

TABLE 1: Concentrations of anaesthetic agents used on leeches

Agent	Concentration(s)	Total number of leeches used
Benzocaine	100 mg/litre	2
	200 mg/litre	2
	400 mg/litre	3
Soda water	120 ml/litre	2
	200 ml/litre	1
	500 ml/litre	12
	Neat (100%)	5
Lignocaine	1 ml/100 ml water plus incremental amounts	1

were kept singly or in groups of two or three in glass jars with a little gravel on the floor and small quantities of pond weed (*Elodea canadensis*). The lids of the jars were covered with gauze. The water was changed weekly. The temperature in the leech room was 20° to 24°C.

Separate jars, thoroughly cleaned and rinsed with distilled water, were used as anaesthetic chambers. The following agents were investigated: (a) Benzocaine, a standard anaesthetic agent for amphibians and fish; this has to be dissolved in 2 to 3 ml acetone before being used since it is not water soluble. (b) Commercial soda water, a potent source of carbon dioxide. (c) Two per cent lignocaine (Xylocaine; Astra), a local analgesic.

In each case the agent was added to the water in the anaesthetic jar before the introduction of the leech. Different concentrations were used as shown in Table 1.

The response of the leeches was assessed on the basis of: (1) Whether they remained attached to the side or bottom of the jar; (2) the speed and extent of their swimming movements; (3) muscle tone (ie, the extent to which the leeches, when removed, were turgid or flaccid); (4) whether the caudal sucker was functioning, and (5) their response to stimulation, ie, touching and handling.

Following anaesthesia the leeches were returned to jars of untreated water. Recovery was judged to have occurred when they were behaving normally. No leech was reused until at least a week had elapsed since its previous exposure.

Benzocaine proved useful at higher concentrations. Following immersion in 100 mg/litre leeches showed slowing and, in many cases, regurgitation of blood, but the effect was not sufficient to permit swabbing and clinical examination. There was a more marked response to 200 mg/litre but this was still inadequate. After 25 minutes in 400 mg/litre the leeches had stopped moving, were no longer attached to the side of the tank and could be readily handled and manipulated. Recovery of two leeches was complete after 177 minutes but the third took several hours.

Leeches reacted immediately to soda water, even at low concentrations (120 ml/litre), with rapid swimming movements. At higher concentrations (200 ml/litre) they started to climb out of the anaesthetic chamber. A 50 per cent solution (500 ml/litre) resulted in similar rapid movements but within two minutes the leeches were slowing and after five to 10 minutes they could usually be removed, handled and swabbed with ease. Recovery time varied but all were completely normal after four-and-a-half hours.

Exposure of five leeches to neat soda water caused intense activity and attempts to escape: the latter appeared to delay the onset of anaesthesia but after two hours all five were flaccid and not attached to the jar. One of these animals failed to recover (the only fatality to date) but the other four recovered completely over a 36-hour period.

Lignocaine was used on only one leech; 1 ml in 100 ml water appeared to have little effect and, therefore, incremental amounts were added – a further 1 ml at 10, 17, 22, 24, 28 and 31 minutes after the initial dose, 2 ml after 35 minutes and 5 ml after 37 minutes. The leech appeared to dislike the agent and as the concentration was increased made violent swimming and contracting movements and attempted to escape. At the end of the 45 minutes, when the leech had

been exposed to a total of 14 ml lignocaine, there was no evidence of effective immobilisation and the study was discontinued. Lignocaine was not tried again.

Following these findings, 50 per cent soda water was routinely used for chemical immobilisation of medicinal leeches and consistently proved successful and safe. To date no fatalities have occurred at this concentration. The addition of oxygen to the water appears to accelerate recovery but this has not yet been fully investigated. The tendency of leeches to climb out of their jar, apparently in order to avoid the soda water, could pose problems but is fairly easily overcome by having a perforated lid or by covering the top of the jar with gauze.

It is assumed that soda water acts by liberating carbon dioxide which renders the leeches immobile by producing anoxia and acidosis. Whether this induces analgesia is unclear and for painful procedures it may be more humane to employ benzocaine.

There is a paucity of information on the anaesthesia of invertebrates (Cooper 1985) and, since these animals are not covered by the Cruelty to Animals Act 1876, experimental work involving them is frequently performed without adequate concern for their welfare. Further research is needed on this subject.

**Acknowledgements.**—The authors are grateful to staff of the Animal Care Unit at the Royal College of Surgeons for help with the investigations and for their conscientious care of the leeches. Dr R. Sawyer of Biopharm (UK) Ltd kindly read and commented on this manuscript before it was submitted for publication.

**References**

COOPER, J. E. (1985) Manual of Exotic Pets. Eds J. E. Cooper, M. F. Hutchison. Cheltenham. British Small Animal Veterinary Association. p209

ELLIOTT, J. M. & MANN, K. H. (1979) A Key to the British Freshwater Leeches. Scientific Publication No 40. Freshwater Biological Association. p10

RAO, P., BAILIE, F. B. & BAILEY, B. N. (1985) *The Practitioner* **229**, 901

SAWYER, R. T. (1986) Leech Biology and Behaviour. Oxford, Oxford University Press

TURK, J. L. & ALLEN, E. (1983) *Annals of the Royal College of Surgeons of England* **65**, 128

WHITLOCK, M. R., O'HARE, P. M., SANDERS, R. & MORROW, N. C. (1983) *British Journal of Plastic Surgery* **36**, 240

**Squamous cell carcinoma in an Indian rhinoceros**

S.N. Naik, C.S. Ishwad, M.S. Karawale, M.V. Wani

*Veterinary Record* (1986) **118**, 590-591

MALIGNANT tumours are found in both domesticated and wild animals (Moulton 1961, Naik and others 1969). The incidence of cancer is reported to be low in wild animals, probably because of their short lifespan, but zoo animals with increased longevity are known to suffer from cancer (Snyder and Ratcliffe 1963). The epidemiological study of cancer in zoo animals provides a good basis for understanding the environmental and biological factors responsible for carcino-

S.N. Naik, C.S. Ishwad, Comparative Oncology Unit, Cancer Research Institute, Bombay 400 012, India  
M.S. Karawale, M.V. Wani, Zoological Gardens, Bombay 400 027, India

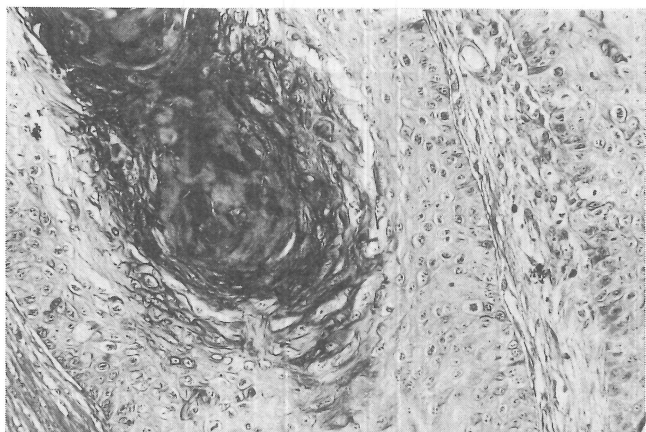


FIG 1: Squamous cell carcinoma showing mitotic cells in the periphery and keratinised pearls in the centre. Haematoxylin and eosin  $\times 120$

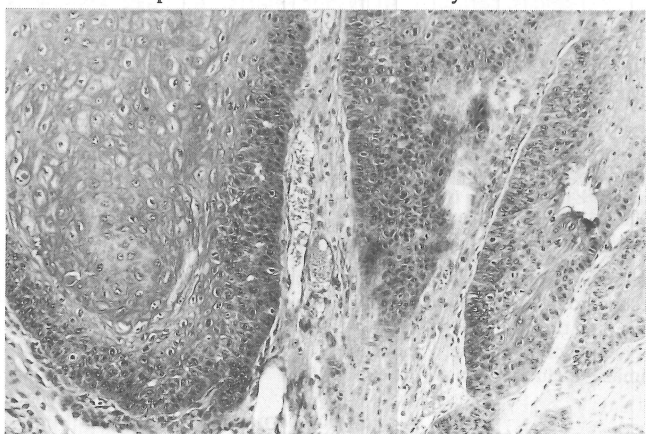


FIG 2: Invasive cords of squamous cell carcinoma showing mitotic cells. Haematoxylin and eosin  $\times 75$

genesis. A case of squamous cell carcinoma of the skin in an old Indian rhinoceros (*Rhinoceros unicornis*) maintained at the Municipal Zoological Gardens, Bombay, is reported here.

A male rhinoceros was brought to Bombay Zoo in 1952. When the animal was about 50 years old and weighed about 3000 kg an ulcer developed on the skin over the nose adjoining the horn. The ulcer extended all round the horn until the horn fell off. The growth then invaded deeply into the skin during a period of 14 to 16 months and did not respond to any treatment. Upon examination, veterinary and medical oncologists suspected that the lesion was malignant. The animal was tranquillised and a biopsy was taken for histopathological studies. Because the lesion was wide and inoperable it was dressed with an ointment containing an anti-cancer drug, but there was no noticeable improvement.

The wound had a cauliflower-like appearance with an undulating surface and a thickened border. The animal gradually lost weight, became emaciated and died about two-and-a-half years after the first appearance of the ulcer. The post mortem examination was carried out 18 hours after death but no clear metastases were evident.

The biopsy tissue was processed and slides were prepared and stained with haematoxylin and eosin. The sections revealed well differentiated areas of squamous cell carcinoma with keratinisation in the centre which stained brightly with eosin (Fig 1). There were columns of squamous cells invading the dermis and subcutis (Fig 2). The tumour cells were pleomorphic with centrally situated large vesicular nuclei some of which showed mitotic divisions (Figs 1 and 2). The proliferation of squamous cells to stratification and keratin pearl formation were clear indications of their attempt to differentiate. From the epidermis the growth invaded through the dermis and subcutis as far as the nasal bone which was seen at post mortem examination to have been eroded.

Carcinomas of the skin are known to take a long time to metastasise, like bovine ocular squamous cell carcinomas,

which vary from non-invasive to malignant, as observed by Russell and others (1956). Similarly the horn core cancer, a squamous cell carcinoma in Indian zebu cattle, takes a long time to metastasise (Naik and others 1969).

The Indian rhinoceros has a single horn made up of filamentous tubules of keratin which are secreted by the skin and cemented together to form a hard projection. This projection is fixed to the skin and does not have a bony support like the horn of ruminants. The ulcer on the skin adjacent to the base of the horn may have developed owing to the constant trauma and irritation caused by the horn striking against hard objects. The ulcer then became a squamous cell carcinoma which invaded around and beneath the horn until the horn separated and exposed a broad wound.

Over most of its body the Indian rhinoceros has very thick skin arranged in characteristic massive folds, but on the nose the skin is thin. Cancer of the thick skin is probably very rare.

This is thought to be the first report of a squamous cell carcinoma of the skin in the rhinoceros.

**Acknowledgements.** — The authors are grateful to Dr M.G. Deo, the research director, Cancer Research Institute, for his encouragement, to Dr D.J. Jussawalla, professor of oncology and the chairman of the planning and development committee, Tata Memorial Centre, for his advice in the treatment of the case and to Mr J.C. Garkhel, superintendent of the Zoological Gardens, for his help.

## References

- MOULTON, J.E. (1961) *Tumours in Domestic Animals*. Berkeley, University of California Press
- NAIK, S.N., BALAKRISHNAN, C.R. & RANDELIA, H.P. (1969) *British Veterinary Journal* 125, 222
- RUSSELL, W.M., WYNNE, E.S. & LOQUVAM, G.S. (1956) *Cancer* 9, 1
- SNYDER, R.L. & RATCLIFFE, H.L. (1963) *Annals of the New York Academy of Science* 108, 793

## Assessing pneumonia in pigs

FOUR methods of assessing the prevalence and extent of pneumonia in pigs were compared. The techniques included: assessing the proportion of lung involved and calculating the mean; counting the number of lungs in each sample that had a greater than a predetermined 'amount' of pneumonia and calculating the prevalence of the sample thus affected; scoring only the lung from each herd sample that was maximally affected by pneumonia; and allocating the lungs to categories depending on the extent of the lesions. The first method was the most informative procedure but also the most time consuming while the fourth was less precise and more difficult to interpret. The second and third techniques were both less time consuming and almost as informative as an indicator of the extent and prevalence of pneumonia as the first method.

MORRISON, R. B., HILLEY, H. D. & LEMAN, A. D. (1985) *Canadian Veterinary Journal* 26, 381

## Q fever from wild rabbits

FOUR cases of atypical pneumonia are described in humans in which there was history of exposure to wild rabbits. In each patient the titre to the phase II *Coxiella burnetii* antigen rose significantly. Ten (45 per cent) of 22 snow shoe hares caught in the area where one of the patients snared his rabbits had antibodies to *C. burnetii*.

MARRIE, T. J., SCHLECH, W. F., WILLIAMS, J. C. & YATES, L. (1986) *Lancet* i, 427