, 633–639.

Mulcahy, G. and Quinn, P. J. (1986). A review of immunomodulators and their applications in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics* **9**, 119.

Naylor, P. H. and Hadden, J. W. (2003). T cell-targeted immune enhancement yields effective T cell adjuvants. *International Immunopharmacology* **3**, 1205–1215.

Nielsen, H. and Bonde, J. (1986). Immunostimulation of blood monocyte function by RU 41 740 (Biostim) in patients with chronic bronchitis. *Journal of Immunopharmacology* **8**, 589–592.

Panigraphy, B., Grumbles, L. C., Miller, D., Naq, S. A. and Hall, C. F. (1979). Antibiotic induced immunosuppression and levamisole induced immunopotentiation in turkeys. *Avian Diseases* **23**, 401–408.

Parish, S. J., McFarlane, R. G., Familton, A. S. and Abell, T. J. (1996). The effect of fenbendazole on the immune system of lambs. *Proceedings of New Zealand Society of Animal Production* **56**, 80–83.

Pelleteir, M., Roberge, C. J., Gauthier, M., Vandal, K., Tessier, P. A. and Girard, D. (2001). Activation of human neutrophils *in vitro* and dieldrin-induced neutrophilic inflammation *in vivo*. *Journal of Leukocyte Biology* **70**, 367–373.

Pelletier, M., Willoughby, D. A. and Giround, J. P. (1978). Modulating effect of levamisole on DNA synthesis in macrophages *in vitro*. *Journal of the Reticuloendothelial Society* **24**, 333–338.

Pery, P., Luffau, G., Charley, J., Petit, A., Rouze, P. and Bernard, S. (1979). Phosphorylcholine antigens from *Nippostrongylus brasiliensis* I. Anti-phosphocholine antibodies in infected rats and localization of phosphocholine antigens. *Annals of Immunology* (*Inst. Pasteur*) **130C**, 879–888.

Prakash, M. S., Rao, V. M. and Reddy, V. (1998). Effect of levamisole on immune status of malnourished children. *Journal of Tropical Pediatrics* **44**, 165–166.

Quinn, P. J. (1990). Mechanism of actions of some immunomodulators used in veterinary medicine. *Advances in Veterinary Science and Comparative Medicine* **35**, 43–49.

Rao, U. R., Chandrashekar, R. and Subrahmanyam, D. (1987). Effect of ivermectin on serum dependent cellular interactions to *Dipetalonema viteae*

microfilariae. *Tropical Medicine and Parasitology* **38**, 123–127.

Razzaq, A. (2000). Immunomodulatory effect of oxfendazole in commercial chicken layers. M.Sc. Thesis, Department of Veterinary Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

Rehman, A. S., Fatima, A. and Jagannath, M. S. (1989). Effect of selenium on primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus. *Journal of Nutrition* **118**, 229.

Reiss, C. S., Herrman, J. M. and Hopkins, R. E. (1987). Effect of anthelmintic treatment on the immune response of mice. *Laboratory Animal Science* **37**, 773–775.

Renoux, G. (1986). The Ten Commandments for immunotherapeutic drugs at the example of sulfer-containing agents. *Comparative Immunology Microbiology and Infectious Diseases* **9**, 121–129.

Renoux, G. and Renoux, M. (1974). Modulation of immune reactivity by phenylimidazothiazole salts in mice immunized by sheep red blood cells. *Journal of Immunology* **113**, 779–790.

Renoux, G. and Renoux, M. (1977). Brucellosis, immunosuppression and levamisole. *Lancet*, 372.

Rojas, A. F., Mickiewica, E., Feierstein, J. N., Glait, H. and Olivari, A. J. (1976). Levamisole in advanced human breast cancer. *Lancet* **1**, 211.

Roth, J. A., Kaeberle, M. L. and Hubbard, R. D. (1984). Attempt to use thiabendazole to improve the immune response in dexamethasone – treated or stressed cattle. *Immunopharmacology* **8**, 121–128.

Saini, S. S., Sharma, J. K., Joshi, V. B. and Kwarta, M. S. (1991). Effect of levamisole on humoral response of cow-calves vaccinated with *Brucella abortus* strain 19 vaccine. *Indian Journal of Animal Research* **25**, 69–72.

Sajid, M. S. (2004). Studies on the immunomodulatory effect of ivermectin in rabbits. M.Sc. Thesis, Department of Veterinary Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

Sangster, N. C., Rickard, J. M., Hennessy, D. R., Steel, J. W. and Collins, G. H. (1991). Disposition of oxfendazole in goats and efficacy compared with sheep. *Research in Veterinary Science* **51**, 258–263.

Sanders, M. A., Bruce, A., Stephen, B., Nancy, I., Kerkvliet, N. I. and Kaminski, N. E. (1991). Symposium on indirect mechanisms of immune modulation. *Fundamental and Applied Toxicology* **17**, 641–650.

Savanur, N. H., Honnegowda., Krishnappa, G., Shastri, K. N. V. and Narayana, K. K. (1996). Effect of ivermectin on lymphocyte status in rabbits. *Indian Veterinary Journal* **73**, 501–503.

Savanur, N. H., Honnegowda., Shastri, K. N. V., Krishnappa, G. and Narayana, K. K. (1995). Effect of ivermectin on immune response in rabbits inoculated with *Salmonella* antigen. *Indian Veterinary Journal* **72**, 221–223.

Sceto, C., Gillespie, K. M. and Mathieson, P. W. (2000). Levamisole induces interleukin-18 and shifts type 1/type 2 cytokine balance. *Immunology* **100**, 217–224.

Schiller, J. H., Lindstrom, M., Witt, P. L., Hank, J. A., Mahvi, D., Wagner, R. J., Sondel, P. and Borden, E. C. (1991). Immunological effects of levamisole *in vitro*. *Journal of Immunotherapy* **10**, 297–306.

Schimied, L. M. and Rosenbusch, C. (1973). Effects del levamisole sobre el indice de sueroprotection en privacunation antiaftosa. *Revue de Medicina Veterinaria* **54**, 467–471.

Singh, B. P., Singhal, L. K. and Chauhan, R. S. (2001). Fenvelerate-induced cell mediated immunological alterations in chicken. *Journal of Immunnology and Immunopathology* **3**, 59–62.

Singh, G., Singh, S. P., Sharma, L. D. and Ahmad, A. H. (2003). Fenvalerate-induced immunosuppression in buffaloe calves. *Journal of Immunology and Immunopathology* **5**, 70–72.

Singh, K. K. and Jha, G. J. (1996). Effect of chronic fenvalerate (synthetic pyrethroid) intoxication on the immune response of goats to *Brucella abortus* strain 19 vaccine. *Journal of Comparative Pathology* **115**, 299–303.

Sloan, T., Docg, D. and Joyce, P. (1991). Identification of phosphorylcholine containing antigens of *Fasciola hepatica* – successful tolerisation against this epitope in experimental animals. *Parasite Immunology* **13**, 447–455.

Soboslay, P. T., Luder, C. G., Hoffmann, W. H., Michaelis, I., Helling, G., Heuschkel, C., Dreweck, C. M., Blanke, C. H., Pritze, S., Banla, M. and Schulz-Key, H. (1994). Ivermectin-facilitated immunity in onchocerciasis; activation of parasite-specific T_h 1-type responses with subclinical *Onchocerca volvulus* infection. *Clinical and Experimental Immunology* **96**, 238.

Soboslay, P. T., Dreweck, C. M., Hoffmann, W. H., Luder, C. G., Heuschkel, C., Gorgen, H., Banla, M. and Schulz-Key, H. (1992). Ivermectin-facilitated immunity to onchocerciasis. Reversal of lymphocytopenia, cellular anergy and deficienct cytokine production after single treatment. *Clinical and Experimental Immunology* **89**, 407–413.

Stankiewicz, M., Cabaj, W., Jonas, W. E., Moore, L. G., Millar, K. and Ng Chie, W. (1995). Influence of ivermectin on cellular and humoral immune responses of lambs. *Veterinary Immunology and Immunopathology* **44**, 347–358.

Stankiewicz, M., Cabaj, W., Jonas, W. E., Moore, L. G. and Ng Chie, W. (1994). Oxfendazole treatment of non-parasitized lambs and its effect on the immune system. *Veterinary Research Communications* **18**, 7–18.

Stevenson, H. C., Green, I., Hamilton, J. M., Calabro, B. A. and Parkinson, D. R. (1991). Levamisole: known effects on immune system, clinical results, and future applications to the treatment of cancer. *Journal of Clinical Oncology* **9**, 2052–2066.

Symoens, J. and Rosenthal, M. (1977). Levamisole in the modulation of the immune response: the current experimental and clinical state: a Review. *Journal of the Reticuloendothelial Society* **21**, 175–221.

Symoens, J., Cree, J. D. and Van Bever, W. (1979). Levamisole. In *Pharmacological and Biochemical Properties of Drug Substances*, Vol. 2. (ed. Goldberg, N. E.), pp. 407–464. Washington Academy of Pharmacologists Association, Washington, DC.

Tall, B. G., Van Tinteren, H. and Zoetmulder, F. A. (2001). Adjuvants 5-FU plus levamisole in colonic or rectal cancer: improved survival in stage II and III. *British Jouranl of Cancer* **85**, 1437–1443.

Theinpont, D., Vanparijs, O. F. J., Raeymaekers, A. H. M., Vanderberk, J., Demoen, P. J. A., Allewijn, F. T. N., Marsboom, R. P. H., Niemegeers, C. J. E., Schellekens, K. H. L. and Janseen, P. A. J. (1966). Teteramisole (R8299), a new, potent broad spectrum anthelmintic. *Nature, London* **209**, 1084.

Uhlir, J. and Volf, P. (1992). Ivermectin: its effect on the immune system of rabbits and rats infested with ectoparasites. *Veterinary Immunology and Immunopathology* **34**, 325–336.

Van Der Maaten, M. J., Schmerr, M. J. F., Miller, J. M. and Sacks, J. M. (1983). Levamisole does not effect the virological and serological responses of bovine leukemia virus-infected cattle and sheep. *Canadian Journal of Comparative Medicine* **47**, 474–479.

Vercruysse, J., Dorny, P., Berghen, P. and Frankena, K. (1987). Use of an oxfendazole pulse release bolus in the control of parasitic gastroenteritis and parasitic bronchitis in first season grazing calves. *Veterinary Record* **121**, 297–300.

Wade, M. G., Foster, W. G., Younglai, E. V., McMahon, A., Leingartner, K., Yagminas, A., Blakey, D., Fournier, M., Desaulniers, D. and Hughes, C. L. (2002). Effects of subchronic exposure to a complex mixture of persistent contaminants in male rats: systemic, immune and reproductive effects. *Toxicological Sciences* **69**, 286–287.

Webster, L. T. (1985). Drugs used in the chemotherapy of helminthiasis. In *The Pharmacological Basis of Therapeutics*, 7th Edn (ed. Gilman, A. G., Goodman, L. S., Rall, T. W. and Murad, F.), pp. 1007–1028. MacMillan, New York.

Wenger, J. D., Forsyth, K. P. and Kazura, J. W. (1988). Identification of phosphorylcholine epitope-containing antigens in *Brugia malayi* and relation of serum epitope levels to infection status of jirds with brugian filariasis. *American Journal of Tropical Medicine and Hygiene* **38**, 133–141.

Wigginton, J. M. (1995). Reversal of ferritin-mediated immunosuppression by levamisole: a rationale for its application to management of the acquired immune deficiency syndrome (AIDS). *Medical Hypotheses* **44**, 85–88.

Zheng, H. J., Tao, Z. H., Cheng, W. F., Wang, S. H., Cheng, S. H., Ye, Y. M., Luo, L. F., Chen, X. R., Gan, G. B. and Piessens, W. F. (1991). Efficacy of ivermectin for the control of microfilaremia recurring after treatment with diethylcarbamazine. II. Immunological changes following treatment. *American Journal of Tropical Medicine and Hygiene* **45**, 175–181.