XYLAZINE STUDY REPORT

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INTRODUCTION

At the last Deer Branch conference, Walker and Middleberg (1988) presented a paper on "Post velveting deaths in stags" which reported on a number of deaths which had occurred in their practice in the previous season. They also reported on a small slaughter trial in which stags were killed at varying times after velveting, and on a survey of 11 practices which sought to assess the incidence of post velveting stag deaths and to record data on the procedures used prior to death and the presumptive diagnosis. The important features that emerged from their studies were:

i) the time of death ranged from 1 to 3 hours to several days after velveting,

ii) all stags which died post-velveting had received xylazine and local anaesthetic, and some had also received yohimbine (Recervyl),

iii) deaths occurred after the use of both "Rompun" and "Thiazine" brands of xylazine,

iv) the reported mortality rate was around 1.7 per 1,000 stags velveted (30/17256),

v) there appeared to be more deaths in 1987-88 than in previous seasons,

vi) very few animals were necropsied but a few "typical" cases revealed few signs other than froth in the trachea, bronchi and bronchioles on gross examination. Histopathological findings were mainly confined to the lungs which showed generalised oedema, thicker than normal intralobular septa, with severe infiltration of degranulated eosinophils and perivascular eosinophils,

vii) one of the stags slaughtered 10 days post-velveting in the small slaughter trial also showed heavy eosinophil infiltration in the lungs, the rest were normal.

Walker and Middleberg (1988) reviewed these findings and concluded that the most likely cause of stag deaths was some form of hypersensitivity. Other possible causes of stag deaths after velveting include:

regurgitation and inhalation pneumonia,
blood loss/hypovolaemic shock,
hyperthermia and
endotoxic shock resulting from bowel stasis.

We will discuss each of these possible causes of stag deaths and present some experimental data to support the arguments.

HYPERSENSITIVITY

Background: A number of anaesthetics, including morphine derivatives, thiopentone, propanidid, althesin, suxamethonium and flunitrazepam have been shown to occasionally cause adverse effects in humans and other animals especially if given intravenously (Clark et al. 1978; Lorenz and

Doeniche 1978; Robinson et al., 1988). These adverse drug reactions are probably pharmacological in nature and are the direct result of the drug, or an interacting mixture of drugs causing the release of histamine and other vasoactive substances from mast and/or basophil cells in the vascular system (Watkins et al., 1978). Althesin, propanidid and thiopentone all cause significant changes in blood white cell counts and plasma histamine levels in virtually 100% of humans receiving them but it is the nature of the threshold barrier between subclinical and clinical histaminoid response which may be of particular importance (Watkins et al., 1978). In addition a wide range of other factors including chemicals, drugs, toxins and physical factors such as temperature or irritants and lymphokines can result in non-immune degranulation (Fadal, 1985).

Mast cells are sessile and are distributed throughout tissues but are present in particularly high numbers in lung tissue, whereas basophils are mainly observed in blood but will migrate into areas of inflammation especially in delayed-type hypersensitivity responses. Both types of cells contain differing amounts of various vasoactive amines and mediators including histamine, serotonin, dopamine, heparin, leukotrienes and eosinophil chemotactic factors. Release of these substances causes transient hypotension, tachycardia and bronchospasm, while severe cases can develop anaphylactic shock. Leukotrienes, or "slow-reacting substances of anaphylaxis" (SRS) produce slow, prolonged contractile effects on peripheral airway tissues. They also increase systemic vascular permeability, contribute to airway oedema, the accumulation of mucus in broncho-constricted airways and sensitise the lung to a wide variety of non-specific stimuli (Fadal, 1985). For example the lungs may become more sensitive to dust or other such irritants.

"Late-phase" reactions mediated by SRS are characterised by eosinophil and neutrophil infiltration and fibrin deposition in affected organs. They usually occur 4 to 8 hours after immediate hypersensitivity and persist for 24 to 48 hours or longer. The intensity of the late-phase reaction usually parallels the intensity of the immediate reaction. Eosinophil chemotactic factor released by degranulating basophils and mast cells attracts eosinophils which are thought to have a moderating effect by releasing antihistamines as well as other substances. This may localize the event and reduce the systemic effects of histamine release. On the other hand, eosinophils may add to "allergic" injury because the basic protein of eosinophils is toxic to the epithelium of the respiratory tract and eosinophils can also produce SRS (Fadal, 1985).

The distribution of drugs in the body may also contribute to adverse reactions. Because all venous drainage is pumped through the lungs prior to distribution around the body, the lung can have a buffering or filtering action on venous drug levels. Basic lipophilic amine drugs (eg lignocaine, propanolol, imipramine and methadone) are highly concentrated in the lung. In most cases this is regarded as a protective mechanism but it may not be desirable in cases where the drug or drug-carrier complexes modulate uptake and/or release of vasoactive substances and hormones (Tucker, 1978).

Evidence for hypersensitivity to xylazine: The histological findings of pulmonary oedema and eosinophilic infiltration reported by Walker and Middleberg (1988) are consistent with hypersensitivity while the time course of "1 to 3 hours to several days" is consistent with "late effect" of SRS. Xylazine is a basic lipophilic drug and is likely to be concentrated in the lungs. Acute pulmonary oedema was reported in 8 of 50 sheep sedated with xylazine (Rompun, 0.15 - 0.5 mg/kg intramuscularly) in Sweden (Uggla and Lindqvist, 1983). Signs of wheezy dyspnoea occurred

after 15 minutes. One sheep died, some recovered spontaneously and others recovered after the administration of atropine. Last year a stag at Invermay died 20 minutes after Rompun/Fentaz (500 mg xylazine + 10 mg fentanyl citrate + 80mg azaperone) sedation and the only histopathological signs were slight congestion and oedema in areas of the lung, with peribronchiolar and perivascular hypercellularity predominantly involving eosinophils (Mackintosh, unpub).

In order to investigate further the effect of xylazine on stags at velveting a stag velveting/xylazine trial was conducted at Invermay last year (Mackintosh et al,. in prep). A summary of the most significant effects is presented here (Table 1).

Table I Time/effect summary of responses of stags to xylazine (Thiazine 5% solution, 0.9 mg/kg) sedation (XS) or physical restraint (PR) and velveted (V) or non-velveted (NV).

0.5h	Hb, RCC, PCV, MCV, Basophils, Lymphocytes, Platelets, PV, Fib, K^+, Anion gap, RBC-K^+ all fell in XS stags TCO_2 rose in XS stags Cortisol rose higher in PR stags Neutrophils rising in all stags
1.0h	Hb, RCC, PCV, MCV, Lymphocytes, Platelets, PV, Fib, CPK, Anion gap, RBCK^+ significantly lower in XS stags Basophils returned to normal TCO_2 significantly higher in XS stags Cortisol rose higher in PR stags Neutrophils rising in all stags
6.0	Eosinophils declined in all stags but significantly more in PR Neutrophils rose significantly more in PR stags CPK peaked at 6 hours in all groups SGOT elevated in all groups Temperatures significantly higher rise in XS stags Cortisol rose higher in PR stags
24h } 48h	CPK and SGOT elevated in all stags} PCV, Fibrinogen high in all stags } Hb and PCV lower in V stags } Haptoglobin higher in XS stags

One of the most intriguing effects was the significant fall in basophil number recorded at 0.5 hour after xylazine administration and then its return to normal by 1.0 hour. At the moment one can only speculate whether this fall in basophils was due to their destruction/degranulation or migration out of central circulation and whether their return was due to replacement from bone marrow reserves, regranulation or re-entry into central circulation. Concurrently, lymphocyte numbers also fell dramatically (30% fall) in xylazine-treated stags at 0.5 hours and then gradually returned to normal over the next 24 - 48 hours. Factors that rapidly affect circulating numbers of lymphocytes include vasoactive modulators such as prostaglandin, histamine, bradykinin and serotonin, all of which are released from degranulating basophils and mast cells (Schalm et al., 1987).

Haptoglobin, an acute inflamatory protein, was significantly higher in xylazine-treated stags 48 hours after treatment indicating that some tissue damage had taken place.

In the above trial, 8 stags received xylazine and none of them showed any subsequent adverse clinical effects. However, the above subclinical changes in haematological and biochemical parameters suggest that xylazine affects most stags in a minor way and one can speculate that these changes may occasionally trigger a serious reaction.

Other in vivo effects of xylazine in intact and splenectomised deer are reported elsewhere (Cross et al., 1988; Cross et al., 1989). More recently we have conducted some preliminary in vitro studies (Cross & Mackintosh, unpub) to investigate the effect of xylazine on deer blood. The results have shown that mixing equal volumes of 2% xylazine and fresh heparinised deer blood caused lysis of white cells which commenced in less than one minute and affected most of the cells within 5 minutes. The 2% xylazine was isosmotic and the cause of the lysis is being further investigated.

Other possible causes of death:-

REGURGITATION AND INHALATION PNEUMONIA

Prolonged lateral recumbency and heavy sedation with xylazine or xylazine mixtures may result in regurgitation of ruminal contents, especially if ruminal tympany develops, or if the rumen is very full of ingesta as is often the case with stags in late spring when feed intakes are high. Xylazine can also cause excessive saliva production in ruminants and premedication with atropine has been recommended (Knight, 1980). However, the most common sequels to regurgitation are either death without the animal regaining consciousness due to obstruction of the airway, or the animal becomes very sick after a day or two and may die in 2 or 3 days from acute necrotising pneumonia. On necropsy the diagnosis of inhalation pneumonia is quite straightforward and is unlikely to be mistaken for a hypersensitivity reaction.

Therefore, it is possible that some of the stag deaths have been a result of inhalation pneumonia, but this is probably not the major cause of the "Stag death syndrome".

BLOOD LOSS AND HYPOVOLAEMIC SHOCK

It is generally recognised that velvet antler removal in stag usually results in relatively little blood loss, especially if effective tourniquets are applied for 5 to 10 minutes after velveting. It is possible that there may be a deficiency in the blood-clotting mechanism in some stags but this has not been reported and is unlikely to be a significant problem. If death occurred due to poor haemostasis, then excessive blood around the head or in the environment is likely to have been in evidence at necropsy and reported.

HYPERTHERMIA

It is generally recommended that velvet antler is removed during the cool part of the day and shade from hot sun should be provided for sedated animals. Xylazine has been reported to interfere with temperature regulation in cattle (Young, 1979; Fayad et al 1989), eland and impala (Drevemo and Karstad, 1974) and fallow deer (Sancken and Fischer, 1988). In ambient temperatures of 25° - 28°C, cattle injected with xylazine (Rompun 0.2 mg/kg IM) had temperature rises of up to 1.9°C (ie to over 40.4°C) which peaked 4 to 5 hours after injection and did not return to normal within 18 hours of injection. Heat-stressed heifers sedated with

xylazine showed marked hyperthermia, suppressed respiration rate and took over twice as long to stand than heifers sedated in thermoneutral conditions. On the other hand, in temperatures of 18 - 26°C the rectal temperatures of eland and impala dropped from 39° to 36 - 37°C when sedated with xylazine. In ambient temperatures of 12 - 18°C, 2 of 4 fallow deer immobilised with xylazine showed hypothermia while 2 showed hyperthermia, with temperature peaks of 39.5 and 40.4°C, 2 to 4 hours after injection.

In the previously mentioned xylazine trial in adult red stags at Invermay, 5 of the 8 xylazine (Thiazine 0.9 mg/kg IM) sedated stags showed an initial fall in temperature in the first hour before their temperature rose and peaked at 6 hours. Rectal temperatures of the other 3 stags rose to a maximum at 1 hour. It has been suggested that xylazine interferes with the temperature regulating mechanisms in the hypothalamus.

The release of an endogenous pyrogen, tentatively identified as Interleukin - 2 (Stites et al 1984), from macrophages in association with tissue damage or endotoxins could also lead to elevated temperatures. Such a factor could also be associated with the observed haematological changes and elevated acute phase proteins seen in deer sedated with xylazine. It has been reported (Fadal, 1985) that high temperatures can cause non-immune degranulation of mast cells and basophils and this may help trigger a hypersensitivity reaction.

ENDOTOXIC SHOCK RESULTING FROM BOWEL STASIS

Xylazine sedation in cattle has been reported to cause watery, haemorrhagic diarrhoea or loose faeces 12 to 24 hours later (Knight, 1980). This has been postulated to result from rumen and intestinal stasis causing overgrowth of pathogenic gut flora and inflammation. It is possible that endotoxic shock may result from xylazine sedation in deer, although this is likely to be rare.

In the stag velveting/xylazine trial at Invermay there were no changes in faecal consistency over the 48 hour trial. However, yohimbine (Mackintosh and van Reenan, 1984) was administered around one hour after the xylazine, and this may have reduced any possible overgrowth of bacteria by restoring rumen and gut motility.

CONCLUSION

Not all the stags which had died in association with xylazine sedation at the time of velveting have been necropsied and consequently it is not possible to attribute all the deaths to any one cause. However, it is clear that a proportion of the dead stags presented similar pathological pictures of lung oedema and eosinophilic infiltration.

These findings are consistent with "hypersensitivity" to xylazine or some other ingredient, impurity or preservative.

The majority of deer show statistically significant but clinically inapparent haematological and biochemical changes in response to xylazine sedation. However it is likely that in a small number of cases an adverse drug response to xylazine is triggered, involving the degranulation of mast cells and basophils. The cause of this "trigger" which results in a severe clinical response to the drug is not known. Nevertheless one can speculate that possible causes may include:

(a) combination with some other pharmacological compound(s),
(b) hyperthermia,
(c) excessively dusty condition,

(d) some innate susceptibility of the animal,
(e) prolonged recumbency, passive congestion in the lungs and oedema,
(f) endotoxic shock and/or septicaemia or pre-existing lung infection or inflammation.

RECOMMENDATIONS

Prevention: Until the aetiology is determined it may be wise to take the following precautions,

(i) Anaesthetise stags in cool conditions and provide shade until they are fully recovered.

(ii) Reverse the sedation with yohimbine as soon as practicable to get the animal on its feet, reverse bowel and ruminal stasis, prevent ruminal tympany and regurgitation and minimise pulmonary congestion. The animal is also more likely to seek shade if necessary.

(iii) Avoid excessively dusty conditions which might lead to inhalation of dust by recumbent stags.

(iv) Ensure that each bottle of xylazine is kept cool and uncontaminated.

There is some evidence that the administration of atropine, by slow intravenous injection as soon as the stag is immobilised, should reduce salivation, increase bronchiolar dilation, prevent or reverse pulmonary oedema and increase heart rate. The suggested dose rate is up to 2.0 mg./kg. However, further investigations should be undertaken before this regime can be recommended for routine use.

Treatment: A stag showing clinical signs of respiratory distress, depression or recumbency within 12-24 hours of velvet antler removal could well be suffering from drug hypersensitivity and it is likely to die if untreated. There is good evidence that certain non-steroidal anti-inflammatory agents should be effective in treatment this condition.

Sodium meclofenamate (Meclomen) and meclofenamic acid (Arquel) antagonise the pharmacological effects of bradykinin and SRS in sheep (Alexander et al., 1970) and cattle (Booth, 1982) and have been used to prevent respiratory distress of calves and horses in anaphylactic shock. They are derivatives of anthranilic acid, an amine analogue of salicylic acid and are thus related to aspirin, acetylsalicylic acid. Aspirin is also moderately effective in alleviating anaphylaxis in horses and calves, and is considered a useful adjunct in the treatment of acute pneumonia in cattle, at a dose rate of 50-100 mg/kg. The major problem with the use of aspirin for treating ruminants is the slow rate of absorption following oral administration (half-time absorption 2.91 hours). However, sodium salicylate (40-50 mg/kg) may be given by intravenous injection (Booth, 1982).

The salicylates reduce inflammatory respnses by their inhibition of prostaglandin synthesis from arachidonic acid. It is known that $PGF_2\alpha$ is a potent bronchoconstrictor and that an anaphylactoid type of reaction develops following administration of minute dosages.

SRS (leukotrienes) are also derived from arachidonic acid by an alternative pathway. Steroidal anti-inflammatory agents block synthesis of arachidonic acid which in turn prevents synthesis of leukotrienes. Thus short acting corticosteroid therapy may be indicated.

Flunixin meglumate (Finadyne) is also a potent non-steroidal anti-inflammatory which acts by inhibiting prostaglandin synthesis. Intravenous administration of Finadyne at a rate of 2.2 mg/kg may help to alleviate signs of drug hypersensitivity.

Antihistamines, such as mepyramine maleate, are probably of limited usefulness in treatment hypersensitivity reactions because histamine is only one component of the response. However, they may assist when used in combination with the non-steroid anti-inflammatory agents referred to above.

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