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Effect of the anticoagulant, pindone, on the breeding performance and survival of merino sheep, *Ovis aries*

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Abstract

The effect of the anticoagulant, pindone, on the breeding performance and survival of relatively free-ranging merino sheep was assessed. Pindone (2-pivalyl-1, 3-indandione) was administered orally as a single (10, 3, or 2 mg pindone kg^{-1} over three consecutive days) or multiple exposure (dosing regime repeated after a further 8 days). Prothrombin times (PT) increased up to 4-fold in treated sheep, and haemorrhage occurred in some instances, particularly with the double dose treatment. Deaths of sheep also occurred, usually when the sheep were placed under added stress, particularly that associated with shearing. The breeding performance of pregnant ewes dosed with pindone was reduced, mainly due to an increase in stillborn and nonviable lambs (i.e. deaths within 2 days of birth). The motility of sperm in treated rams was also affected. Pindone persisted in the blood (maximum, 13.2 mg L^{-1}) for up to 14 days after the last dose, and the half-life ($t1/2$) was estimated at ~5 days depending upon the dosing regime. Other tissue residues ranged from 17 (fat) to 39 (liver) mg kg^{-1}. The implications of these findings for ongoing responsible use of pindone (anticoagulants) in pest control programs are also discussed.

Keywords: Anticoagulant; Breeding performance; Pindone; Pest control; PT; Residue; Sheep; Survival

1. Introduction

Pindone (2-pivalyl-1, 3-indandione) was originally developed as an anticoagulant rodenticide against commensal rodents (Oliver and Wheeler, 1978; Lund, 1988; Twigg and Kay, 1995). However, more recently, its use has been extended to include feral European rabbits (*Oryctolagus cuniculus*; Oliver and Wheeler, 1978; Wheeler and Oliver, 1978; Oliver et al., 1982), and it has been investigated as a possible toxin for controlling introduced Australian brushtail possums (*Trichosurus vulpecula*) in New Zealand (Eason and Jolly, 1993; Eason et al., 1993). Pindone acts by blocking the synthesis of vitamin K-dependent clotting Factors II, VII, IX and X through the inhibition of the enzyme, epoxide reductase. Failure to synthesise these factors ultimately results in potentially fatal coagulopathy (Mount, 1988; Martin et al., 1991). The effects of pindone poisoning can often be reversed by administration of vitamin K_1 (Beauregard et al., 1955; Martin et al., 1994).

Because the one-stage prothrombin time (PT) requires functional Factor VII, it provides a sensitive index of the toxicity of the anticoagulant rodenticides (Doerr et al., 1975; Mount, 1988; Martin et al., 1991, 1994). A marked elevation in PT precedes the onset of clinical signs (bleeding) of anticoagulant toxicity. Changes in PTs have been used in the diagnosis, and in monitoring the treatment, of anticoagulant poisoning (Mount, 1988), including the assessment of the relative toxicity of pindone to a number of vertebrate species (Doerr et al., 1975; Martin et al., 1991; 1994).

Although sodium fluoroacetate (1080) is the preferred poison for rabbit control in Australia, pindone is registered for controlling these pests in most Australian states as it provides a useful alternative in situations where 1080 cannot be used (Twigg and King, 1991; Williams et al., 1995). Pindone is also occasionally used to control introduced rats

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(*Rattus* spp.) on offshore islands (Martin et al., 1994). However, there has been limited investigation into possible nontarget effects resulting from pindone-baiting programs, the persistence of pindone in poisoned animals, or the level of pindone residues in tissue. Despite earlier contentions (e.g. Oliver and Wheeler, 1978; Martin et al., 1991), pindone is now believed to represent a greater potential hazard to nontarget wildlife than previously thought (e.g. Martin et al., 1994). As rabbits were found to be quite sensitive to pindone (chronic LD_{50} of 0.52 mg kg^{-1} daily for 7 days (Oliver and Wheeler, 1978)), and as the sensitivity of domestic species to pindone was believed to be low (Oliver and Wheeler, 1978; Martin et al., 1991), the initial pindone-based rabbit control strategy planned to use poison bait (oats with 2.8 g pindone g^{-1}, and poisoned oats mixed with filler (unpoisoned oats) at 1:11, laid in trails at ~17 kg km^{-1}) in the presence of domestic livestock. Sheep (*Ovis aries*) seemed to survive dose rates of up to 16 mg kg^{-1} day^{-1} and, although PTs increased approximately 2-fold, they had returned to normal within 7 days (Oliver and Wheeler, 1978). However, the chronic, medium-term effects of sublethal doses on such species have not been determined, and more recent research (e.g. Martin et al., 1994) suggested that such a use strategy may not be prudent.

In this paper, we describe the effect of orally administered pindone on merino sheep (*O. aries*), particularly on their PTs, its impact on the breeding performance of ewes and rams, the residue levels in blood and other tissue, and the impact of shearing on the survival of pindone-treated sheep. We also make additional recommendations regarding the safe and effective use of pindone products (and hence by inference, other anticoagulants) in vertebrate pest control programs.

2. Materials and methods

2.1. Pindone administration

The dosing regime selected was chosen as a worst case scenario with respect to the amount of pindone that individual sheep could ingest as a result of being exposed to pindone-based (poisoned oats) rabbit control programs in Western Australia. One 'dose' was spread over three consecutive days, commencing with a relatively high dose but with decreasing amounts each day (i.e. 10 mg kg^{-1}, 3 mg kg^{-1}, or 2 mg pindone kg^{-1}). This approximated the rate at which sheep could eat pindone-oats from bait trails, based on our earlier field observations (i.e. bait availability decreases with time). The total dose equates to ~19% of the acute LD_{50}. For the double exposure treatments, this dosing regime was repeated 8 days after the initial exposure (see below). The pindone (2-(2,2-dimethyl-1-oxopropyl)-1*H*-indene-1,3(2*H*)-dione) was dissolved in maize oil and dosing solutions were such that each sheep received a similar volume of pindone-oil solution. Dose volumes were usually around 15.0, 4.5, and 3.0 mL, respectively, which were less than 1% of body mass. These solutions were administered orally using a Vaxmaster drench gun with backpack.

An earlier trial with three groups of nine merino ewes indicated that the method of dosing (on poisoned oats (mean (S.D.) peak PT time 37.4±5.2 s), in a gel (35.3±2.5 s),or in maize oil (36.9±3.3 s)) did not affect the observed peak in PT (ANOVA F=1.39, df=2,26, P>0.05). The observed time for the peak in PT to occur was also similar between these treatments (Days 5–7, 5–7 and 5–8, respectively).

2.2. Effect on prothrombin time (PT)

As PT can be used as a measure of anticoagulant poisoning (Mount, 1988), changes in PTs (i.e. blood clotting times) were used as an index of the toxicity of pindone to sheep. Prothrombin times were determined in two separate trials with merino sheep using two slightly different reagents: (1) Dade's 'Activated Thrombroplastin' (Quick et al., 1935; Quick, 1972), and (2) 'Simplastin' (General Diagnostics, 'Simplastin'). Both reagents measure the relative activity of Factors I, II, V and X, and particularly Factor VII (Doerr et al., 1975; Mount, 1988). The need for the second reagent arose due to a lack of supply of the Dade's 'Activated Thrombroplastin'. In Trial 2, Dade's 'Activated Thrombroplastin' was used for all days up to Day 11, and Simplastin was used on Days 14 to 32. Dade's 'Activated Thrombroplastin' only was used for Trial 1. The Simplastin procedure gave PTs ~2 s less than did Dade's 'Activated Thrombroplastin'.

Blood samples (4.5 mL) were collected from the jugular vein during the morning, and added to 0.5 mL 3.8% sodium citrate solution (i.e. 9:1 v/v). Samples were centrifuged, the plasma immediately removed to a second tube, and placed in an ice-water bath. Activated thromboplastin was mixed with an equal quantity of 0.2 M calcium chloride (37 °C), and the PTs determined using pre-warmed plasma and a stop watch. Tests were run in triplicate.

After acclimation, all sheep were orally dosed as above in the afternoon with 10 mg kg^{-1}, 3 mg kg^{-1}, or 2 mg pindone kg^{-1} on Days 1, 2 and 3, respectively. Untreated sheep (n=3×2) received similar amounts of maize oil only. Sheep were maintained in small paddocks supplemented with oats and hay. Water was ad libitum.

In Trial 1, 33 nonpregnant merino ewes (35–54 kg; 30 treated, 3 untreated) were used to determine the effect of pindone on blood clotting (i.e. PT) times following the single, 3-day exposure. PTs were determined for all ewes on the day prior to dosing to establish base-line PT levels. Blood samples were collected daily thereafter until PTs had returned to normal levels, which took between 16 and 22 days.

In Trial 2, the effect of two 3-day exposures to pindone (i.e. double exposure) was determined using 33 merino wethers (31–35 kg; 15×2 treated, 3 untreated). PTs were determined for all wethers prior to dosing on Day 0. Blood samples were collected thereafter on Days 4, 7, 11, 14, 18, 21,