OGIMOTO, K. (1994) Antibacterial activity of tilmicosin against *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* isolated from pneumonic lesions in swine. *Journal of Veterinary Medical Science* **56,** 917-921
MARQUARDT, D. W. (1975) An algorithm for least-square estimation of non-linear parameters. *Journal of the Society of Industrial and Applied Mathematics* **11,** 431-441
MODRIC, S., WEBB, A. I. & DAVIDSON, M. (1999) Effect of respiratory tract disease on pharmacokinetics of tilmicosin in rats. *Laboratory Animal Science* **49,** 248-253
MODRIC, S., WEBB, A. I. & DERENDORF, H. (1998) Pharmacokinetics and pharmacodynamics of tilmicosin in sheep. *Journal of Veterinary Pharmacology and Therapeutics* **21,** 444-452
MORCK, D. W., MERRILL, J. K., GARD, M. S., OLSON, M. E. & NATION, P. N. (1997) Treatment of experimentally induced pneumonic pasteurellosis of young calves with tilmicosin. *Canadian Journal of Veterinary Research* **61,** 187-192
MORCK, D. W., MERRILL, J. W., THORLAKSON, B. E., OLSON, M. E., TONKINSON, L. V. & COSTERTON, J. W. (1993) Prophylactic efficacy of tilmicosin for bovine respiratory tract disease. *Journal of the American Veterinary Medical Association* **202,** 273-277
OSE, E. E. (1987) In vitro antibacterial properties of EL-870, a new semi-synthetic macrolide antibiotic. *Journal of Antibiotics* (Tokio) **40,** 190-194
PARKER, R. M. & PATEL, R. K. (1994) Determination of tilmicosin in ovine milk using high-performance liquid chromatography. *Analyst* **119,** 2577-2579
PERITI, P., MAZZEI, T., MINI, E. & NOVELLI, A. (1993) Adverse effects of macrolide antibiotics. *Drug Safety* **9,** 346-364
PIZZIMENTI, F. C., NACCARI, F., ALONZO, V. & ROTIROTI, D. (1987) Evaluation of cephotaxime concentrations in rat cerebral areas and serum by microbiological and HPLC. *Chemioterapia* **6,** 101-104
RAMADAN, A. (1997) Pharmacokinetics of tilmicosin in serum and milk of goats. *Research in Veterinary Science* **62,** 48-50
REEVE-JOHNSON, L., ESPINASSE, J., THOMAS, L. & SUSTRONCK, B. (1997) An assessment of the clinical efficacy of a novel oral formulation of tilmicosin in the treatment of induced respiratory pasteurellosis in young calves. *Journal of Veterinary Pharmacology and Therapeutics* **20** (Suppl 1), 132-133
RIEGELMAN, S. & COLLIER, P. (1980) The application of statistical moment theory to evaluation of in vivo dissolution time and absorption time. *Journal of Pharmacokinetics and Biopharmaceutics* **8,** 509-534
SARGISON, N. D. & SCOTT, P. R. (1995) Evaluation of antibiotic treatment of respiratory disease, including suspected septicemic pasteurellosis in five-week-old lambs. *Agri-Practice* **16,** 25-28
SCOTT, P. R., MCGOWAN, M., SARGISON, N. D., PENNY, C. D. & LOWMAN, B. G. (1996) Use of tilmicosin in a severe outbreak of respiratory disease in weaned beef calves. *Australian Veterinary Journal* **73,** 62-64
SHRYOCK, T. R., WHITE, D. W., STAPLES, J. M. & WERNER, C. S. (1996) Minimum inhibitory concentration breakpoints and disk diffusion inhibitory zone interpretive criteria for tilmicosin susceptibility testing against *Pasteurella* spp associated with bovine respiratory disease. *Journal of Veterinary Diagnostic Investigation* **8,** 337-344
SPSS (1999) Statistical Package for Social Science. Advanced Statistics 9.0. Chicago, SPSS
STEPHENS, C. P., BLACKALL, P. J., WADE, L. K. & LOWE, K. B. (1993) In vitro antibacterial properties of tilmicosin against Australian isolates of *Pasteurella multocida* and *Pasteurella haemolytica* from cattle. *Australian Veterinary Journal* **70,** 391-392
STIPKOVITS, L., MILLER, D. J. S., MOLNAR, T. & GLAVITS, R. (1997) Efficacy of Tiamutin*CTC, lincomycin+CTC, Econor+CTC and tilmicosin in treatment (via feed) of piglets experimentally infected with *Mycoplasma hyopneumoniae, Pasteurella multocida* and *Actinobacillus pleuropneumoniae. Journal of Veterinary Pharmacology and Therapeutics* **20** (Suppl 1), 131
THOMPSON, T. D., LAUDERT, S. B., CHAMBERLAND, S. & LAWRENCE, K. (1994) Micotil – pharmacokinetics of tilmicosin, a semi-synthetic macrolide antibiotic, in acutely pneumonic cattle and primary bovine alveolar macrophages. Proceedings of the 6th European Association of Veterinary Pharmacology and Toxicology, Edinburgh, August 7 to 11. p 31
VOGEL, G. J., LAUDERT, S. B., ZIMMERMANN, A., GUTHRIE, C. A., MECHOR, G. D. & MOORE, G. M. (1998) Effects of tilmicosin on acute undifferentiated respiratory tract disease in newly arrived feedlot cattle. *Journal of the American Veterinary Medical Association* **212,** 1919-1924
WAGNER, F. (1998) The single treatment of bacterial lung diseases, a successful therapy for cattle. *Mühle und Mischfuttertechnik* **135,** 619-620
YAMAOKA, K., NAKAGAWA, T. & UNO, T. (1978) Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *Journal of Pharmacokinetics and Biopharmaceutics* **6,** 165-175
ZIV, G., SHEM-TOW, M., GLICKMAN, A., WINKLER, M. & SERON, A. (1995) Tilmicosin antibacterial activity and pharmacokinetics in the cow. *Journal of Veterinary Pharmacology and Therapeutics* **18,** 340-345

Parasites from Indo-Pacific hump-backed dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) stranded in Hong Kong

Veterinary Record (2001) **148,** 776-780

E. C. M. PARSONS, R. M. OVERSTREET, T. A. JEFFERSON

E. C. M. Parsons, PhD, Seaquest, Tigh na Mara, 10 Main Street, Tobermory, Isle of Mull, Argyll PA75 6NU
R. M. Overstreet, PhD, Department of Parasitology, Gulf Coast Research Laboratory, 703 East Beach Drive, Ocean Springs, MS 39564, USA
T. A. Jefferson, PhD, Southwest Fisheries Science Center, NOAA, NMFS, PO Box 271, La Jolla, CA 92038, USA and Ocean Park Conservation Foundation, Ocean Park, Aberdeen, Hong Kong

Between 1993 and 1998, 28 Indo-Pacific hump-backed dolphins (*Sousa chinensis*) and 32 finless porpoises (*Neophocaena phocaenoides*) stranded in Hong Kong territorial waters were examined postmortem for parasites. The nematode *Halocercus pingi* was discovered in the lungs of one hump-backed dolphin and in 10 finless porpoises, typically within abscesses or granulomata, and they were frequently accompanied by a catarrhal exudate and lesions characteristic of pneumonia. Seven of the 10 finless porpoises were calves with substantial lungworm infections, and three were neonates with visible fetal folds and umbilical remnants, suggesting that *H pingi* is transferred to the neonate before birth or during lactation. Electron micrographs of *H pingi* should allow the nematode to be identified by other researchers. An ectoparasitic stalked barnacle (*Xenobalanus globicipitis*) was recovered from a finless porpoise, the first time that this species of barnacle has been recorded in Hong Kong's territorial waters.

THE Hong Kong special administrative region (SAR) is situated on the south coast of the Chinese province of Guangdong (centered at 22°20'N, 114°10'E). There are records of 16 species of cetaceans from Hong Kong (Parsons and others 1995, Parsons 1997), but only two species reside permanently in the SAR's territorial waters. These are the Indo-Pacific

TABLE 1: Summary of the results of postmortem examinations of finless porpoises stranded in Hong Kong waters between 1993 and 1998 that were parasitised by the nematode *Halocercus pingi*

Year	Number of porpoises Examined	Parasitised	Parasitised porpoises Stranding date	Length (cm)	Age* (GLG)	Code‡	Histological samples taken	Cause of death (Parsons and Jefferson 2000)
1993	2	1	5/6/93	133	5	4	No	Undetermined
1994	0	–	–	–	–	–	–	–
1995	5	4	15/3/95	104·5	<1	2	Yes	Verminous pneumonia
			2/11/95	83	<1†	4	No	Probable verminous pneumonia
			15/12/95	76	<1†	2	Yes	Verminous pneumonia
			25/12/95	143	2	2	Yes	Undetermined
1996	15	4	14/1/96	86	<1	2	No	Probable verminous pneumonia
			15/1/96	67	<1	2	Yes	Boat strike
			8/3/96	137	5	3	No	Uterine infection§
			25/4/96	84	<1	2	No	Probable verminous pneumonia
1997	7	0	–	–	–	–	–	–
1998	3	1	7/1/98	67	<1†	2	No	Probable fishery by-catch

* Age measured in growth layer groups (GLGs). One GLG is considered to be one year of growth (Perrin and Myrick 1980)
† Fetal folds and/or umbilical remnants still visible
‡ Decomposition code (Geraci and Lounsbury 1993)
§ Also parasitised by the ectoparasitic stalked barnacle *Xenobalanus globicipitis* and an unidentified (possibly arthropod) parasite

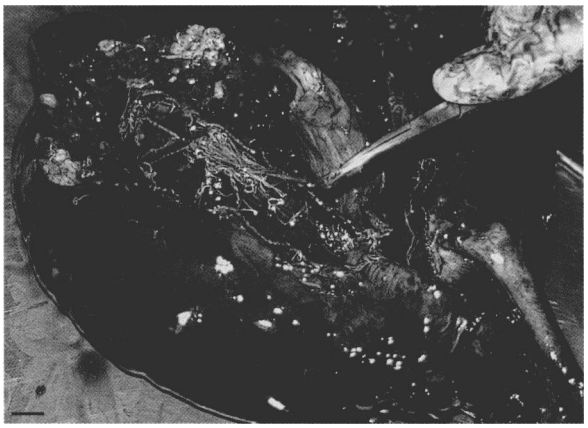

FIG 1: Lung of a finless porpoise with a heavy infection of the nematode *Halocercus pingi* and associated lesions. Bar = 1 cm

hump-backed dolphin (*Sousa chinensis*) and the finless porpoise (*Neophocaena phocaenoides*) which, before 1993, had not been studied in the region. Several research projects have since been conducted upon the two species, including detailed postmortem analyses of stranded individuals (Parsons 1997, 1998, Jefferson 2000, Parsons and Jefferson 2000). This paper summarises the parasitological data gathered from these studies.

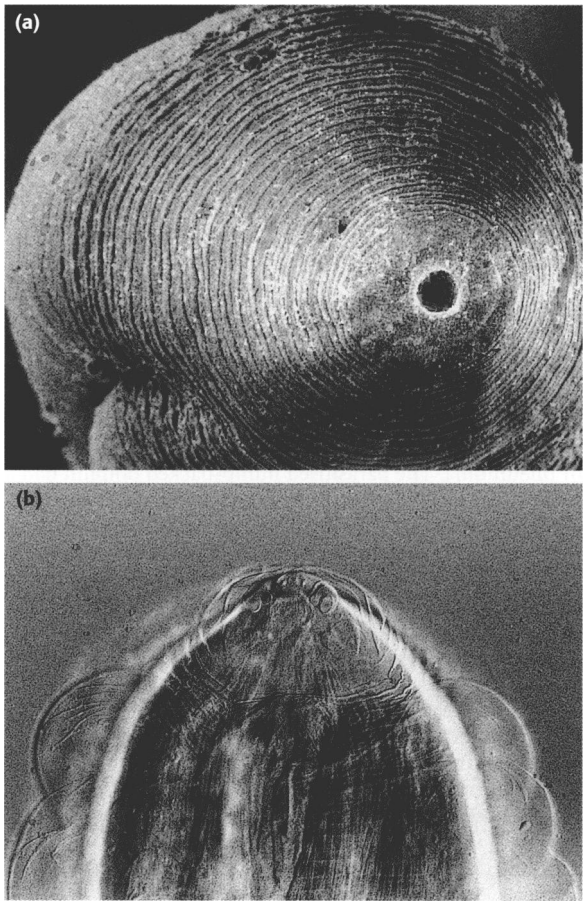

FIG 2: Scanning electron microscope view of the anterior end of *Halocercus pingi*. (a) Papillae present but distinct. (b) Lateral view of relatively large double papillae in an outer circle and six smaller ones in an inner circle. The oesophagus of the portions of the specimens examined was 199 to 213 µm long by 35 µm wide (the estimated total length was within the previous reported ranges of 150 to 183 µm for males and 255 to 364 µm for females)

Knowledge of the parasites of Indo-Pacific hump-backed dolphins is limited to a record of the cyanamid *Syncyamus aequus* in two animals from South Africa (Ross and others 1994), and two nematodes, *Anisakis alexandri* in the stomach of a hump-backed dolphin from Xiamen (Hsu and Hoeppli 1934), and a nematode, tentatively identified as *Halocercus pingi*, in the liver of one animal, possibly from south-east Asia (Gibson and Harris 1979).

There is more information about the parasites occurring in finless porpoises. The nematodes *Crassicauda fuelleborni*, *Stenurus auditivus*, *Stenurus nanjingensis*, *Pharurus asiaorientalis*, *Pseudostenurus auditivus* and *Pseudostenurus sunameri* have been extracted from the sinuses and cranial air spaces of finless porpoises and *H pingi* has been recorded in lung tissue (Hoeppli and others 1929, Wu 1929, Hsu and Hoeppli 1934, Yamaguti 1951, Neiland and others 1970, Dailey and Brownell 1972, Petter and Pilleri 1982, Tao 1983). In addition, four trematodes (*Campula folium*, *Nasitrema spathulatum*, *Nasitrema sunameri* and *Orthosplanchus elongatus*) and one cestode (*Diphyllobothrium furhmanni*) have been recorded from this species (Hsu 1935, Ozaki 1935, Neiland and others 1970, Dailey and Brownell 1972).

MATERIALS AND METHODS

Twenty-eight Indo-Pacific hump-backed dolphins and 32 finless porpoises were examined postmortem (Parsons and Jefferson 2000) by the methods described by Geraci and Lounsbury (1993) and Jefferson and others (1994). The parasites were fixed in a standard 10 per cent formalin solution and examined by scanning electron microscopy. Tissue samples taken for histopathological analysis were fixed in 10 per cent formalin solution or 90 per cent ethanol solution and processed by the Hong Kong Agriculture and Fisheries Department Veterinary Laboratory and the Comparative Pathology Laboratory, the University of Miami (Parsons and others 1999). The ages of the stranded cetaceans were estimated by decalcifying, sectioning and staining teeth by the procedures described by Myrick and others (1983) and Hohn and Lockyer (1995).

RESULTS

The most common parasites were lungworms. Nematodes, identified as *H pingi*, were excised from the lungs of one juvenile hump-backed dolphin. The same nematodes were discovered in 10 of the 32 finless porpoises (Table 1), often in

FIG 3: Scanning electron microscope views of the posterior end of male *Halocercus pingi*. (a) Ventral view of rugose alae and intra-alar region, much reduced caudal bursa and distal portion of spicules. (b) Dorsolateral view showing bipapillate dorsal ray, lateral rays and ventral ray, and protruding spicules. (c) Lateral view of anus and non-protruded spicules. (d) Lateral view showing entire protruded spicules (right spicule 800 µm, left spicule 570 µm). (e) Fold in spicule. (f) Lateral view of anal area showing entire gubernaculum

very high densities (Parsons and Jefferson 2000). The nematodes were typically found within abscesses or granulomata ranging in diameter from 1 to 2 mm to 30 mm (Fig 1). Seven of the parasitised porpoises were calves and they constituted 54 per cent of all the finless porpoise calves stranded in Hong Kong during the study period. Three of them had visible umbilical remnants, fetal folds and unfused skulls, indicating that they were probably less than a few weeks old, but they had heavy infections of parasites in the lungs, suggesting that the parasites had been acquired before, or immediately after, birth (Parsons and others 1999).

The specimens from the lungs of the finless porpoises were clearly different from *Halocercus lagenorhynchi*, but presumably conspecific with *H pingi*. Because of uncertainties in the descriptions of the spicules and bursal rays of *H pingi*, Dougherty (1944) thought that *H pingi* might be a junior synonym of *H lagenorhynchi*. Because the illustrations of *H pingi* by Wu (1929) and others are conventionalised and difficult to relate to actual specimens, Figs 2 to 5 are included to aid future workers in distinguishing this species.

The parasitic infections in the finless porpoises were accompanied by either a foamy or a catarrhal exudate in the airways, and the animals frequently had lesions characteristic of pneumonia. The parasitised hump-backed dolphin had a focal suppurative and necrotising pneumonia; some pinpoint haemorrhages were detected in the lung tissue and there were eosinophils and macrophages in the surrounding air spaces with occasional multinucleate cells; the macrophages were foamy, and some contained eosinophilic granules in the cytoplasm (Parsons and Jefferson 2000).

Verminous pneumonic lesions were also apparent in four finless porpoises, one of which had a trachea containing foamy exudate, a heavy parasitic load with many abscesses in the lungs, and areas of atelectasis and emphysema (Parsons and Jefferson 2000). However, it also appeared to have suffered a traumatic blow, probably from a boat, and this blow was the likely cause of death, rather than the verminous pneumonia. It is possible that the parasitic infection may have debilitated the animal and reduced its ability to evade boat traffic.

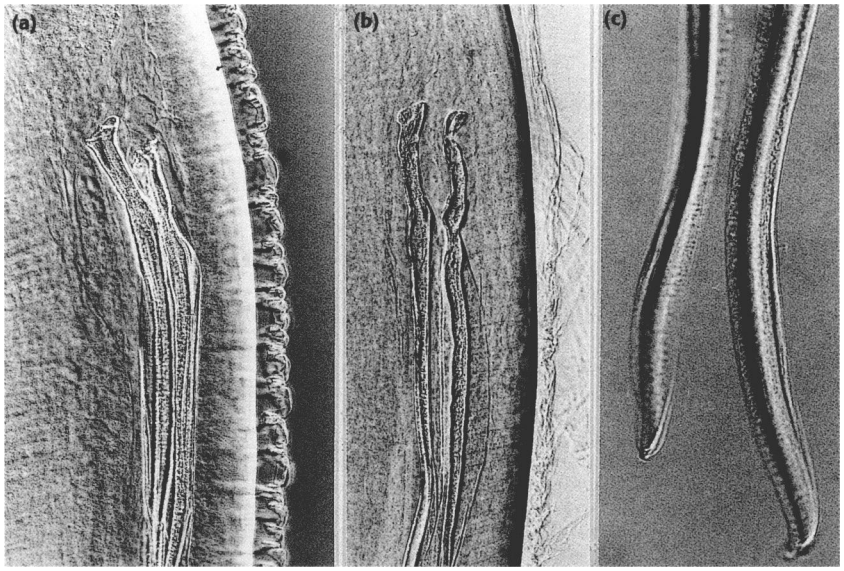

FIG 4: Scanning electron microscope view of the spicules of *Halocercus pingi*. (a) Proximal portion of both non-protruded spicules. (b) Proximal portion of single spicule. (c) Distal portions of both spicules

Unidentified nematode parasites were found in 1 to 2 cm nodules on either side of the ventral midline of the peritoneum of one finless porpoise. A female porpoise had a swollen and necrotic uterus, 4 to 5 cm thick, which was densely infected with cysts containing an unidentified (possibly arthropod) parasite (Parsons and Jefferson 2000). The trailing edge of the flukes of this animal was also heavily encrusted with the stalked barnacle *Xenobalanus globicipitis*, the first time that this barnacle had been recorded in Hong Kong.

The only platyhelminths were several unidentified trematodes discovered in the orbit of a hump-backed dolphin.

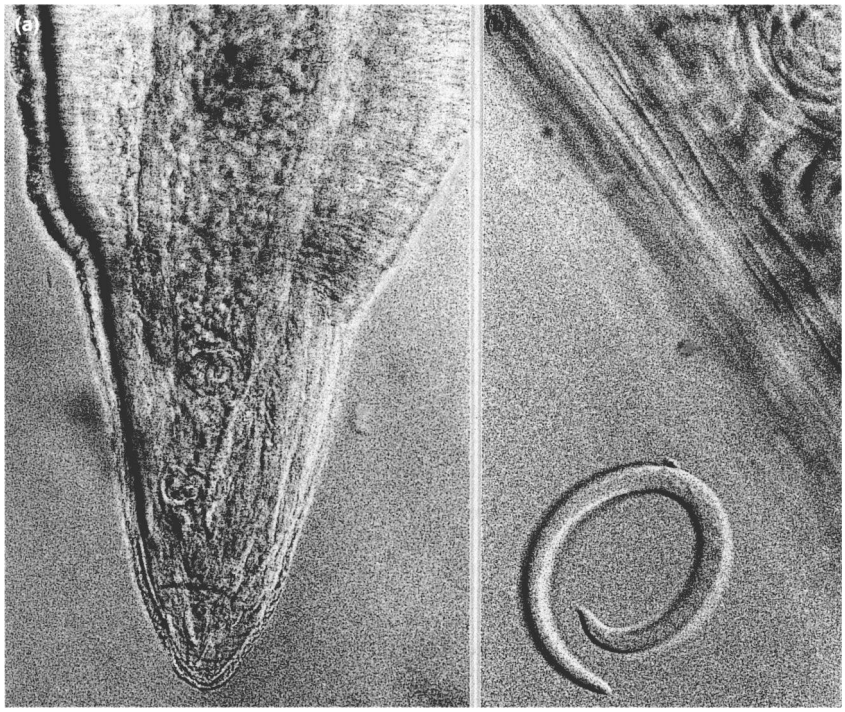

FIG 5: Scanning electron microscope view of a female *Halocercus pingi*. (a) Posterior end; one of different shapes in cold-fixed specimens. (b) Extruded larva and others still in uterus

DISCUSSION

Among these cetaceans, the predominant parasite was the nematode *H pingi*, which was isolated from 10 of 32 stranded finless porpoises. This nematode has also been described in other populations of Chinese and Taiwanese finless porpoises (Tao 1983, Gao and Zhou 1993) and the species thus appears to be particularly vulnerable to this type of parasite. The occurrence of the nematode in very young animals suggests that the parasite may either be able to cross the placenta and enter the lungs of the fetus or be transmitted through the mother's milk.

The immune system of neonatal animals is not fully developed, and they rely upon passive immunity derived from the transfer of antibodies in the colostrum to counteract infections. This underdeveloped immune system may be one reason why so many of the neonatal finless porpoises had been parasitised. However, in Hong Kong, stranded neonatal finless porpoises have been reported to contain high levels of organochlorine pollutants (Parsons and Chan 1998, Minh and others 1999), chemicals which are immunosuppressive (Safe 1984, Vos and Luster 1989, Busbee and others 1999) and may therefore further reduce their resistance to the nematodes.

ACKNOWLEDGEMENTS

The authors wish to thank Reimi Kinoshita, Les Simms, Pam Monson and Ronnie Palmer of the Gulf Coast Research Laboratory, and the staff of the Veterinary Department, Ocean Park, for their assistance and advice during this study. I. Beasley, M. Torey, G. Yang, L. Porter, D. Choi, Y. K. Chan, C. C. Lay and several others assisted with the postmortem examinations of the stranded animals. The authors also thank the two anonymous reviewers for their constructive comments on earlier versions of the paper. This project was funded by the Agriculture, Fisheries and Conservation Department of the Hong Kong SAR Government.

References

BUSBEE, D., TIZARD, I., STOTT, J., FERRICK, D. & OTT-REEVES, E. (1999) Environmental pollutants and marine mammal health: the potential impact of hydrocarbons and halogenated hydrocarbons on immune systems dysfunction. In Chemical Pollutants and Cetaceans. The Journal of Cetacean Research and Management Special Issue 1. Eds P. J. H. Reijnders, A. Aguilar, G. P. Donovan. Cambridge, International Whaling Commission. pp 223-248

DAILEY, M. D. & BROWNELL, R. L. (1972) A checklist of marine mammal parasites. In Mammals of the Sea, Biology and Medicine. Ed S. H. Ridgway. Springfield, Charles C. Thomas. pp 528-589

DOUGHERTY, E. C. (1944) The lungworms (Nematoda: Pseudaliidae) of the Odontoceti. Part 1. *Parasitology* **36,** 80-94

GAO, A. & ZHOU, K. (1993) Notes on classical literatures and contemporary researches on the finless porpoise (*Neophocaena phocaenoides*). *Acta Theriologica Sinica* **13,** 223-234

GERACI, J. R. & LOUNSBURY, V. J. (1993) Marine Mammals Ashore: A Field Guide for Strandings. Galveston, Texas A & M Sea Grant Publications. p 305

GIBSON, D. I. & HARRIS, E. A. (1979) The helminth-parasites of cetaceans in the collection of the British Museum (Natural History). *Investigations on Cetacea* **10,** 309-324

HOEPPLI, R., HSU, H. F. & WU, H. W. (1929) Helminthologische britrage aus Fukien und Chekiang. *Archiv für Schiffs Tropenhygiene* **33,** 44

HOHN, A. A. & LOCKYER, C. C. (1995) Protocol for obtaining age estimates from harbour porpoise teeth. In Biology of the Phocoenids. Reports of the International Whaling Commission Special Issue 16. Eds A. Bjørge, G. P. Donovan. Cambridge, International Whaling Commission. pp 494-496

HSU, H. F. (1935) Contributions a l'Etude de cestodes de China. *Revue Suisse de Zoologie* **42,** 447-570

HSU, H. F. & HOEPPLI, R. (1934) On some parasitic nematodes collected in Amoy. *Peking Natural History Bulletin* **8,** 155-168

JEFFERSON, T. A. (2000) Population biology of the Indo-Pacific hump-backed dolphin in Hong Kong waters. *Wildlife Monographs* **144,** 1-65

JEFFERSON, T. A., MYRICK, A. C. & CHIVERS, S. J. (1994) Small cetacean dis-

section and sampling: a field guide. National Oceanic and Atmospheric Administration Technical Memorandum NMFS 198. La Jolla, National Marine Fisheries Service
MINH, T. B., WATANABE, M., NAKATA, H., TANABE, S. & JEFFERSON, T. A. (1999) Contamination by persistent organochlorines in small cetaceans from Hong Kong. *Marine Pollution Bulletin* **39,** 383-392
MYRICK, A. C., HOHN, A. A., SLOAN, P. A., KIMURA, M. & STANLEY, D. D. (1983) Estimating age of spotted and spinner dolphins (*Stenella attenuata* and *Stenella longirostris*) from teeth. National Oceanic and Atmospheric Administration Technical Memorandum NMFS 30. La Jolla, National Marine Fisheries Service
NEILAND, K. A., RICE, D. W. & HOLDEN, B. L. (1970) Helminths of marine mammals, I. The genus *Nasitrema*, air sinus flukes of delphinid Cetacea. *Journal of Parasitology* **56,** 305-316
OZAKI, T. (1935) Trematode parasites of Indian porpoise *Neophocaena phocaenoides* Gray. *Journal of Science Hiroshima University (Zoology)* **3,** 115-138
PARSONS, E. C. M. (1997) Hong Kong's cetaceans: the biology, ecology and behaviour of *Sousa chinensis* and *Neophocaena phocaenoides*. PhD thesis, University of Hong Kong
PARSONS, E. C. M. (1998) Strandings of small cetaceans in Hong Kong's territorial waters. *Journal of the Marine Biological Association of the United Kingdom* **78,** 1039-1042
PARSONS, E. C. M., BOSSART, G. D. & KINOSHITA, R. E. (1999) Postmortem findings in a finless porpoise (*Neophocaena phocaenoides*) calf stranded in Hong Kong. *Veterinary Record* **144,** 75-76
PARSONS, E. C. M. & CHAN, H. M. (1998) Organochlorines in Indo-Pacific hump-backed dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) from Hong Kong. In The Marine Biology of the South China Sea III. Ed B. Morton. Hong Kong, Hong Kong University Press. pp 423-437
PARSONS, E. C. M., FELLEY, M. L. & PORTER, L. J. (1995) An annotated checklist of cetaceans recorded from Hong Kong's territorial waters. *Asian Marine Biology* **12,** 79-100
PARSONS, E. C. M. & JEFFERSON, T. A. (2000) Post-mortem investigations on stranded dolphins and porpoises from Hong Kong waters. *Journal of Wildlife Diseases* **36,** 342-356
PETTER, A. J. & PILLERI, G. (1982) *Pharurus asiaorientalis* new species, metastrongylid nematode, parasite of *Neophocaena asiaorientalis* (Phocoenidae, Cetacea). *Investigations on Cetacea* **13,**.141-148
ROSS, G. J. B., HEINSOHN, G. E. & COCKCROFT, V. G. (1994) Humpback dolphins *Sousa chinensis* (Osbeck, 1765), *Sousa plumbea* (G. Cuvier, 1829) and *Sousa teuszii* (Kukenthal, 1892). In The Handbook of Marine Mammals, Vol 5: The First Book of Dolphins. Eds S. H. Ridgway, R. Harrison. London, Academic Press. pp 23-42
SAFE, S. (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanisms of action. *CRC Critical Reviews in Toxicology* **13,** 319-395
TAO, J. (1983) A new species and a new record of nematodes from porpoise, *Neophocaena phocaenoides*. *Acta Zootaxonomica Sinica* **8,** 350-353
VOS, J. G. & LUSTER, M. I. (1989) Immune alterations. In Halogenated Biphenyls, Terphenyls, Napthalenes, Dibenzodioxins and Related Products. Eds R. D. Kimborough, A. A. Jensen. Amsterdam, Elsevier. pp 295-324
WU, H. W. (1929) On *Halocercus pingi* n sp, a lungworm from the porpoise, *Neomeris phocaenoides*. *Journal of Parasitology* **15,** 276-279
YAMAGUTI, S. (1951) Studies on the helminth fauna of Japan. Part 46: nematodes of marine mammals. *Arbeiten auf der Medizinischen Fakultät zur Okayama* **7,** 295-306

SHORT COMMUNICATIONS

Attaching and effacing lesions in the intestines of two calves associated with natural infection with *Escherichia coli* O26:H11

R. F. GUNNING, A. D. WALES, G. R. PEARSON, E. DONE, A. L. COOKSON, M. J. WOODWARD

Escherichia coli O26 has been associated with diarrhoea in young calves for many years (Sojka 1965, Sherwood and others 1985, Wray and others 1993, Beutin and Muller 1998). In naturally infected calves, attaching and effacing (AE) lesions have been reportedly associated with this serogroup in the large intestine of a calf less than one month old (Mainil and others 1987) and in an eight-day-old calf (Iijima and others 1990). Experimentally, AE lesions have been reported in the large intestine (Mainil and others 1987) and the small and large intestines (Wray and others 1989) of calves inoculated with *E coli* O26:H11. In human beings *E coli* O26:H11 appears to be the predominant verocytotoxin-producing *E coli* (VTEC) of this serogroup and has been associated with diarrhoea, bloody diarrhoea and haemolytic uraemic syndrome (HUS) (Bettelheim 2000). This short communication describes AE lesions in the small and large intestines of two calves naturally infected with *E coli* O26:H11.

Two severely diarrhoeic calves (calf 1 and calf 2), both aged two weeks, were submitted live for postmortem examination from separate farms where several calves were similarly affected. One of the outbreaks included dysentery and the calf from that farm (calf 2) had blood in its faeces.

A zinc sulphate turbidity test was performed on blood from calf 2. Samples of the small intestine of calf 1, and the small and large intestines of calf 2, were collected for routine bacteriological examination. Selected colonies of *E coli* were subcultured onto Dorset egg slopes for further typing, to test for the presence of verocytotoxins and the detection of genes encoding virulence determinants by PCR, as described by Pearson and others (1999). Intestinal contents from both calves were examined by ELISA for rota- and coronavirus and by a Ziehl-Neelsen staining method for cryptosporidium. Samples of duodenum, jejunum, ileum and colon from both calves, and samples of caecum and rectum from the dysenteric calf (calf 2) were fixed immediately after euthanasia in 10 per cent neutral buffered formalin. Tissues were processed routinely to paraffin wax, and 4 µm sections were stained with haematoxylin and eosin. For peroxidase-anti-peroxidase (PAP) immunostaining, additional 4 µm sections of the small and large intestines were mounted on organosilane-coated slides and incubated overnight at 4°C with a commercially available rabbit anti-O26 antiserum (Prolabs), at a dilution of 1/2000. Goat anti-rabbit antiserum (Sigma), rabbit PAP (DAKO) and diaminobenzidine were then applied sequentially. Sections incubated with normal rabbit serum (diluted 1/500 and 1/1000) and rabbit anti-O157 antiserum served as controls. For electron microscopic studies small areas of the ileum of both

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R. F. Gunning, BVetMed, NDA, MSc, MRCVS, Veterinary Laboratories Agency – Langford, Langford House, Langford, Bristol BS40 5DX
A. D. Wales, BVSc, MRCVS, **G. R. Pearson,** BVMS, PhD, FRCPath, MRCVS, Department of Pathology and Microbiology, **E. Done,** MA, VetMB, MRCVS, Department of Clinical Veterinary Science, University of Bristol, School of Veterinary Science, Langford BS40 5DU
A. L. Cookson, BSc, PhD, **M. J. Woodward,** BSc, PhD, Veterinary Laboratories Agency – Weybridge, New Haw, Addlestone, Surrey KT15 3NB

Correspondence to Dr Pearson