# Papers and Articles

## Immobilisation of free-ranging wild animals using a new drug

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**Field trials were conducted with the potent morphine-like analgesic, R33799 (Janssen Pharmaceutica; Beerse, Belgium) in South African national parks on 217 free-ranging wild animals, representing 20 different species. The drug was found to be effective and safe for a wide range of ungulates and pachyderms but Burchell's zebra (*Equus burchelli*) did not react to expected dosage levels. A suggested dosage regime for 19 species is given. Recommended optimal dosage rates varies from about 1 μg per kg for pachyderms to about 10 μg per kg for most of the larger ungulates. Xylazine and azaperone were found valuable adjuncts to R33799 in dosage ratios of 10:1 and 30:1 respectively.**

IMMOBILISATION of wild animals is used not only to facilitate translocation but has become indispensible for veterinary, biological and ecological research (Hofmeyr 1973, Harthoorn 1976).

Projectile syringes and apparatus for their projection opened up possibilities for chemical immobilising agents. Earlier trials involved a wide range of muscle relaxants, reflex inhibitors, central nervous system depressants, hypnotics, analgesics and neuroleptics. Their effects (Pienaar 1973a) were rather inconsistent and most had critical dosage margins. The lack of instant reversibility by antidote was also a disadvantage.

After introduction of the highly potent, thebaine derived analgesic, oripavine hydrochloride (etorphine, or M-99; Reckitt & Colman), in 1963 and of the piperidine derivative, fentanyl (R4263; Janssen), the first successful series of field trials on a wide spectrum of wild ungulates was set up (Pienaar 1973a). Efforts were directed at perfecting dosage rates for particular species and at finding the correct neuroleptic additive for the existing narcotic-analgesic mixtures.

Janssen Pharmaceutica, Belgium, synthesised and screened for analgesic activity a series of N-4-substituted 1-(2-arylethyl)-4-pipendinyl-N-phenylpropanaruides (Van Daele and others 1975, Van Bever and others 1976). Some of these compounds were found to be extremely potent analgesics characterised by unusually high safety margins, surpassing the performance of fentanyl under laboratory conditions (Van Bever and others 1976). The finding of tests carried out in the national parks of South Africa on R33799, a 4-substituted derivative of fentanyl, are documented.

### Materials and methods

The newly developed piperidine derivative R33799, was used as the basic immobilising agent. R33799 is chemically closely related to fentanyl. The drug was supplied in 1 ml ampoules as a pharmaceutical solution of 10 mg per ml. For more accurate dispensing when small quantities were needed, the drug was diluted to 5 mg per ml or 1 mg per ml.

Other drugs that were used in conjunction with R33799 included the following:

*Azaperone* (Flupyridol; Janssen). This drug was used for its known neuroleptic properties and is a butyrophenone derivative. Pharmaceutical solutions containing 100 mg per ml were prepared in the manner described previously by Pienaar (1968).

*Xylazine hydrochloride* (Rompun; Bayer). This thiazine derivative was used for its known sedative, analgesic (central acting) and synergistic effects on etorphine and fentanyl in capturing nervous and aggressive antelope species (Smuts 1973). The drug substance was made up to concentrations of 100 to 500 mg per ml in the solvent provided with the drug.

*Naloxone hydrochloride* (Narcan; Endo Laboratories). This is a potent narcotic antagonist synthesised from oxymorphone hydrochloride, a narcotic analgesic. It was used for its antagonistic properties to etorphine and fentanyl and its wide safety range (Blumberg and others 1965, Martin 1967, Smuts 1975).

*Cyprenorphine* (M285; Reckitt & Colman). This morphine derivative was used for its potent antagonistic action against morphine and morphine-like analgesics and its wide therapeutic index (Harthoorn 1973).

The immobilising drugs were administered either by 3 ml capacity Van Rooyen projectile syringes fitted with a retractable barb needle (Van Rooyen and De Beer 1973) or a 1 ml capacity spring-loaded projectile syringe fitted with a barbed needle (De Vos and others 1973). A Van Rooyen modified shotgun was used to propel the larger syringes, while a hypodermic-projectile firing gun (Cap Chur Gun; Palmer) was used for the 1 ml syringes.

Operations were restricted to the Kruger, Kalahari Gemsbok, Mountain Zebra, Addo Elephant and Bontebok National Parks in South Africa. Drug reactions were tested on 20 species of animals as listed in Table 1. The subject animals were free-ranging and were darted from a ground vehicle or helicopter. They were immobilised and caught either to translocate as part of an animal reduction programme, or for marking, to assist various ecological study projects, or solely to test drug dosages and combinations.

To test the potency of R33799 and the correct dosage rate for each species a relatively high dose was used initially. Where possible this dose was gradually brought down until the desirable effect was obtained. After the first few trial runs and judging from experience with the related compound, fentanyl, an approximation of the relative strength was obtained. Corrective allowances were made with subsequent dosages.

Necropsies were performed on those animals that died during the operations. Estimates of the weight of immobilised animals were derived from a combination of experience and known adult live-weight records by Wilson (1968) and Von La Chevallerie (1970). Induction time is taken as the time for an animal to become immobilised after injection.

### Results and discussion

A total of 217 free-ranging wild animals, representing 20 different species, were immobilised with the trial mixtures (Table 1).

Providing the animals were kept reasonably free from external stimuli very little excitement was noticed during the induction phase, even when R33799 was used without tranquillisers. Adverse reactions were however witnessed occasionally, especially in nervous or highly excitable animals. R33799 therefore seems to have retained the typical morphine-like reaction of causing excitement, which is apparently minimised by its powerful action and rapid absorption. In the field this problem was largely overcome by the use of tranquillisers.

**TABLE 1: The results of chemical immobilisation using R33799 in 20 free-ranging wild animal species**

| Species | Sample number | Estimated body weight | | R33799 dosage rate | | Neuroleptic dosage rate | | | | Induction time | | Average antidote dosage rate M285 or Narcan | | Recovery time* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Xylazine | | Azaperone | | | | | | | |
| | | Range kg | Mean kg | Range µg per kg | Mean µg per kg | Range µg per kg | Mean µg per kg | Range µg per kg | Mean µg per kg | Range min | Mean min | µg per kg | µg per kg | Range min | Mean min |
| African elephant (*Loxodonta africana africana*) | 9 | 2500-6200 | 4700 | 0·8-2 | 1·3 | — | — | — | — | 10-30 | 16 | 10·6 | 10·6 | 4-15 | 6·5 |
| Square-lipped (white) rhinoceros (*Ceratotherium simum simum*) | 4 | 1500-2000 | 1767 | 0·6-1·3 | 0·8 | — | — | — | — | 7-20 | 12·3 | 17 | 11·3 | 3-15 | 8 |
| Black rhinoceros (*Diceros bicornis bicornis*) | 12 | 500-1250 | 962·5 | 0·8-2 | 1·2 | — | — | — | — | 3·5-25 | 10·2 | 20·8 | 20·8 | 1-5 | 3·2 |
| Giraffe (*Giraffa camelopardalis giraffa*) | 5 | 600-1100 | 780 | 2·3-3·3 | 2·7 | 36·4-53·3 | 47 | 128·2 | 128·2 | 0·3-11 | 7·3 | 38·5 | — | 3-8 | 5 |
| African buffalo (*Syncerus caffer caffer*) | 6 | 80-750 | 400 | 3·3-6·7 | 5·2 | 33·3-62·5 | 43·3 | — | — | 2·5-7 | 5 | 29·3 | 29·2 | 1-5 | 3·4 |
| Eland (*Taurotragus oryx oryx*) | 71 | 80-800 | 432·4 | 6·3-20 | 12·4 | 156·3-625 | 284·8 | — | — | 2-13·5 | 5·6 | 46·3 | 34·7 | 0·8-10 | 2·6 |
| Roan antelope (*Hippotragus equinus equinus*) | 1 | 200 | 200 | 15 | 15 | — | — | — | — | 4·5 | 4·5 | 60 | — | 2·5 | 2·5 |
| Blue wildebeest (*Connochaetes taurinus taurinus*) | 4 | 120-220 | 187·5 | 4·6-25 | 10·7 | — | — | 113·6-416·7 | 186·7 | 3-10 | 5·8 | 26·7 | 16 | 1·5-7 | 3·6 |
| Black wildebeest (*Connochaetes gnou*) | 12 | 120-160 | 147·5 | 9·4-20 | 11·6 | 101·7-203·4 | 135·6 | — | — | 3·5-13 | 7·1 | 33·9 | 33·9 | 0·5-8 | 3·8 |
| Red hartebeest (*Alcelaphus buselaphus caema*) | 54 | 90-160 | 125 | 6-19·5 | 12·2 | 71·4-115·4 | 80 | 90·9-272·7 | 144 | 2-20 | 8·2 | 64 | 64 | 2-6 | 3·3 |
| Tsessebe (*Damaliscus lunatus lunatus*) | 3 | 70-140 | 100 | 12·8-28·6 | 20·9 | 178·6-357·1 | 267·9 | — | — | 4-4·5 | 4·3 | 80 | 100 | 0·3-7 | 4·1 |
| Blesbok (*Damaliscus dorcas phillipsi*) | 2 | 80-95 | 87·5 | 10·5-12·5 | 11·5 | — | — | — | — | 1-4 | 2·5 | 57·1 | — | 1·5-2 | 1·8 |
| Common waterbuck (*Kobus ellipsiprymnus ellipsiprymnus*) | 2 | 150-250 | 200 | 10-12 | 11 | — | — | — | — | 5-9 | 7 | 50 | 40 | 2-2·5 | 2·3 |
| Greater kudu (*Tragelaphus strepsiceros strepsiceros*) | 2 | 120-200 | 160 | 10-12·5 | 11·3 | — | — | — | — | 2·5-6·5 | 4·5 | — | 18·8 | 1·3-6 | 3·6 |
| Gemsbok (*Oryx gazella gazella*) | 3 | 70-180 | 126·7 | 12·5-28·5 | 18·1 | 156·3-600 | 250·6 | — | — | 2·5-8 | 5·7 | — | 78·9 | 2·5-4·5 | 3·3 |
| Impala (*Aepyceros melampus melampus*) | 14 | 25-48 | 32·7 | 4-20 | 9·2 | — | — | — | — | 2-3 | 4·9 | 15·3 | 61·2 | 1-4·5 | 2·1 |
| Springbok (*Antidorcas marsupialis marsupialis*) | 5 | 22-35 | 28 | 8·4-15 | 11 | — | — | — | — | 2-11 | 4·9 | 20·2 | — | 1-5 | 2·8 |
| Steenbok (*Raphicerus campestris campestris*) | 2 | 12 | 12 | 25 | 25 | 416·7 | 208·3 | — | — | 5·7-7·2 | 6·5 | — | 416·7 | 4·2-7·8 | 6 |
| Warthog (*Phacochoerus aethiopicus sundevalli*) | 3 | 50-65 | 55 | 10-20 | 13·8 | — | — | — | — | 3·5-4·5 | 4 | — | 181·8 | 2·5-5 | 3·5 |
| Burchell's zebra (*Equus burchelli antiquorum*) | 3 | 300-330 | 310 | 15·2-33·3 | 21·7 | — | — | — | — | 35† | — | — | 125 | 1 | 1·2 |
| Total | 217 | 12-6200 | 538·9 | 0·6-33·3 | 11·3 | 33·3-625 | 164·7 | 90·9-416·7 | 153 | 0·3-30 | 6·3 | 38 | 77·7 | 0·3-15 | 3·6 |

*Recovery time = time from administration of antidote to recovery to a standing position.
†Only one zebra was immobilised, but in a standing position. The others showed no visible reaction to lower dosages of R33799.

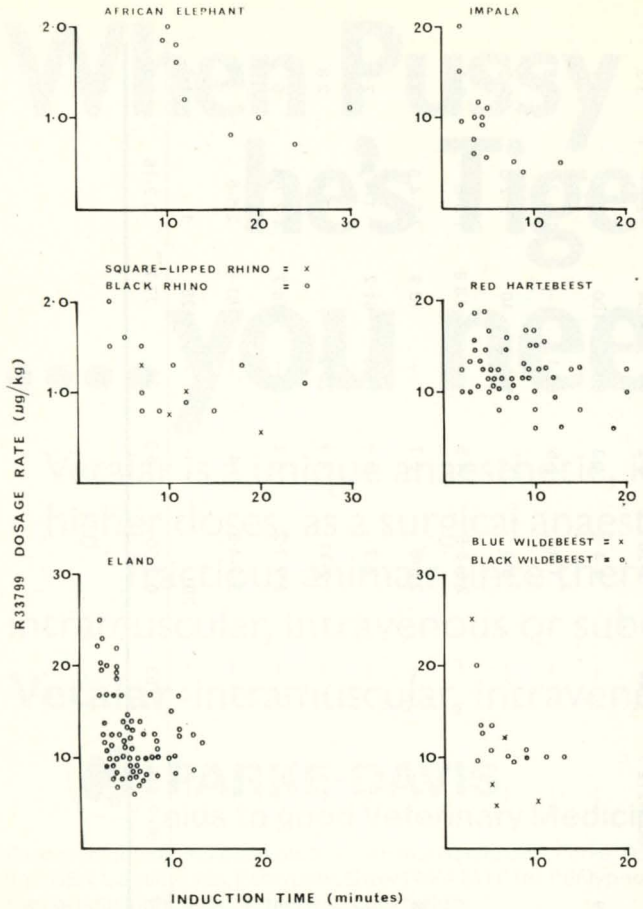


FIG 1: Scatter diagram of the relationship between R33799 dosage rate and induction time in eight free-ranging wild animal species

In a review of chemical immobilisation Harthoorn (1976) stressed that immobilisation techniques had been designed to produce as short an induction as possible, commensurate with safety. As indicated by Table 1, R33799 induces relatively short induction times in the species tested, dependent on the dosages used, as shown by the dosage rate/induction time relationship as depicted by Fig 1. The analysis shows that above a certain level, which differs with the different species, the dosage rates produce less of a variation in the induction phase and are consistently short (Fig 1). Below this level the induction times were subject to considerable variation with no clear cut pattern emerging. Variation in induction time can also be influenced by other variables such as site of administration (neck, shoulder, hip, lumbar regions, etc), drug deposition site (intramuscular, subcutaneous, into fascia between muscle layers, intra-abdominal, etc), amount of drug-loss through bleeding seepage from the dart puncture wound, excitability of the subject animal, physical exertion of the animal before and after darting, etc. High dosages seem to overcome the effect of these variables and produce consistent short times for the induction phase.

When R33799 was used in optimal amounts, the induction phase usually terminated in analgesic hypnosis, characterised by sternal recumbency. Lateral recumbency was, however, seen in most of the cases where high doses were used. Except in the elephant, lateral recumbency presents a distinct disadvantage in the field because of the possibility of bloating or aspiration asphyxia.

In most cases when working with smaller or optimal doses of R33799, some mobility of the spinal column was still retained. This invariably resulted in restricted movements of the head and a squirming movement of the body when handled and complicated manipulative procedures considerably. The addition of xylazine, however, effectively produced the required level of muscle relaxation necessary for handling procedures.

Negligible toxic effects or adverse reactions were seen when R33799 was used at optimal dosage rates. Even at extremely high dosage rates, respiratory depression was rare and when it occurred, it was so mild that reversal of the drug action was not considered necessary.

Mild muscular tremors in a few cases where high doses of R33799 were used without an additive were the only evidence of extrapyramidal symptoms in ungulates. Under the same circumstances however the warthog showed more violent and uncontrollable "dancing" movements of the hind legs. Such symptoms were easily controlled by the administration of small amounts of azaperone or xylazine.

In some cases where extremely high dosages of R33799 were used, a residual state of central nervous depression was noticed, which was not entirely reversible with antidote injection. The animals appeared apathetic and could only be aroused by harsh external stimuli. This was only seen in a few cases in blue wildebeest, tsessebe and steenbok that were subjected to gross overdosage. A similar reaction is sometimes produced by high dosages of the related compound, fentanyl (Harthoorn 1973). Also, morphine antagonists act by competing with the morphine-like analgesics for the receptor sites and so do not normally produce a 100 per cent reversal (Harthoorn 1976). Residual effects are likely to be large with a powerful drug like R33799, especially when high dosages are used.

Allotrophagia was marked in ungulates when high or optimal dosages of R33799 was used. Even in upright or sternal recumbency some animals still exhibited this reaction.

Out of the 217 animals immobilised with R33799, a total of 10 (4·6 per cent) died. Drug action was not held responsible for these deaths. Over-exertion during the predarting stage accounted for seven, aspiration asphyxiation for two, while one fell down a cliff after being darted.

It is concluded that R33799 has a very wide therapeutic index. During the field trials dosage rates varying from 0·6 to 28·6 µg per kg were successfully used (Table 2). Even within one species a dosage rate varying from 12·5 to 28·5 µg per kg was used without ill effect. And a dose of 1 mg may be effectively used to immobilise animals as divergent in size as a 2000 kg square-lipped rhinoceros or a 200 kg blue wildebeest.

R33799 appears more potent than fentanyl or etorphine hydrochloride. Taking previous prescribed dosages of fentanyl and etorphine hydrochloride into account (Pienaar 1973, Harthoorn 1973, Harthoorn 1976) the relative potencies for the immobilisation of the larger free-ranging wild animal species seem to be about 20:15:1 for R33799, etorphine hydrochloride and fentanyl respectively. However, this is a subjective assessment, based on field experiences.

R33799's potency and solubility means only small drug volumes are necessary. When used with xylazine which has been made up and used as 500 mg per ml solutions, dart syringes never need to exceed 1 ml in capacity. This means speedier absorption, less tissue damage, greater accuracy over longer distances and possible standardisation of dart syringes for all terrestrial game animals. Animals as divergent as a 6000 kg elephant, 800 kg eland and 10 kg steenbok can now be immobilised with the same capacity dart syringe, with only the lengths of needles varying.

The action of R33799 can rapidly and effectively be reversed with the usual morphine antagonists. Naloxone hydrochloride and cyprenorphine were initially used in dosage rates as advocated by Smuts (1975), Pienaar (1973) and Harthoorn (1973, 1976) for etorphine hydrochloride. However, significantly higher doses of the antagonist were required to counteract the action of R33799. When optimal dosages of R33799 were used the reversal was found to be near complete, leaving the animal in a state of effective awareness of his surroundings—a definite advantage in a natural environment. As stated earlier, very high dosages of R33799 leaves the animal in a mild state of depression which definitely increases the vulnerability of such an animal to predators.

Acting by substrate competition, it follows that the dose of

TABLE 2: Recommended optimal dosage regime of R33799 and additives for the immobilisation of 19 free-ranging wild animal species

| | | Analgesic (narcotic) R33799 | | Additive Xylazine | | Additive Azaperone | | Analgesic antagonist Naloxone | | Analgesic antagonist Cyprenorphine | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adult body weight kg | Dosage rate µg per kg | Total dose* mg | Dosage rate µg per kg | Total dose* mg | Dosage rate µg per kg | Total dose* mg | Multi-plication factor | Total dose† mg | Multi-plication factor | Total dose† mg |
| African elephant | 5000 | 1 | 5 | — | — | — | — | 6-10 | 40 | 10-12 | 50 |
| Square-lipped rhinoceros | 2000 | 1 | 2 | — | — | 100 | 200 | 6-10 | 16 | 10-12 | 20 |
| Black rhinoceros | 1000 | 1·5 | 1·5 | — | — | 150 | 150 | 8-10 | 10 | 10-12 | 12 |
| Giraffe | 1000 | 3(1·5)§ | 3(1·5)§ | 40 | 40 | 100 | 100 | 3-4 | 12 | 4-5 | 15 |
| African buffalo | 700 | 5 | 3·5 | 50 | 35 | 150 | 105 | 2-3 | 10 | 3-4 | 12·5 |
| Eland | 800 | 8 | 6·4 | 200 | 160 | 300 | 240 | 2-3 | 15 | 3-4 | 20 |
| Roan antelope | 250 | 10 | 2·5 | 100 | 25 | 300 | 75 | 2-3 | 7 | 3-4 | 10 |
| Blue wildebeest | 220 | 8 | 1·75 | 80 | 17·5 | 240 | 52·5 | 2-3 | 5 | 3-4 | 6 |
| Black wildebeest | 150 | 8 | 1·2 | 80 | 12 | 240 | 36 | 2-3 | 3·5 | 3-4 | 5 |
| Red hartebeest | 140 | 10 | 1·4 | 100 | 14 | 300 | 42 | 2-3 | 4 | 3-4 | 5 |
| Tsessebe | 140 | 10 | 1·4 | 100 | 14 | 300 | 42 | 2-3 | 4 | 3-4 | 5 |
| Blesbok | 90 | 10 | 0·9 | 100 | 9 | 300 | 27 | 2-3 | 2·5 | 3-4 | 3·5 |
| Common waterbuck | 240 | 10 | 2·4 | 100 | 24 | 300 | 72 | 2-3 | 7 | 3-4 | 8 |
| Greater kudu | 260 | 10 | 2·6 | 100 | 26 | 300 | 78 | 2-3 | 7 | 3-4 | 8 |
| Gemsbok | 180 | 10 | 1·8 | 100 | 18 | 300 | 54 | 2-3 | 5 | 3-4 | 7 |
| Impala | 50 | 7 | 0·35 | 100 | 5 | 300 | 15 | 3-4 | 1·4 | 4-5 | 1·7 |
| Springbok | 35 | 10 | 0·35 | 100 | 3·5 | 300 | 10·5 | 3-4 | 1·4 | 4-5 | 1·7 |
| Steenbok | 12 | 10 | 0·12 | 100 | 1·2 | 300 | 3·6 | 3-4 | 0·4 | 4-5 | 0·5 |
| Warthog | 80 | 12·5 | 1 | 100 | 8 | 300 | 24 | 3-4 | 4 | 4-5 | 5 |
| Burchell's zebra‡ | | | | | | | | | | | |

*Analgesic or additive total dose = Analgesic rate x body-weight.
†Analgesic antagonist total dose = Analgesic total dose x multiplication factor.
‡R33799 is not recommended for the zebra. Etorphine is recommended as the principal immobilising agent.
§Figures in parenthesis denote non-casting dosage levels for giraffe.
The dosage listed are for free-ranging wild animals. Wild animals adapted to captive conditions usually require lower dosages.
When a short induction period is required, the R33799 dosage rate can be drastically increased.
R33799 can be used without an additive. In cases of excitable species and warthog a mixture or "cocktail" is recommended. Xylazine is preferred.
Although diprenorphine (Reckitt and Sons, Ltd, England) is not listed as an antagonist, it can be used with equal success in dosages slightly less than cited for cyprenorphine.
Nalorphine hydrobromide (Lethidrone; Burroughs Wellcome & Co) can also be used as an antagonist.

antagonist is a function of the weight of the animal rather than the original dose of the immobilising compound (Harthoorn 1976). Both weight of animal and dosage rate of R33799 was therefore taken into consideration in the suggested dosage regime for antagonists (Table 2).

Additives such as azaperone and xylazine may reduce the incidence of excitement during the induction phase as well as extrapyramidal effects. Muscular relaxation brought about by xylazine especially was also used effectively to increase the tractability of the animal. These additives were found to be synergistic to R33799. Ample justification for combining R33799 with xylazine or azaperone therefore exists.[1] Care should be taken, however, to use medium to light dosage rates only if proper resuscitation on injection of the antidote is to follow. The approximate dosage ratios which were found most suitable for this purpose in ungulates were: R33799: xylazine::1:10; R33799: azaperone::1:30 (Table 2).

From reaction patterns observed and an analysis of the results, as depicted by Fig 1, an optimal dosage regime has been drawn up for 19 species (Table 2). These species cover a sufficiently wide spectrum for the dosage rates for related species to be deduced by extrapolation.

Eland, kudu, gemsbok and waterbuck belong to the group of excitable animals which is generally considered difficult to capture successfully with chemical compounds (Ebedes 1969, Pienaar 1973, Smuts 1975). It was therefore unusual to witness an average induction time of 5·6 minutes for 71 free-ranging eland immobilised with R33799 (Table 1). Similar remarks apply to gemsbok, which were rendered tractable in 5·7 minutes (Table 1). Also, kudu and waterbuck were caught without the addition of neuroleptics. The kudus exhibited very little evidence of excitability during the induction stage. The induction phase of the two waterbuck which were caught without neuroleptics was however characterised by excitability. Both went down rather violently, turning over on their backs and flailing with their legs. The righting reflex seemed to be drastically impaired. When put into a sternal position, however, they were tranquil and tractable.

In pachyderms, significantly short induction times were achieved with high dosages, especially in the elephant. Previously, etorphine hydrochloride was considered the only suitable drug for the capture of these species (Harthoorn 1977).

In the case of giraffe, the object with the drug dose is to stop the animal not to cast or put it down (Harthoorn 1973). Excellent results were obtained in the present study, however, by putting giraffe down completely. Both dosage levels are quoted in Table 2.

At a dosage rate of 7 µg per kg of R33799, without the addition of neuroleptics, a very satisfactory reaction pattern was established in the impala. Even with a gross overdosage of 20 µg per kg a rapid but safe response was elicited. The respiration was strong throughout. This is rather significant since the impala is sensitive to etorphine (Pienaar 1973).

Warthogs seem to be more resistant to the action of R33799, requiring relatively higher dosages for effective immobilisation than the other species cited. The addition of xylazine or azaperone or other suitable tranquillisers is strongly recommended to counteract any extrapyramidal symptoms which tend to occur.

It was only with the extremely high dosage rate of 33 µg per kg of R33799 that a zebra could be captured. Even in this case the animal remained standing while the antidote was administered. Like the related compound fentanyl, R33799 is therefore not recommended for immobilisation of zebra. The drug of choice for this species remains etorphine, at a dosage rate of 2 to 4 mg for an adult animal, as advocated by Pienaar (1973).

The results show that R33799 is a powerful, yet safe morphine-like analgesic for pachyderms and ungulates. Other advantages are its wide therapeutic index, wide spectrum of action and reliability.

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# Factors affecting the survival of *Treponema hyodysenteriae* in dysenteric pig faeces

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*Treponema hyodysenteriae* **was found to survive for periods of up to 48 days in dysenteric pig faeces stored at temperatures between 0°C and 10°C inclusive. Survival was reduced to seven days at 25°C and did not exceed 24 hours at 37°C. Dilution 1:10 with tapwater appeared to enhance survival to a maximum of 61 days at 5°C but further dilution reduced it. Drying and exposure to disinfectants rapidly eliminated** *T hyodysenteriae* **from dysenteric faeces. Phenolic and sodium hypochlorite disinfectants were most effective. The use of these findings in the formulation of control programmes for swine dysentery is discussed.**

SWINE dysentery occurs in susceptible pigs following the ingestion of faeces from infected pigs (Whiting and others 1921) containing the causal agent, *Treponema hyodysenteriae* (Harris and others 1972). Many outbreaks of swine dysentery result from the mixing of infected and susceptible pigs in such a way that the latter have already access to freshly voided infected faeces. In some cases, however, boots, clothing or implements (Terpstra and others 1968) may be contaminated and may spread infection. In other cases, susceptible pigs may be exposed to infection by contact with drainage channels or drinking water contaminated with infected faeces. Finally, infection may persist in contaminated depopulated pens or buildings and infect susceptible pigs used for restocking.

The length of time for which infectivity survives is in dispute and may be less than seven days in depopulated, contaminated pens (Terpstra and others 1968) although Harris and Glock (1975) recommended that farms should remain depopulated for 60 days in attempts to eradicate swine dysentery from infected units.

This study was carried out in order to provide information about the ability of *T hyodysenteriae* to survive in dysenteric faeces under conditions likely to be found on farms. The effects of temperature, dilution with tapwater, drying and exposure to disinfectants on the survival of *T hyodysenteriae* in dysenteric faeces were studied.

## Materials and methods

Dysenteric faeces were obtained from pigs experimentally infected with pure cultures of *T hyodysenteriae* isolate S73/2 (Taylor 1976). Faeces samples were obtained direct from the rectum and processed within one hour of collection. Upon arrival at the laboratory, wet preparations of each sample were examined by phase contrast microscopy for the presence of spirochaetes with the morphology of *T hyodysenteriae*. Their identity was confirmed on air-dried acetone-fixed smears by a specific fluorescent antibody test. The antiserum used was prepared against *T hyodysenteriae* and was absorbed with the non-pathogenic spirochaete PWS/A (Hunter and Saunders 1977).

*T hyodysenteriae* was isolated by streaking faecal samples on 7 per cent horse blood agar containing 400 µg per ml of spectinomycin (Spectam injectable; Abbott) according to the method described by Songer and others (1976). Cultures were examined after 48 hours' incubation at 42°C in an atmosphere of 95 per cent hydrogen, 5 per cent carbon dioxide. If colonies of *T hyodysenteriae* were not seen, the cultures were re-incubated for a further 48 hours. Colonies of *T hyodysenteriae* were identified by their characteristic morphology (Taylor and Alexander 1971, Harris and others 1972). In cases where doubt arose about the identity of the spirochaetes cultured, subcultures were made on horse blood agar without spectinomycin and tested with the specific fluorescent antibody test to confirm that they were *T hyodysenteriae*.

The effects of temperature on the survival of *T hyodysenteriae* were examined by placing eight 3 to 5 g aliquots of dysenteric faeces in closed sterile universal bottles and storing them at the following temperatures: 0°C, 5°C, 10°C, 20 to 22°C (room temperature), 25°C, 37°C, 42°C and 56°C. Samples were cultured daily until negative results were obtained on three successive days and were returned to their storage temperature within 15 minutes of removal.

Two dysenteric faecal samples were diluted with tapwater (pH 6·5 to 6·6) to final concentrations of 1:10, 1:50, 1:100 and 1:500. A total of 15 ml of each diluted sample was stored at 5°C and 10°C and cultured daily for comparison with undiluted portions of the faeces; the latter were placed in sterile universal bottles stored at the same temperatures. In a second study, a 1:10 dilution was prepared from a portion of faecal samples 1 to 6 used in the temperature storage experiment described above and 15 ml aliquots of each sample were stored at 0°C, 5°C, 10°C, 20 to 22°C and 25°C. The undiluted faeces samples stored at the same temperatures acted as controls for this study.

The effects of drying were studied by spreading 3 to 5 g aliquots of dysenteric faeces on sterile plastic petri dishes and exposing them to the air in a refrigerator at a temperature of 5°C. A control aliquot was placed in a closed, sterile universal bottle in the same refrigerator and both samples were cultured immediately and at 12 hourly intervals thereafter until two negative results had been obtained. Cultures were inoculated with dried faeces alone and dried faeces resuspended in deionised water to the consistency of a thick paste.

The effect of disinfectants on the survival of *T hyodysenteriae* in dysenteric faeces was studied by placing 2 to 4 g of dysenteric faeces in a weighed sterile universal bottle to which three parts of freshly prepared diluted disinfectant was added. Disinfectant was diluted to the concentration required with sterile deionised water. A phenolic disinfectant (Young's White Septol 'B', 50 per cent phenol) Formalin BP (40 per cent formaldehyde W/V), sodium carbonate decahydrate and sodium hypochlorite solution ("Chloros", 10 per cent chlorine by weight) were used.