Australian VETERINARY JOURNAL

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• *Live sheep exports in disarray* • *Work related injuries* • *National registration of veterinarians* • *Nominations open for AVA awards* • *Lobbying for a national approach to feral pigs* • *Pet Dental Health Month proves a success* • *Botulinum toxin to treat blepharospasm* • *Applying science to welfare of hens* • *Iridovirus-associated mortality of Murray cod* • *Toxoplasmosis in dolphins* • *Phalaris poisoning in horses*

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AVA involved on multiple levels during sheep export predicament

A shipment of 57,000 Australian sheep, rejected by Saudi Arabian authorities for being infected with scabby mouth at unacceptably high levels, was the centre of a firestorm of controversy in September, and the AVA was involved on several fronts.

The drama began when the Cormo Express, which loaded the sheep in Fremantle in early August, delivered them to Jeddah and a Saudi Arabian vet inspected them.

He said some 6 per cent were suffering from scabby mouth, over the 5 per cent limit allowable under the agreed protocols.

The AQIS approved vet on board said the percentage was closer to 0.35 per cent.

The reason for the disparity is still not known. Attempts to negotiate alternative unloading destinations failed, and the owner was reported to have been ready to give the sheep away.

What the Federal Agriculture Minister, Warren Truss, described as "delicate negotiations" proceeded with up to ten countries in the region, all in the glare of unfavourable publicity.

When the shipment was initially rejected, the Minister decided to suspend the Saudi Arabian live export trade until an "effective mechanism to ensure the safe entry of livestock into Saudi could be negotiated".

AVA President Dr Jo Sillince said that the Cormo Express affair has generated a lot of discussion within the community and the veterinary profession.

"The AVA has been involved at various levels of discussions on this issue," she said.

"Initially a media release supporting the government's decision to suspend the live sheep trade with Saudi Arabia was sent out. At the same time, members were reminded that AVA does have a policy about live animal exports."

AVA's policy states: The AVA supports the export trade of live sheep and cattle provided:

- the Livestock Export Accreditation Program (LEAP) Australian Livestock Export Standards, developed by Livecorp on behalf of the meat and livestock industry, are strictly enforced and subject to regular review in collaboration with the veterinary profession, and
- the industry makes further concerted effort to safeguard the welfare of animals up to and at the time of slaughter in importing countries.

"The AVA has its current live animal transport policy re-listed for Policy Council this month to determine whether we are on the right track or whether this needs revisiting," Dr Sillince said.

Dr Sillince encouraged AVA members who believed they could contribute constructive recommendations to convey them to her or to AVA Veterinary Director Dr Kevin Doyle before Policy Council met, in order that they could be taken into consideration.

The AVA has been in almost daily contact with the Minister's office and with the Department to stay abreast of government information on this issue.

At the same time, the Association gained a commitment from the Government to be part of its review team in an official capacity.

From AVA ranks an ad hoc group has also been formed comprising cattle veterinarians, sheep veterinarians, and invited others to examine LiveCorp's guidelines for the trade and determine what improvements could or should be recommended to government.

"The ad hoc group was quickly organised as we wanted to get some discussion in time for Policy Council," said Dr Sillince.

Police forcibly remove protester Sonja Gasser, chained by her neck to a gate blocking the path of trucks carrying sheep for export in the Victorian port of Portland on 24 September. Protesters had attempted to block loading of the export ship Al Kuwait, bound for the Persian Gulf amid growing controversy over the live sheep trade. A shipload of Australian sheep still remains stranded in the Persian Gulf. (AAP Image/Josh Nash)

Discussions have also taken place between the AVA, Middle East experts and members who have travelled on the Cormo Express to ensure that it is aware of all sides of the issue and is aware of any cultural and political implications.

The AVA has also held discussions concerning vaccination, Scabby Mouth and possible options. The issue is also under discussion by the AVA Animal Welfare Committee.

As *AVJ* went to press the fate of the sheep was still officially unresolved, but it was reported that Australian livestock exporters had stepped in to buy back the sheep with the idea of giving them to Iraq for slaughter. It was reported that about 7 per cent of the animals had died. The other 93 per cent were said to have gained several kilos in weight, and to have had adequate supplies of water and fodder and generally cooler weather conditions at sea.

Political repercussions have included moves to ban the live export of sheep from Tasmania, and protests preventing a ship from docking in Portland (VIC) to take on board sheep bound for the Middle East.

The RSPCA and some other animal welfare lobbyists advocated killing and disposing of all sheep on board.

But the AVA issued a media release, very widely reported, which pointed out that such a move would be both an animal welfare and environmental disaster.

Instead the AVA offered the services of the AVA President and a specialist sheep veterinarian to the Government to advise on the actual state of animal welfare on the ship.

"Only the AVA has the expertise and independence to give advice on the conditions and welfare of the sheep on board the Cormo Express," said Dr Sillince.

The live export trade to Saudi Arabia was worth about $195 million in 2002/2003. The federal government said that sheep mortalities to all destinations had fallen from 1.34 per cent in 1999 to a YTD figure in August 2003 of 0.64 per cent. In late 2002, the government announced a formal process to improve animal welfare – the Action Plan for the Livestock Export Industry.

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CONTRIBUTIONS
News and general correspondence
News items and general correspondence should be submitted to the Managing Editor, AVA House, 134-136 Hampden Rd, Artarmon NSW 2064, Australia or PO Box 371 Artarmon NSW 1570 Australia. Telephone (02) 9411 2733, fax (02) 9411 5089, email editor@ava.com.au Web address www.ava.com.au

Scientific section
Articles for the Scientific Reports section of the *AVJ* should be submitted to The Scientific Editor, AVA House, 272 Brunswick Road, Brunswick Victoria 3056. Telephone (03) 9387 2982, fax (03) 9388 0112, email avjsc@ava.com.au

Clinical section:
Articles for the Clinical Section should be submitted to The Editor, Clinical Section, AVA House, 272 Brunswick Rd, Brunswick Victoria 3056. Telephone (03) 9387 2982, fax (03) 9388 0112, email avjsc@ava.com.au

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telephone (02) 9460 7955, fax (02) 9460 8632
email: mcmedia@mcmediasales.com.au

This month's cover photograph was taken in July and shows a chicken looking through a wire fence. (AAP Image/David Ewing).

Record-breaking Sydney Uni students wash dogs to raise funds

Tired, but proud – and exceptionally clean! These University of Sydney vet science students are the new dog washing champions of the world, 133 dogs ahead of the nearest competition. [Photo: Kristen Clarke]

Sydney Olympic Park was the scene for a record-breaking effort when 12 intrepid Veterinary Science students from the University of Sydney washed 848 dogs in eight hours to enter the Guinness Book of Records.

They broke the world record for dog washing as part of vets@work week. The previous record of 715 dogs, also washed by 12 people in eight hours, was set by a Dutch team.

The event was held to raise funds for the University of Sydney Veterinary Science Foundation.

"The team worked tirelessly to wash so many dogs and we would like to thank the dog owners of Sydney for being such good sports and bringing their dogs to Sydney Olympic Park and taking part," said Dr Jennie Churchill, Director of the Veterinary Science Foundation.

As well as the world record attempt, the event included free health pet checks, the chance to meet celebrity TV vet Dr Harry Cooper, exhibitions by NSW Police dogs, demonstrations of microchipping, and the opportunity see a range of different dog breeds.

The Veterinary Science Foundation is raising money to build a state-of-the-art teaching hospital and small animal clinic at the University of Sydney.

Nominations called for AVA awards

The AVA each year recognises services to the profession and the Association through a number of awards and prizes. Submissions in support of nominations for the latest round are requested to reach National Office by December 8, 2003.

Among the prestigious AVA prizes and awards are:

- The Gilruth Prize: for meritorious service to veterinary science in Australia
- The Kesteven Medal: for distinguished contributions to international veterinary science (issued jointly with the Australian College of Veterinary Specialists)
- Fellows/Life Fellows: for outstanding service by members to the AVA
- Meritorious Service Awards: for special long-term service to the AVA through branches or divisions, or through SIGs.

More details: Angela Jackson at National Office, tel (02) 9411 2733, fax (02) 9411 5089, or email: avahq@ava.com.au.

Two recent legal judgements throw light on employers' obligations to injured workers

by Michelle Gilliver-Smith

Managing injured workers is a difficult and sometimes troubled process. Employers have to comply with tough legislation that ensures injured employees are given alternative duties and assisted in their rehabilitation.

Two recent decisions help to provide some useful and up-to-date guidance on the subject:

- Perlidis v Brambles Security Services Limited. This was a 2003 decision of the NSW Anti-Discrimination Tribunal that examined Brambles' duty to assist an injured employee to perform his work upon his return.
- Cosma v Qantas Airways (2002). In this decision, the full court of the Federal Court considered whether Qantas was entitled to terminate the employment of an employee who had little prospect of returning to his pre-injury role, despite the fact that the employee had been working in alternative roles for many years.

Employers' obligations to injured workers can be found in Federal (Workplace Relations Act) and State legislation (Anti-discrimination and Occupational Health and Safety Acts). Anti-discrimination provides that it is unlawful for an employer to discriminate against a person on the ground of a disability or injury by dismissing that person or subjecting them to any other detriment.

It is not, however, unlawful to discriminate against a person on the ground of their disability if they (because of that disability) would be unable to carry out the "inherent requirements" of the employment. Or, in order to carry out those requirements, if they required facilities that are not required by persons without the disability, such that the provision of those facilities would impose an unjustifiable hardship on the employer.

In the Brambles case a security guard was unable to lift heavy loads because of a back injury. Brambles eventually dismissed the employee on the basis that he could no longer perform the inherent requirements of the job. Brambles argued that they had an occupational health and safety obligation to prevent the employee from sustaining further injury. The tribunal found the employer's act of dismissal discriminatory and dismissed Brambles argument.

Brambles did not try to accommodate the employee and therefore could not argue that the provision of assistance to the employee would place an unjustifiable hardship on the employer.

Alternative employment

Cosma was employed as a porter at Qantas. He was given alternative duties after his return to work with a shoulder injury. As part of his rehabilitation he was assigned to different duties of a clerical and administrative nature. He performed these duties for five years. Qantas then terminated his employment when they found that he would not be able to return to his pre-injury role.

The central issue for the court was the employee's ability to perform the alternative clerical duties. The employee argued that he could perform the inherent requirements of the "particular employment", that is, the clerical duties. Qantas, on the other hand, argued that the employee could not perform the inherent requirements of the pre-injury position of the porter.

The full court confirmed the original decision of Heerey J. of the Federal Court and determined that "particular employment" referred to the job that the employee was required to perform. The employee could not, and had no prospect of being able to, perform the inherent requirements of his original position as a baggage porter. Qantas had no obligation to provide him with endless "alternative employment".

Qantas was therefore not held in breach of the Disability Discrimination Act in terminating his employment.

Implications

So what are the implications for you as employers and employees?

Anti-discrimination legislation does not impose a requirement that employers provide endless alternative employment positions to injured workers. An employer will not be found to have unlawfully discriminated against an employee if the employee cannot perform the inherent requirements of the job they were employed to do any longer because of injury.

Employers should always keep in mind that the decision to dismiss an employee with an injury cannot be made until all efforts to rehabilitate the employee, and to explore all facilities and modes of assistance that may enable them to perform the job, have been exhausted.

When an employee is injured in your workplace it is absolutely crucial that the injury, the consequences and the rehabilitation are managed carefully and in accordance with the many laws governing this area.

*** Michelle Gilliver-Smith MIRHRM is AVA Industrial Officer, Wentworth Human Resources Pty Ltd**

For more information and assistance on this or any other HR matter, contact Michelle, Melissa or Gemma at the AVA HR Hotline on 1300 788 977 or e-mail avahrhotline@wentworth-hr.com.au.

A quite unusual lobbying win for AVA

It is unusual that a Federal Agriculture Minister phones the AVA president personally to clarify statements made in the media. After the Acting Minister, Ian Macdonald, spoke in the media to the effect that there were "dishonest vets in the live animal transport industry", AVA went on the warpath to protect the reputations of the AVA members who have been doing a fine and ethical job in this sometimes emotion-arousing industry.

After a few rounds with the minister's office while the minister was said to be uncontactable, the AVA advised that we would issue a press release unless we heard from him – and sent off the draft as proof. Within minutes his office phoned and a conversation with the minister followed.

The minister provided transcripts to demonstrate that he had not made such radical statements as reported. He provided the transcripts of the radio interview and a clarifying press release. One key issue in obtaining the press release was that AVA had supported the minister's stand just the previous week. The reputation of the AVA members was salved, but more importantly the message is there that AVA will protect its members' reputations. We can now work more closely with the government on the review of transportation standards.

viewpoint

AVA President Jo Sillince

The take-home message here?

- If the government does something to support AVA policy then congratulations are in order – it builds the relationship for the future
- AVA will not let the reputations of members be impugned; that is part of our relevance for members
- AVA will not be pushed around by ministers' offices
- AVA will progress policy whenever and wherever possible
- AVA is focused on a continuous improvement theme in relation to livestock transport – unlike some other groups just calling for a ban on the trade. Thus AVA is able to provide constructive and positive input to see standards improve
- AVA is rapidly becoming a more relevant force with government in driving AVA policies forward.

... and more on live transport

During the recent sheep shipment events, a member phoned me out of the blue to note that he had travelled on "sheep ships" and had some recommendations as to specific standards that might change. The outcome is a small and informal task force specifically to review the standards and to make recommendations for change – a good example of one member changing the AVA. All members, and particularly those who have travelled with sheep, are invited to look through the standard and make specific recommendations for continuous improvement.

RSPCA supports AVA stance on rodeos

After an event in Queensland, the RSPCA generated extensive media coverage as it called for veterinarians to be present at all rodeos. This is a statement of AVA policy and is a marked change for RSPCA, which had previously called for the banning of rodeos. The message from this is that continuous improvement themes are often more successful than absolutes in the world of policy. AVA has had a win on this one.

Raids on farms: a real industry service or just media hype?

Recent raids on poultry farms in two States have highlighted clearly the question of whether the end justifies the means.

I don't think there would be a single veterinarian who would oppose the investigation and severe prosecution of any farmer (or other person) who was guilty of animal cruelty. However, when there are legal and legitimate ends to achieve this, are raids supportable?

In one case, illegal "raiders" entered highly bio-secure farms, removed about 15 birds that had "deformities", turned on high-powered lights and filmed the houses and the birds. In the other case, the illegal entrants killed about 500 birds with golf clubs, creating terror and panic in the flock.

In both cases the birds would have been exposed to people who were not their stockmen, thereby stressing the birds. In both cases there were multiple untrained persons in the sheds, creating the genuine risk of crowd panic and "stack ups" as they are known in the industry. This is like a human crowd panic; birds run to the end of the shed (as they are not in cages) and pile up on each other, creating mass death through suffocation. That is clearly to the detriment of animal welfare.

In both cases entries were without usual bio-security procedures – no clean outer clothing, no farm boots, no checking that the entrants did not have birds of their own at home. Thus, virtually by definition, these people were introducing novel bacteria and viruses, potentially endangering the whole flock. Is this to the benefit of animal welfare?

I support the RSPCA's work on the Poultry Code of Conduct released last year. I support the Victorian research and release of the Animal Welfare Audit kit. I support the State standing committees on animal welfare, the National Consultative Committee on Animal Welfare, and I have no objection to key customers (like restaurant chain McDonald's) demanding that suppliers meet their codes of animal welfare.

I support the proper investigations carried out by State departments of agriculture and RSPCA when there is reason to believe there is cruelty. With all these legal means to report, investigate, prosecute, expose and improve animal welfare, is illegally raiding farms and deliberately endangering all the birds to "rescue" just a few still reasonable methodology – or is it fast becoming just a media stunt?

I'll leave that to you to decide.

Progress made on national registration

The AVA has met twice with the Productivity Commission about the important issue of national registration, as well as responding to both the mutual Recognition Agreement between the States and the Trans Tasman Mutual Recognition Agreement with an explicit AVA submission.

While the commission is seeking to improve mutual recognition, AVA has made the case for national registration using several models. We have demonstrated the particular needs of the profession for movement and flexibility. Issues like the small size of the profession, its diversity and large numbers of fields of endeavour; locums; trans-border practices; visiting specialists and those with special interests (some of which are not represented in some regions); national organisations (commercial and other); and the need to respond to emergency animal diseases have all been advanced.

AVA has also noted that the State attorneys-general are looking at a form of agreement for lawyers allowing them to practise for short periods in States other than their "home" without further registration, eg. for court cases. Apparently there are many details to be worked out, such as indemnity insurance, investigations, trust fund rules and so on.

This may provide a model, as might the car registration model and the APVMA registration of drugs model, which have also been addressed with the commission. AVA has also participated in discussions with the Australian Veterinary Boards Council on national registration and some Divisions have raised it with Boards.

Dr Kevin Doyle,
Veterinary Director

Policy and lobbying initiatives and a new Assistant Veterinary Director

comment

AVA CEO Margaret Conley

One of the most important services that AVA provides for members is to promote its policies to governments and decision-makers. We have a senior staff member, Dr Kevin Doyle, who as AVA's National Veterinary Director leads the policy implementation area for the Association.

As many AVA members know, Kevin is on many expert and other committees in either a personal capacity or as an AVA representative. He is in essence responsible for ensuring that AVA policies are represented to government and that the AVA is involved in consultation processes that may lead to changes in legislation or the introduction of new bills.

Kevin is currently following up the recommendations of the Review of Rural Veterinary Services released in February. Among these was the recommendation that an Australian Veterinary Reserve be established to assist the Federal Government should a serious disease outbreak occur.

Kevin is also involved with the Therapeutics Advisory Committee in areas such as the review of Virginiamycin, the Macrolides Review, Ceftiofur Review, and National Drugs and Poisons Schedule Committee scheduling. Other areas of representation include membership of the Primary Industries Standing Committee (PISC deals with issues concerning antibiotic resistance), Infopest, the AVA Animal Welfare Trust and Animal Welfare Advisory Committee, Livestock Exports and the Bartlett Bill (private member's bill put before the Senate dealing with Animal Welfare issues).

Dr Mike Bond (R) joined AVA in September and will work closely with National Veterinary Director Dr Kevin Doyle

AVA is also part of a group seeking to eradicate the risks from feral pigs that includes a strategic partnership with the National Farmers Federation, Cooperative Research Centres and other groups, and is lobbying with the NFF on this matter.

In addition, the AVA is part of the consultation process with Biosecurity Australia in developing draft policies on animal quarantine issues and is participating on a committee choosing practices to be involved with the Australian Quarantine and Inspection Service veterinary scholarship scheme.

As you can gather, Kevin has a huge remit. So I'm delighted to welcome Dr Mike Bond, who started with the AVA on September 9 and will work two days a week in the position formerly occupied by Bernard Robinson. Mike was previously Chief Veterinary Officer for Western Australia and more recently was involved in consulting both here and overseas. He is widely experienced in government and management and has a background in pathology.

Mike is working on the AVA Animal Welfare Advisory Committee and is assisting AQIS with its rural veterinary scholarship scheme. He is also working on therapeutics.

This is just the tip of the iceberg and much more will be happening over the next few months. We will continue to keep you updated on the various issues and promote AVA policy to governments and through the media.

This is a good time to also tell you that membership renewal notices will be posted in October. With input from members, we have revamped the forms and produced an easy-to-read brochure detailing the tangible membership benefits and services available. Please take time to read it and renew your membership before January 1, 2004. If you have any questions about membership, contact the National Office staff on 1300 137 309.

The waiting room display created for Pet Dental Health Month 2003 by Chermside Veterinary Hospital, in Brisbane. Chermside was the National Winner of the prize awarded by Hills Pet Nutrition for most creative and original display. There were more than 120 entries in the competition.

REPORT: Pet Dental Health Month 2003

Pet Dental Health Month was held again in August and well over 800 practices were involved, proving that every year the degree of awareness about the importance of oral health for pets is reaching a higher level.

Many thanks to the AVA, ASAVA, Hills Pet Nutrition and Pfizer Animal Health for their assistance in promoting PDHM to veterinarians and the public. This year's theme was based upon an easy three-point plan recommended to clients by the Australian Veterinary Dental Society for better oral care of pets. Clients were advised to:

1. Visit their vet for a pet dental check up
2 Start an oral care routine for their pet
3. Offer their pet a dental-friendly diet to help prevent gum disease.

AVDS, in conjunction with AVA and ASAVA, focused this year's theme to further educate the pet-owning public on the ease of dental disease prevention.

The media again picked up on PDHM with numerous newspaper articles, radio interviews and television segments looking at the importance of oral care for the overall well being of pets.

All participating practices received dental kits to promote pet oral care to clients. A number of practices put in a lot of hard work to produce spectacular waiting room displays. Hills Pet Nutrition also ran a contest for the best PDHM waiting room display.

Congratulations to all those practices that put in a lot of effort in making PDHM a runaway success again this year. The input from Sarah Webster of Hills Pet Nutrition and Matthew Cragg of Pfizer Animal Health was invaluable.

PDHM will be on again in August 2004 and I look forward to the participation of veterinarians from all over Australia.

– Dr Tony Caiafa, Convenor of PDHM 2003

AVA lobbies for nationally coordinated approach to feral pig threat

The AVA teamed up in late August with the Cattle Council of Australia and National Farmers Federation to brief Federal parliament on the need for a nationally coordinated approach to controlling Australia's estimated 23 million feral pigs. At stake, the three groups said, was not just the protection of Australian agriculture but also potentially the health of the human population.

The briefing to MP's pointed out that feral pigs present a number of hazards, such as: stock losses due to predation on livestock; severe damage to the environment and to biodiversity, particularly in rainforests and some national heritage areas; and the potential spread of endemic, emerging and exotic animal diseases and zoonoses.

The MP's were also informed that the entry of exotic diseases such as classical swine fever, Nipah virus or Foot-and-Mouth Disease could destroy Australia's export industries with a consequent devastating effect on the nation's economy. The Productivity Commission has, for example, estimated that the total cost to the national economy of a Foot-and-Mouth Disease outbreak to be up to $13 billion.

Control of feral pigs currently is largely seen as a State responsibility. But although about $2.5 million is being spent each year by the States, with likely an equivalent amount spent by landholders, the uncoordinated efforts to date have made few gains. Moreover, since feral pigs are no respecters of state boundaries, the current state-based control approach will most probably continue to fail.

AVA and CCA told the MP's that a workshop on the problem in Cairns in June had concluded that eradicating the threat from feral pigs was a long term goal that will require the development of more effective control techniques and technology. But the workshop said an immediate objective should be to minimise the threat by developing and implementing a national strategy and action plan to manage feral pigs via currently available techniques.

The current programs of the Vertebrate Pests Committee could make a firm base to proceed from. All stakeholders needed to be involved in a coordinated way: federal and state governments, landholders, local authorities, and other parties including environmentalists and animal welfare groups.

AVA and CCA proposed that a motion be tabled at the Primary Industries Ministerial Council and the Natural Resource Management Ministerial Council calling for a nationally coordinated approach to the issue of feral pigs, along with appropriate time frames (for example, July 1 2004) set as start dates.

The two bodies emphasised that what they were calling for was better coordination and utilisation of existing funds, rather than new money.

President-elect addresses BVA

AVA President-elect Dr Norm Blackman was one of two speakers on rural veterinary practice and its challenges at the British Veterinary Association's Congress in Edinburgh in September. Dr Blackman was able to give an Australian insight based on the Rural Vets Review at the session, which formed part of the BVA's Contentious Issues program.

The AVA distributed a media release at the end of August supporting the Federal Government's decision to suspend live sheep exports, following an outbreak of scabby mouth in a shipment which had been refused entry to the port of Jeddah in Saudi Arabia until the animals were cleared.

The release said AVA supported the government's decision to suspend livestock exports to Saudi Arabia until the welfare of the animals could be assured.

"The AVA is pleased with the decision and supports the government in its negotiations with Saudi Arabia in developing agreed guidelines to allow the trade to continue," said Dr Jo Sillince, AVA President.

The release noted that AVA recognised that the live sheep and cattle export trade makes a billion dollar contribution each year to the sheep and cattle industries, and to rural and regional communities across Australia.

The Australian and 3AK Melbourne reported the AVA's support on this issue.

The following week Senator Ian Macdonald, Acting Minister for Agriculture, was reported by ABC Radio in Far North Queensland and Western Australia as stating that there were dishonest veterinarians in the cattle industry who would be pursued by the Federal Government. The AVA took exception to this and contacted the minister's office immediately to seek an explanation.

After repeated requests, and an indication that a media release criticising the minister would be sent out, the minister gave a personal explanation to Jo Sillince. The minister also issued a media release explaining his comments. The full release with details was circulated to members and is available on the AVA website www.ava.com.au.

Meanwhile, a media release announcing the new AVA Special Interest Group in Exotic and Unusual Pets drove the media into a frenzy. We have two new media stars in Dr Brendan Carmel – for his radio interviews in Adelaide, Sydney and Melbourne – and Dr Mark Simpson for his interviews and photo with the *Newcastle Herald*. The bulk of the media was covered by Jo Sillince and she reported a few "strange" interviews, especially with one breakfast radio crew in Melbourne.

Media activity for August

AVA issued four releases during August. These included a warning to pet owners that they should not have pets treated by people who were non-veterinarians. This followed evidence that owners were taking their pets to people who practise in homeopathy, naturopathy or herbal remedies rather than to qualified veterinarians.

The release pointed out that there were a number of qualified veterinarians around Australia who practised both Western and Eastern medicine. This generated 14 press articles, three radio mentions and nine radio interviews from regional, rural and metropolitan media.

The AVA PetPEP program in NSW issued two media releases about its program at The Entrance and Corrimal East Primary Schools. Dr Bruce Cartmill, NSW Division President, and Michelle Lee, NSW AVA PetPEP co-ordinator, received coverage in local newspapers, radio and TV. This is an excellent result for PetPEP and we expect that the other States will attract similar coverage as we make further inroads into the media.

MEDIA UPDATE

AVA also received mentions in the following publications:

The Advertiser (Adelaide) – feline AIDS
Readers Digest – getting the best vet for your pet
The Weekend Australian – live animal exports
Wimmera Mail Times – profile of an AVA member
Burnie Advocate – plans in Tasmania to deal with an outbreak of disease
Courier Mail (Brisbane) – pets and problems
The West Australian – reports on the high suicide rate among vets
Satellite Newspaper (Goodna-Redbank, QLD) – AVA PetPEP program
Sunday Tasmanian – tail docking issue

Dr David Marshall, WA Division President, spoke on 6PR in Perth about the high suicide rates among veterinarians. This issue arose from a study undertaken by the University of Western Australia. The study was not limited to this issue but also looked at levels of injury, stress and exposure to disease. More details of the study will be released as the research data undergoes further analysis.

Horse industry better prepared for exotic diseases

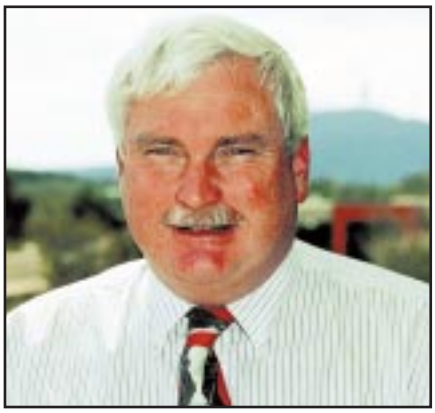
Representatives of the horse industry recently took part in a national workshop in Melbourne to prepare people for liaison roles that would be pivotal in an effective response to an emergency animal disease outbreak.

The Horse Industry Liaison Officer course was a competency-based training module designed to prepare people for the role of industry liaison officer (ILO) in a local disease control centre. Such centres would become the hub of local activity in combating an emergency animal disease.

The two-day course was conducted by Dr Terry Thomas, veterinarian and emergency disease response trainer for Animal Health Australia and Dr Patricia Ellis from the Australian Racing Board, and sponsored by AHA.

Dr Mark Caves, official Veterinarian with the NSW Thoroughbred Racing Board, said the course had brought the NSW horse sector a step closer to being able to effectively handle an EAD outbreak.

"The course was very informative and gave a very good overview about what an industry liaison officer could expect to confront," he said. "The scenario exercises were particularly insightful and led to some very spirited debate about the practicalities one would face. It has been very helpful in stimulating our planning for how the thoroughbred industry in NSW will cope with an outbreak, and how important it is to start communicating the need for planning right now."



Dr Terry Thomas: liaison role a pivotal one in any EAD outbreak.

Dr Thomas said ILOs would perform a vital role in handling a disease outbreak. "Emergency diseases can't be controlled without the cooperation of governments at every level, all of the emergency services required, and livestock industry participants," he said. "The ILO position sits in the middle of the equation, requiring detailed livestock knowledge, close contacts within the affected livestock community, and skills of communication and leadership."

During an outbreak, ILOs would perform an essential role in ensuring that a local horse community was informed about the disease strategy, control plans, and how the response would be carried out. An ILO would also advise the control centre about the effects of a disease response on the local industry and assist in developing and implementing plans for disease eradication or control.

"The extent of disruption to racing, breeding, tourism and other horse activities will be largely influenced by the way an outbreak is managed."

AVA PetPEP Update

AVA PetPEP education officers have their day in the sun. Left to right they are: Rowena Bell (Tasmania), Sophie Kenny (Victoria), Caitlyn Feldmann (Queensland & NT); and Michelle Lee (NSW & ACT)

by Caitlyn Feldmann

Welcome to the latest AVA PetPEP update! As well as the many successful visits by AVA PetPEP affiliated vets to their local schools, other good things have been happening, too.

In Tasmania, the AVA and Glenorchy City Council (near Hobart) have teamed up for the first time to deliver the AVA PetPEP to local primary schools holding Pet Expos.

AVA's Tasmanian Education Officer, Rowena Bell, says that the opportunity for a partnership with the Glenorchy City Council was fantastic:

"The AVA and Council actually have similar objectives, which are to teach children how to understand and appreciate their responsibilities towards pets," she notes.

On behalf of the AVA PetPEP team, we'd like to extend our warmest thanks to all the veterinarians and veterinary nurses who have supported the program during 2003. Their visits to schools and ongoing support and encouragement are what make the AVA PetPEP program so special.

If your practice would like to start visiting schools or to increase its involvement with AVA PetPEP, then simply contact the Education Officer from your State. Each officer assists with the co-ordination of the program by linking vets with schools in their State.

You can contact AVA PetPEP on 1800 282 738, or contact the Education Officer in your state on these email addresses: Rowena Bell (TAS) petpeptas@ava.com.au; Sophie Kenny (VIC) petpepvic@ava.com.au; Caitlyn Feldmann (QLD/NT) petpepqld@ava.com.au; Michelle Lee (NSW/ACT) petpepnsw@ava.com.au.

AVA PetPEP convenors are also available in Western Australia and South Australia. They are veterinarians who devote their time to assisting with the program. Just call the new National Coordinator, Caitlyn Feldmann, who will assist you in locating the convenor closest to you. Caitlyn can be contacted on: 1800 282 738 or email: petpep@ava.com.au .

• Don't forget, it's subscription time again. Your AVA membership renewal form has a new box for AVA PetPEP subscription levies. Please show your support by contributing the $165 levy and join the growing list of practices contributing to, and benefiting from, AVA PetPEP. Only one vet in each practice needs to subscribe to gain this benefit.

The form also asks for a $25 contribution. This assists PetPEP with staffing, postage, printing and supporting the "grass roots" operations of the program. Your assistance in maintaining this funding is greatly appreciated.

AVA member wins presenter role on leading TV show

AVA member Dr Chris Brown is the new presenter joining the next series of the popular animal TV show Harry's Practice on the Seven Network.

Readers may recall from our July edition that Chris was the winner of the AVA HR service putting competition at the Cairns Conference in May. He makes a return to AVJ this month to talk about the new role he has taken on in television.

Chris, 25, described landing the role on Harry's Practice as a whirlwind experience. It took only two and a half weeks from being "discovered" by an agent – in a way that would not be out of place in Hollywood – to doing his first shoot.

"I was out one night with some friends when I was approached by a guy who turned out to be an agent, and he asked me to contact him," said Chris. "About two weeks later I did a screen test and was offered the chance to do some presenting on Harry's Practice. I took the opportunity because I wanted to see where it would take me."

Chris jokes that when he did the screen test he was told he could spin stories better than anyone they'd ever seen. He says his style is different to Harry's and it will be good to have a young male vet on TV as there are not many around. He hopes he can inspire other young men to consider becoming veterinarians.

"The best thing about being a veterinarian is that you can go and work with a variety of animals in different areas," he says. "I like the diversity that the profession offers. The bad thing about the job is the money and the hours – everyone expects you to be a vet one hundred per cent of the time."

Chris, who hails from Newcastle (NSW), has two dogs, Rosie, a kelpie/collie cross, and Rusty, a kelpie. They spend their time in Newcastle since apartment living in Sydney means Chris can't have them in Sydney. He has also developed a great liking for turtles since treating some for his friends

Chris's family includes another veterinarian, his father Graeme, who inspired him to follow in his footsteps following the death of a family dog. Chris went to the University of Sydney and graduated with first class honours in 2001. He then took time out teaching surf safety to children before landing a job at a clinic in Neutral Bay.

In 2000, he got the chance to work alongside his father who is studying for a PhD. They travelled 300km north-west of Alice Springs to a settlement where dogs outnumber people about four to one. They also spent time in other isolated communities treating animals and getting to know the people.

Word about their work spread and last October they headed out past Bourke into far-west NSW for a similar clinic, desexing, worming and vaccinating cats and dogs. Their makeshift surgery was set up at a sheep station where only ceiling fans were available to combat the 35-degree heat.

New TV vet star Dr Chris Brown and his cherished dog Rosie

A fairly recent AVA member, Chris says the main thing that attracted him to the Association was the opportunity to stay in touch with other veterinarians and receive up-to-date scientific knowledge. "I really want to do things in a modern way and keeping up with changes in veterinary medicine is important to me."

Chris makes his debut on Harry's Practice in October.

- Jenny Palmer

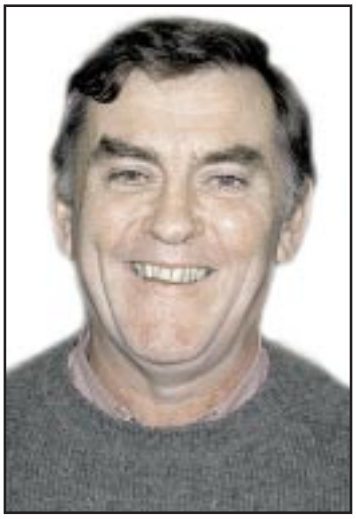
PetPEP patron now a star in Beverly Hills

In other TV news, Chris Brown's fellow AVA member Dr Katrina Warren is set to reprise her role on a bigger stage, namely US television.

Katrina, who co-hosted Harry's Practice for six years after getting her TV break on Totally Wild, is now the star of a TV program called Beverly Hills Vet which premiered in early September on Animal Planet, the US cable channel.

According to the publicity material, she "prescribes cures for animals with unimagineable oddities and meets their equally eccentric families. The biggest challenge Katrina faces is figuring out if some pets' real problem is the one they can't shake ... their owners." The AVA, and many Australian schoolchildren, have benefited from Katrina's energy and skills for several years in her capacity as Patron of AVA PetPEP.

Origin of SARS remains a mystery



Laurie Gleeson: farm animals apparently not implicated.

Work in China seems to indicate that farm animals were not implicated in the outbreak of severe acute respiratory syndrome earlier this year and the UN Food and Agriculture Organisation says the source of the SARS coronavirus remains obscure.

FAO's official statement was based on a report by Dr Laurie Gleeson, a senior Australian veterinarian and infectious diseases expert from CSIRO's Australian Animal Health Laboratory who visited China recently on a three-week mission to track down the possible source of the virus.

Dr Gleeson reviewed laboratory and field data obtained from animal sources – both domestic species and wildlife – by Chinese investigators during and after the massive spread of SARS among the human population in China. Identifying an animal reservoir would be of great importance for future prevention measures in China or elsewhere if such an association could be made.

He met with Chinese and other scientists investigating the SARS virus to help interpret their findings and recommend possible further studies. Chinese and Canadian researchers have sampled or tested more than 600 farm animals including chickens, ducks, pigs and rabbits, but have not detected evidence of SARS coronavirus infection.

"Based on preliminary laboratory testing, a number of animal species are under investigation as a possible source for the virus, including the palm civet, racoon dog, a species of fruit bat, and one species of snake," Dr Gleeson said. "But we still don't know the original source as it is possible that these animals were exposed to the virus in the animal markets."

Dr Gleeson recommended strengthening of epidemiological capability through targeted surveillance studies directed to animal populations in China that were considered to be at high risk of exposure to SARS virus. This would provide information on the roles played by certain animal species and ensure that they were included in an early warning system to detect renewed viral circulation.

His report stressed the urgent need to develop better diagnostic tests in animals and define the relationship between the SARS virus isolated from humans and the slightly different virus isolated from animals. Stepped-up surveillance at farms and slaughterhouses in China was also recommended.

A CSIRO spokeswoman said FAO and the World Health Organisation were exploring ways to follow up on the Gleeson recommendations for planning further studies that target livestock species in high-risk areas, and for standardising and validating laboratory tests for SARS virus in animals, to further the knowledge of SARS coronavirus.

Surveillance of antimicrobial resistance in animals

The Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR), in its report of September 1999, made 22 recommendations across five key areas for an antimicrobial resistance (AMR) management program. One recommendation was to develop a comprehensive surveillance system for resistant bacteria and AMR genes in bacteria from humans and animals.

The Commonwealth Government response, released in August 2000, strongly supported the intent of the JETACAR report and outlined mechanisms for implementing the recommendations.

The Department of Agriculture, Fisheries and Forestry (DAFF) has now developed an action plan for AMR surveillance in animals that has a public health focus. The action plan will be implemented through a pilot surveillance program, funded by DAFF, focusing on cattle, pigs and poultry. Sample collection is to commence in October 2003 and will be completed by June 2004.

Approximately 200-300 samples from each species will be collected and resistance testing undertaken on isolates of Escherichia coli, Enterococcus spp. and Campylobacter spp. In the longer term, the pilot program will be reviewed and amended as necessary for incorporation into the development of an ongoing program of AMR surveillance in animals in Australia.

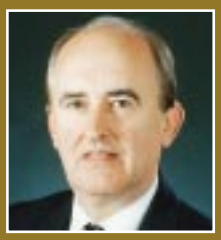
Exercise Minotaur: one year on

National preparedness for any large outbreak of an emergency animal disease (EAD) such as foot-and-mouth disease (FMD) has advanced substantially since Australia's largest disease simulation, Exercise Minotaur, was held one year ago.

Since the exercise, governments and industry have been cooperating closely to strengthen Australia's ability to prevent and, in the worst case, respond to a large outbreak of EAD. These activities include:

- Developing new arrangements and contracts to ensure an adequate supply of FMD vaccine
- Substantially improving diagnostic capacity at the CSIRO Australian Animal Health Laboratory, which is training State and Territory laboratory staff in FMD diagnostic technology
- Reviewing the EAD Response Agreement (the cost-sharing agreement involving all jurisdictions and industry) to cater for the sizeable costs that an outbreak of FMD might incur
- Investigating improvements to animal health information systems
- Progressing epidemiological modelling of FMD scenarios using real geographical and livestock information to explore a range of scenarios and response options to inform decision-making during an emergency response
- Planning and developing a national Rapid Response Team to assist smaller jurisdictions in EAD responses
- Negotiating an updated international agreement to source additional animal health staff to assist a country experiencing a large EAD outbreak
- Strengthening preventive measures such as audits of bans on swill-feeding and local control of feral animals in the States and Territories
- Improving awareness programs for EADs.

World Watch



Commonwealth Chief Veterinary Officer Gardner Murray

Exercise Tethys

On November 17-18, the Commonwealth, State and Territory Governments and industry will participate in the first multi-jurisdictional simulation exercise focused on the aquaculture industry.

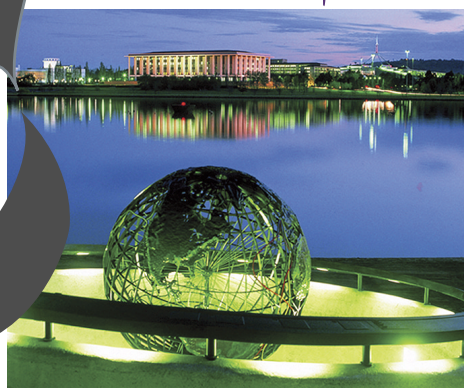
Representatives of industry, government departments and the Murray-Darling Basin Commission have spent six months in extensive planning to design and develop the detailed scenario for the exercise. The scenario is based on a fictitious outbreak of a real disease in a silver perch farm in New South Wales.

Called Exercise Tethys, the simulation aims to address issues of inter-jurisdictional communication and cooperation in response to an EAD incident in aquatic animals.

Canberra attractions:-

Parliament House
Australian War Memorial
Australian Institute of Sport
National Gallery of Australia
National Library of Australia
National Museum of Australia
National Zoo and Aquarium
Tidbinbilla Space Complex
Old Parliament House
Cockington Green
Local Wineries
ScreenSound
Questacon

The Art of Veterinary Science



AVA Annual Conference

2-7 May 2004 - Canberra

Up to 12 concurrent scientific veterinary streams including: small animals, cattle, equine, sheep, practice managers and a range of specialist topics. Field trips including a dive with an endangered shark species.

An exciting social program to include a progressive dinner at Questacon, the National Science & Technology Centre with hands on interactions for all ages. An opportunity for golfers to play the exclusive Royal Canberra Golf Course. Visits to the National Gallery.

Venue is the National Convention Centre boasting a state of the art facility. The conference will host the largest gathering of veterinary industry in Australia exhibiting the latest in technology and equipment.

Join us in Canberra and experience the National Capital

The Art of Veterinary Science
AVA Annual Conference
2-7 May 2004 - Canberra

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Australian Veterinary Association

I read with interest the paper Knemidocoptes intermedius in a wild currawong (Strepera graculina) in the July Journal (*Aust Vet J* 81 7 p411). The paper implies that this is a rare or unusual occurrence in pied currawongs. It is one of the most common reasons for presentation of adult pied currawongs at Taronga Zoo's Wildlife Clinic.

There are a number of cases lodged in the Australian Registry of Wildlife Health at Taronga Zoo. The parasite has also previously been confirmed as Knemidocoptes intermedius. This disease in pied currawongs has previously been reported in literature not cited in this paper.

I would also like to refer to the comment regarding the client's concerns of possible transmission to other birds and the suggestion of treatment. Although this parasite is fairly host specific and unlikely to affect other species, it is likely to be transmitted to other currawongs congregating around the feeder. The higher density of birds around the feeder and the use of a single feeder by many birds of the same and different species facilitate the spread of disease in wild birds.

Treatment of naturally occurring disease in wild animals should only be used if anthropogenic factors have contributed to the higher than usual incidence of the disease. It is far better to remove or modify factors that may contribute to the spread of disease – in this case the feeder. Artificial feeding of birds also results in potential nutritional imbalances and an increase in populations of more dominant species (for example currawongs, noisy minors) that may displace more timid species.

Dr Larry Vogelnest
Senior Veterinarian
Taronga Zoo
Veterinary and Quarantine Centre
Mosman NSW

I am responding to the item in last month's Journal regarding "non-veterinarian therapists" (*Aust Vet J* 81 9 p522). I agree that the AVA should play a major role in educating the general public about different claims made by therapists and that AVA members and veterinarians should be vigilant in ensuring illegal practices performed by some such therapists are reported and stopped in the protection of animal welfare and public interests.

Letters

Editor's note: The *AVJ* welcomes letters from members in all areas of the profession on matters of importance to you. Please keep them brief - to meet our space constraints. Letters will be subject to minimal editing procedures. Subject to letters complying with the *AVJ's* legal responsibilities, they will not be censored. Nor will individuals or groups waging 'campaigns' be permitted to abuse these pages. If submitting a letter intended for publication, kindly identify it as such. Letters to the Editor can be sent by mail, fax or e-mail at the contact points listed at the start of the News Section. Writers may use a pseudonym to protect their identities - but must supply the Editor with verifiable names and points of contact.

However, I would like to clarify the situation regarding animal physiotherapists in Australia as they represent a situation where qualified non-veterinary professionals treat animals in a legal and fully legitimate way.

Animal physiotherapists are fully qualified professional physiotherapists who are represented by a formal special interest group (the Australian Animal Physiotherapy Association or AAPA) through their own professional body, the Australian Physiotherapy Association. They are fully regulated by their own registration board, even when practising on animals, and have a legally protected title and practice. It is, in fact, against the Physiotherapists Act for anyone who is not a registered physiotherapist to call themselves a physiotherapist, perform physiotherapy on animals, or imply that they are qualified to do so.

Animal physiotherapists who are members of AAPA have a stringent code of ethics. It respects the Veterinary Surgeons Act such that animal physiotherapists do not make a veterinary diagnosis and work only via veterinary referral. Further, they have made a commitment to two years of postgraduate masters-level training to convert their skills to animals, with the program starting this year at the University of Queensland.

Continued page 599

Continued from page 597

Veterinarians can become physiotherapists by doing two-year postgraduate masters programs offered by the physiotherapy departments of many universities.
Animal physiotherapists represent a unique group of highly qualified professionals who can increase the scope of veterinary practice without losing the science, mutual professional respect, breaching the law or compromising animal welfare.

Dr Catherine McGowan
Senior Lecturer in Equine Science
Co-ordinator MAnSt (Animal Physiotherapy)
Schools of Animal Studies and Veterinary Science
University of Queensland
Gatton QLD

My thanks to Brian Farrow for his refreshing and timely comments in the editorial (*Aust Vet J* 81 7 p396) on the proliferation of alternate medical approaches to treatment in veterinary science. There are many very sound reasons why our branch of medicine should still be referred to as a science.

P.F. McCormack
Adelaide SA

The "correspondence" in the July issue (*Aust Vet J* 81 7 p431-33) commenting on the letter from Dr P.M. Sidhom (*Aust Vet J* 81 6 p364) disappointed me immensely: they were so full of self justification and excuses for subjecting animals to such brutal treatment in recognition of cultural differences and in pursuit of profit. To quote A. Brightling, "Without question the welfare of cattle slaughtered at the Bassatin abattoir falls well short of accepted community standards in Australia".
If there is even a grain of truth in Dr Sidhom's revelations (why would she invent such horrors?) then there is no justification in subjecting animals to this treatment.
In my opinion this trade should never have been undertaken before establishing that humane slaughter practices were in place, and it should now be halted until this is done.
Given this choice, I would rather perish on the voyage than be tortured on arrival.

Dr Joan Rofe,
Yellingbo VIC

Letters

I wish to add my thoughts to the current discussion about the export of live cattle. In particular, the conflict of interest inherent in the AQIS Third Party Service Program for the Pre-Export Preparation of Livestock Species - Cattle.
Under the scheme, AQIS sets the guidelines under which so-termed "third party" veterinarians involved in the export process operate. It is then up to the exporter to choose which private veterinarian undertakes the inspection and certification of cattle and to pay for that service.
The unfortunate reality is that this process does not allow veterinarians to provide an independent professional opinion as to the suitability or otherwise of cattle for export. The third party veterinarian is in effect an employee - and in some cases advocate - of the exporter and as such cannot do or say anything that interferes with the export process if they wish to continue in the job. It is just too easy for the exporter to obtain inspection and certification services from a more "flexible" veterinarian for the next boat if the current service provider causes problems.
I believe that the program must be changed to remove this conflict of interest if the credibility of veterinarians and the live export industry is to be maintained.

Dr Matthew Bolam,
Nightcliff NT
(recently resigned third party veterinary service provider)

Note to readers:
In the June edition of *AVJ* an article was published concerning the shipment of livestock from Australia to Egypt. As a result of that publication the AVA received a letter from solicitors acting for the owners of the subject vessel, threatening legal action. AVA took advice from its solicitors and solicitors acting for the AVA's insurers, both of whom advised the AVA not to publish any further letters concerning the issue until matters were resolved. AVA acted upon that advice. Consequently several members' letters concerning the issue have not been published in *AVJ* until now.

Australian
VETERINARY
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CLINICAL SECTION

CONTRIBUTIONS INVITED

Practising veterinarians and others are invited to contribute clinical articles to the Australian Veterinary Journal. We will consider material in a variety of formats, including clinically orientated reviews, reports of case series, individual case studies, diagnostic exercises, and letters containing comments or queries. Practitioners are also invited to contribute to the case notes feature, where accepted articles are not peer reviewed but are edited for publication. Contributors should consult instructions to authors and recent issues of the journal for guidelines as to formatting. Over referencing should be avoided: authors should preferably quote only those articles they feel are most likely to be of interest and benefit to readers.

Send all contributions to:
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AVA House, 272 Brunswick Road,
Brunswick, Vic. 3056,
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Trilostane treatment in dogs with pituitary-dependent hyperadrenocorticism

JA BRADDOCK[a], DB CHURCH[b], ID ROBERTSON[c] and ADJ WATSON[a]

Objective To evaluate the efficacy of trilostane in treating dogs with pituitary-dependent hyperadrenocorticism.

Design Prospective clinical trial using client-owned dogs with pituitary-dependent hyperadrenocorticism treated at University Veterinary Centre, Sydney from September 1999 to July 2001.

Procedure Thirty dogs with pituitary-dependent hyperadrenocorticism treated with trilostane, a competitive inhibitor of 3β-HSD, were monitored at days 10, 30 and 90 then 3-monthly by clinical examination, tetracosactrin stimulation testing, urinary corticoid:creatinine ratio measurement and by client questionnaire.

Results Twenty-nine of 30 dogs were successfully treated with trilostane (median dose 16.7 mg/kg; range 5.3 to 50 mg/kg, administered once daily); one responded favourably but died of unrelated disease before full control was achieved.

Conclusion Trilostane administration controlled pituitary-dependent hyperadrenocorticism in these dogs. It was safe, effective and free of side-effects at the doses used. Most dogs were initially quite sensitive to the drug for 10 to 30 days, then required higher doses until a prolonged phase of stable dose requirements occurred. Urinary corticoid:creatinine ratio was useful in assessing duration of drug effect. Some dogs treated for more than 2 years required reduction or temporary cessation of drug because of iatrogenic hypoadrenocorticism.
Aust Vet J 2003;81:600-607

ACTH	Adrenocorticotropic hormone
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
3β-HSD	3β-hydroxysteroid dehydrogenase
IV	Intravenous
LDDST	Low-dose dexamethasone suppression test
PDH	Pituitary-dependent hyperadrenocorticism
UCCR	Urinary corticoid:creatinine ratio
UVCS	University Veterinary Centre, Sydney

Pituitary-dependent hyperadrenocorticism is one of the most commonly recognised endocrine diseases of mature dogs. Medical treatment with mitotane or ketoconazole can be unsatisfactory because of drug side-effects or lack of efficacy, and trilostane has been suggested recently as an alternative.[1] Trilostane is a synthetic, hormonally inactive steroid that competes with pregnenolone as a substrate for 3β-HSD, thereby inhibiting conversion of pregnenolone to progesterone,[2] and non-selectively inhibiting steroid hormone production in adrenal, gonadal and placental tissues.

Trilostane has been used in human medicine to treat hyperadrenocorticism of various causes,[3-8] hormone-dependent breast[9] and prostate cancer,[10] hyperaldosteronism[11-14] and several other disorders. It has shown promise for modifying cortisol concentrations in animal models,[7,15] and has been used in the dog[1,16-20] and the horse.[21]

[a]Faculty of Veterinary Science, The University of Sydney, New South Wales, 2006
[b]Department of Veterinary Clinical Sciences, The Royal Veterinary College, Hatfield, Herts, AL9 7TA, United Kingdom
[c]Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150

The therapeutic use of trilostane in dogs has been reported in three abstracts from the UK. The first[1] concerned treatment of hyperadrenocorticism in four dogs with PDH and one with an adrenal tumour: all responded well, with resolution of clinical signs and no adverse effects during 3 to 7 months of observation, although endocrine test results were not described. The second report[16] involved treatment of four dogs with PDH and two with functioning adrenal tumours: all improved clinically; satisfactory endocrine test results were achieved in four dogs but were not maintained; three of these four relapsed clinically and the other dog died of unrelated disease at 20 days. The third study[17] reported the duration of effect of trilostane in suppressing cortisol production in 10 dogs. Baseline cortisol concentrations were reduced for up to 13 h after trilostane administration, but mean cortisol concentrations over 24 h did not differ from pre-treatment values. However, cortisol responses to administered ACTH were blunted for up to 20 h after receiving trilostane. The degree of adrenal suppression varied between dogs, and it was unclear whether the duration of effect also varied.

Preliminary results on the use of trilostane in a large multicentre clinical trial in the UK and Ireland have recently been reported,[18] and a more detailed report involving 78 PDH-affected dogs was published subsequently.[19] In that study a range of trilostane doses was used initially (mean 5.9 mg/kg; range 1.8 to 20 mg/kg) and adjusted according to ACTH stimulation test results in almost half the dogs. For 23 dogs requiring an increased trilostane dose, the final mean dose was 11.4 mg/kg. For those that required a decreased dose (nine dogs) the mean dose was 3.2 mg/kg. Two other dogs receiving 6.5 and 11 mg/kg respectively ceased trilostane treatment after 2 and 3 months due to low cortisol concentrations that did not increase in response to ACTH administration. These dogs were not treated further and signs of hyperadrenocorticism did not return. Trilostane was considered by these investigators to have an efficacy "comparable with that of mitotane" in abolishing hyperadrenocorticoid signs, with reduction or resolution of polyuria/polydipsia and polyphagia in 70% of dogs with these signs and improvement or resolution of skin problems in 62% of dogs affected. Furthermore, there was a significant reduction in mean post-ACTH cortisol concentrations over time, with 59 of 73 dogs having post-ACTH cortisol concentrations below 250 nmol/L by the second re-examination; it was not stated whether suppressed response to ACTH stimulation was sustained at this level subsequently. Eleven others had cortisol concentrations below 250 nmol/L "at some time during treatment". Overall, the drug appeared to be safe and well tolerated, although two dogs developed hypoadrenocorticoid signs and one died despite treatment, and another two died for apparently unrelated reasons within 1 week of commencing trilostane. A few dogs with functioning adrenal tumours were also treated,[18] with successful reduction in post-ACTH cortisol concentrations and long survival times. It was concluded that trilostane provided a suitable alternative for treating canine hyperadrenocorticism, with a low prevalence of side-effects.

Another smaller study[20] similarly found trilostane to be effective and safe in 11 dogs with PDH and reported ultrasonographic changes in adrenal parenchyma and increased adrenal gland size during therapy.

The study reported here, undertaken at the same time as the aforementioned European trials but with different design and dosing protocol, provides an interesting comparison with findings from the other studies. It includes long term follow-up of the patients and information on the use of UCCR to monitor responses and determine optimal dosing regimens.

Materials and methods

Thirty dogs with naturally-occurring PDH were selected from patients at the UVCS between September 1999 and December 2000. Five were referred because previously diagnosed PDH had not responded well to standard therapy and 25 were referred as untreated cases. Dogs selected for inclusion had no evident concurrent systemic disease, other than stable diabetes mellitus or stable valvular cardiac disease, and owners that agreed to participate in the trial. The study comprised a period of data collection for each dog from the time of enrollment in the study (at variable times after 1.9.99) until 15.7.01. After this date one of the authors (JB) continued to manage the treatment of these dogs up until the time of writing, and some observations made during this follow-up time have also been included in this report.

The diagnosis of PDH was based on appropriate historical and/or physical findings,[22] supported by LDDST results indicating failure of normal pituitary-axis feedback, as detailed below. All dogs underwent abdominal ultrasound examination to exclude the presence of an adrenal tumour and to screen for other clinically silent intra-abdominal conditions. Ultrasonographic changes compatible with steroid hepatopathy and/or bilaterally symmetrical enlargement of adrenal glands (≥ 5 mm wide at either pole of either gland) were considered consistent with PDH, but their absence did not preclude inclusion.

As an additional test to confirm pituitary-dependent disease, endogenous plasma ACTH concentration was measured in most (28/30) dogs prior to starting treatment. For dogs previously untreated for PDH, samples for endogenous ACTH measurement were collected with the 0-h sample of the LDDST or pre-trial tetracosactrin response test. In the other five, pre-treatment endogenous ACTH concentration data were used. An endogenous ACTH concentration of greater than 45 ng/L was considered consistent with PDH. The response to tetracosactrin was tested in 29/30 dogs prior to treatment with trilostane.

All dogs previously treated for PDH had ceased treatment at least 3 weeks before inclusion in the study. Hyperadrenocorticoid signs were evident, and loss of control of PDH was confirmed by tetracosactrin response testing in all cases.

Trilostane (Modrenal, Stegrum Pharmaceuticals, Billinghurst, UK) was given once daily to all dogs for 3 months. The medication, supplied as 60 and 120 mg capsules, was repacked into 30 and 90 mg capsules where necessary. Initially, dogs weighing less than 5 kg received 30 mg, those 5 to 19.9 kg received 60 mg, and dogs 20 kg and over received 120 mg. No instructions were given initially on the time of day to give medication, or whether to administer it with food.

Owners were asked to return dogs on days 10, 30, and 90 of treatment, and thereafter every 3 months. They were asked to measure the dog's water intake for 24 h prior to each visit and to provide a morning urine sample from the dog for UCCR determination. Not all owners were able to collect urine for each recheck. At each visit, owners completed a questionnaire about the dog's general health, thirst, appetite, activity, panting, and abnormal events such as vomiting or behavioural changes. The first author (JB) examined all dogs, assessed medication usage, and performed a tetracosactrin response test, usually between

10 am and 1 pm. One dog relocated to WA during the trial was examined by a colleague there.

A dog's trilostane dose was adjusted if the adrenal response test indicated inadequate or excessive control, using the reference values in Table 1. Dose increments or decrements were 60 mg for dogs weighing more than 10 kg and 30 or 60 mg for smaller dogs, depending on the degree of adrenal suppression or release desired. If the dose was adjusted, re-examination, tetracosactrin response testing and UCCR determination were requested within 2 to 4 weeks. This process was repeated until a satisfactory tetracosactrin response test result was obtained.

Approximately 6 months into the trial, it became apparent that the time between drug administration and tetracosactrin response testing on that day influenced results. As the aim was to determine the optimal effect of the drug in treating PDH, owners of dogs with as yet uncontrolled PDH were asked to administer trilostane in the morning, so that endocrine testing could be performed within 12 h (typically within 6 h) of dosing, at the time of perceived maximal activity of the drug.

After 90 days of treatment, the efficacy of trilostane in relieving clinical signs and the response to tetracosactrin was evaluated: continuation or cessation was recommended based on this assessment, but the decision was left to the owner. Neither choice offered a financial advantage for the owner since the cost of treating and monitoring the dog for the first 6 months of treatment were waived, regardless of whether the dog stayed on the trial drug or was changed to a different treatment (usually mitotane).

Test protocols and sample collection

LDDST — A 0-h blood sample was collected for plasma cortisol determination, after which 0.01 mg/kg dexamethasone was injected via the cephalic vein. Blood samples were collected 4 h and 8 h post-dexamethasone administration for cortisol measurement. Dogs were considered to have failure of normal pituitary-adrenal axis function if plasma cortisol concentration did not suppress appropriately, as outlined in Table 1. Suppression of cortisol concentrations at 4 h (to less than 50% of baseline values or ≤ 20 nmol/L) with escape by 8 h was considered supportive of a diagnosis of PDH.

Tetracosactrin stimulation test — A 0-h blood sample was collected for plasma cortisol determination and then 5 µg/kg tetracosactrin (also known as cosyntropin, a synthetic ACTH analogue) was injected via the cephalic vein. The post-tetracosactrin venous blood sample was collected at 1 h for cortisol measurement.[23] Interpretation of findings was as in Table 1.

Endocrine assays — Plasma cortisol and urinary corticoid assays were performed using a commercial radioimmunoassay kit (Coat-A-Count, Cortisol Radioimmunoassay Kit, Diagnostic Products, Los Angeles, USA), previously validated for canine cortisol.[24] Urine immunoreactive cortisol (urine 'corticoid') was measured using the same commercial radioimmunoassay kit: this has been used previously to measure urine corticoids in dogs.[25] Blood samples (3 to 5 mL) were collected by jugular venipuncture. The heparinised samples were centrifuged and the supernatant frozen at -20°C within 10 min of collection. Urine samples were stored at -20°C. Batched samples were assayed for cortisol at the end of every week. Urine creatinine concentration was determined using 50 µL diluted 1:10 with 450 µL distilled water and processed by a Cobas Mira discrete biochemical analyser (Hoffmann-La Roche, Basel, Switzerland) with creatinine reagents from Trace Scientific, Noble Park, Australia. The UCCR was calculated by dividing the urine cortisol concentration (nmol/L) by the urine creatinine concentration (nmol/L). For endogenous ACTH measurement, 5 mL blood collected by jugular venipuncture into sodium-EDTA was centrifuged immediately for 5 min and separated plasma was stored at -20°C. Batched samples were assayed at the end of each month for ACTH, using a commercial radioimmunoassay kit (RSL ^{125}I ACTH, ICN Biomedical Inc, Costa Mesa, USA), previously validated for dogs.[26]

Statistical analysis — Data were analysed using Statistix Ver 7, Analytical Software, Tallahassee, USA. A Shapiro-Wilk W Test was conducted for normality. As data were not normally distributed, non-parametric analyses were appropriate. A Kruskall-Wallis one way analysis of variance was used to detect overall differences in measured values between days 0, 10, 30, 90, ± 180 and final measurements. Mean ranks were compared to determine the point at which the differences occurred, that is, detecting between-day effects.

Results

Owner compliance with treatment recommendations, based on questioning the owner and assessing the amount of medication used between visits, was considered excellent in all dogs completing the trial.

Recheck times were generally acceptable, with most pets presented within 1 week of the due date. A few exceptions

Table 1. Reference ranges for endocrine tests used in trial.

Tetracosactrin response test		
Untreated hyperadrenocorticoid patient:		
Baseline cortisol	25 - 75 nmol/L	Normal baseline
1-h cortisol	200 - 400 nmol/L	Normal response
1-h cortisol	400 - 600 nmol/L	Hypersecretion, Suspicious of hyperadrenocorticism
1-h cortisol	> 600 nmol/L	Supportive of hyperadrenocorticism
Treated hyperadrenocorticoid patient:		
Baseline cortisol	25 - 75 nmol/L	Normal baseline
Baseline and 1-h cortisol	≤ 15 nmol/L	Excessive control of hyperadrenocorticism
1-h cortisol	25 - 75 nmol/L	Tight control of hyperadrenocorticism
1-h cortisol	75 - 125 nmol/L	Acceptable control of hyperadrenocorticism
Low dose dexamethasone suppression test		
Baseline cortisol	25 - 75 nmol/L	Normal baseline
4-h cortisol	≤ 20 nmol/L	Normal suppression
8-h cortisol	≤ 20 nmol/L	Normal suppression
Urinary corticoid:creatinine ratio		
≤ 15 x 10^{-6}	Normal dog or tight control of hyperadrenocorticism	
≥ 25 x 10^{-6}	Acceptable control of hyperadrenocorticism	

occurred, in which case the visit closest to the due date was used for analysis. Thus data were collated and analysed for days 0 and 10, and for days *nearest to* 30, 90 and 180, along with data from the last recorded visit prior to 15.7.01.

The mean and median ages were 9.5 years (range 5 to 14). There were five entire and six castrated males and 18 spayed and two entire females. The mean body weight was 16 kg (range 4 to 43). Of the five dogs previously treated for PDH, two had received mitotane alone, one received ketoconazole alone, one received mitotane and selegiline at different times, and one received mitotane and ketoconazole at different times. At the end of the study period dogs had been treated for 170 to 600 days (mean 384), or 11,370 dog-treatment days.

The medication was well tolerated in all dogs. There were no reports of troubling side effects until after the study period. The final mean trilostane dosage required to control PDH was 19 mg/kg (median 17). The range of doses required was wide (5 to 50 mg/kg). Larger dogs seemed to require less per kg than did smaller dogs: the 10 dogs on the lowest doses had a mean bodyweight 19.5 kg (median 22.2), while the 10 on the highest doses had a mean 10.6 kg (median 8.7).

Endocrine test results are depicted in Figure 1. Twenty-nine of 30 dogs (97%) had final tetracosactrin responses indicating control of PDH. The remaining dog with inadequate test results was reported to have improved clinically but died of unrelated disease (cervical vertebral neoplasia) before endocrine control could be established.

Mean baseline cortisol concentration at 0 days (pre-treatment) was 171 nmol/L (median 143, range 57 to 402) and the mean 1-h post-tetracosactrin cortisol concentration was 812 nmol/L (median 531, range 127 to 2613). There was an overall downward trend in these values during the trial.

At 10 days, 12/30 (40%) dogs had stimulation test results that indicated control of PDH, including seven (23%) with tight control. The mean baseline cortisol concentration was 105 nmol/L (median 69, range 12 to 329) and mean 1-h concentration was 199 nmol/L (median 169, range 21 to 492).

At 30 days, only nine dogs (30%) were assessed to have controlled PDH, based on stimulation tests, though, of these, eight (27%) were tightly controlled. The mean baseline cortisol concentration was 117 nmol/L (median 123, range 13 to 315) and mean post-tetracosactrin concentration was 188 nmol/L (median 184, range 17 to 456).

At 90 days, 17/30 (57%) dogs had stimulation test results indicating control, of which 11 (37%) were tightly controlled and 2 (7%) were over-controlled. The mean baseline cortisol concentration was 92 nmol/L (median 66, range 12 to 282) and the post-tetracosactrin mean concentration was 140 nmol/L (median 109, range 12 to 417).

At 180 days, 29 dogs remained alive and 23 (79%) were considered controlled, with tight control in 17 (59%) and excessive control in 3 (10%). Mean baseline cortisol concentration then was 53 nmol/L (median 44, range 7 to 134) and mean post-stimulation cortisol concentration was 79 nmol/L (median 41, range 7 to 323).

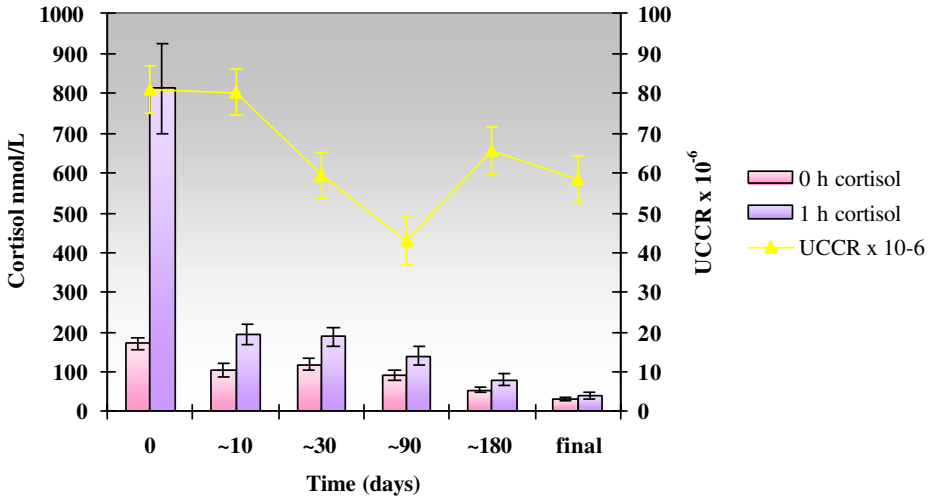

Figure 1. Endocrine test results: mean plasma cortisol concentrations before (0-h) and after (1-h) tetracosactrin administration, and mean urine corticoid:creatinine ratios (UCCR) in 30 dogs with pituitary-dependent hyperadrenocorticism before and during treatment with trilostane. Bars indicate standard error of mean.

The downward trend in cortisol values continued to the final test result obtained for each dog (Figure 1). The mean baseline cortisol concentration at final test was 31 nmol/L (median 27, range 4 to 99) and the mean post-stimulation cortisol was 40 nmol/L (median 29, range 3 to 231). Statistical analysis showed reductions in mean cortisol values from day 0 ($P < 0.01$) for both baseline and stimulated cortisol concentrations at all times tested, except for the day 30 baseline cortisol concentration. There was also a significant and progressive decrease in the response to tetracosactrin stimulation at every test ($P < 0.01$).

As experience was acquired with trilostane, satisfactory endocrine control that required no further dose adjustments was achieved in less time: for the first ten dogs, the average was 89 days (median 79, range 10 to 195), while for the last ten, the mean was 39 days (median 31, range 10 to 113).

In some instances, the UCCR did not parallel stimulation test results or clinical assessment of response. Of the 12 dogs that had controlled PDH at 10 days, and for which a UCCR was obtained, only three (25%) had concordant results (UCCR $\leq 15 \times 10^{-6}$). Corresponding figures at other times were: 1/9 (11%) at 30 days, 4/14 (29%) at 90 days, 8/17 (47%) at 180 days, and 10/23 (43%) at the final test. Statistical analysis showed that UCCR did not change over time ($P = 0.26$), in contrast to the reduction in basal and stimulated cortisol concentrations.

Of the original 30 dogs, 22 (73%) continued on trilostane beyond the formal end of the trial and eight did not, due to death or euthanasia from causes unrelated to PDH (five dogs), cessation of medication by owners for reasons unrelated to dissatisfaction with the medication (two dogs) or loss to follow-up (one dog). This last dog was doing well on day 315.

During the study, three dogs developed concurrent illness unrelated to PDH or its treatment: two with transient gastrointestinal disturbances, one with disorientation and transient azotaemia. Another dog developed diabetes mellitus, which was managed successfully while trilostane was continued.

Four dogs developed clinical signs of hypoadrenocorticism during trilostane treatment, all after completion of the trial. One dog with concurrent stable diabetes mellitus developed inappetence and lethargy after 12 months, but this resolved after reduction in the trilostane dose. These signs recurred 9 months later and tetracosactrin responses revealed adrenal suppression despite 1 week without trilostane. Trilostane was withheld for another 2 months without signs of PDH, but polyuria and polydipsia recurred and adrenal stimulation tests revealed an exaggerated response to tetracosactrin so treatment was resumed at a lower dose.

Another dog developed hypoadrenocorticoid signs after 20 months. Adrenal suppression persisted for 4 months without trilostane, after which an exaggerated responsiveness to tetracosactrin returned. This dog was then stabilised on a lower trilostane dose.

The third dog with hypoadrenocorticism after 19 months of treatment failed to respond to adrenal stimulation testing 3 days after withdrawal of trilostane. It was stabilised subsequently on a reduced trilostane dose, given every 48 h instead of 24-hourly.

The fourth dog had acute signs and laboratory abnormalities of mineralocorticoid and glucocorticoid deficiency after 22 months of treatment. It responded rapidly to appropriate therapy but adrenal function remained suppressed for 6 weeks. Trilostane was subsequently resumed at a reduced dose.

One other dog had its trilostane dose reduced after 21 months because of excessive control demonstrated with tetracosactrin stimulation testing, although it did not show hypoadrenocorticoid signs.

Discussion

Trilostane proved effective in treating PDH in these dogs. There was a rapid response to treatment in all dogs and no troubling side-effects during the trial itself, although four dogs had signs consistent with hypoadrenocorticism later. The dose had to be titrated to effect for each individual, but the dose rates used here should provide a guide for treating other populations of affected dogs.

As experience was gained in using trilostane, the time taken to achieve satisfactory endocrine control was reduced to a mean of 39 days. This time frame is comparable with our experience in initiating mitotane treatment of PDH-affected dogs: these required a mean of 9 days induction treatment and a further 28 to 60 days before they stabilised and an appropriate maintenance dose was determined.[27]

Endocrine testing

Although most reliance was placed on tetracosactrin response testing to assess endocrine control, UCCR was also used. In some dogs, stimulation test results appeared very unsatisfactory despite excellent clinical responses and relatively low UCCRs. However, giving the same trilostane dose in the morning instead of the evening produced adrenal responses, tested within 12 h of trilostane administration, which demonstrated adequate suppression and were consistent with clinical findings. Another apparent discrepancy was noticed in some dogs that had low baseline cortisols and minimal response to stimulation testing, suggesting tight control, but very high UCCRs, suggesting poor or no control. Evaluation of these cases indicated that trilostane had been given in the morning, with stimulation testing performed within 6 to 8 h of dosing. Urine collection for UCCR measurement had generally occurred at or before the time of dosing. Thus, the UCCR was estimated at the end of the 24 h dosing interval when trilostane was having least effect, while response to tetracosactrin was tested at the time of estimated peak drug effect. By altering the time relationship between dosing and testing, it was confirmed that UCCRs were lower when urine was collected nearer the time of dosing, and the plasma cortisols higher when tested later. The conclusions reached were that trilostane suppressed cortisol hypersecretion for less than 24 h, and that increased UCCR indicated escape from endocrine control for several hours prior to urine collection. Others[17] have concluded similarly that trilostane suppresses cortisol production for 24 h or less.

Subsequently, owners were instructed to administer trilostane in the morning and UCCR was used to gauge duration of effect of the drug: dogs with high morning UCCR values were considered likely to have effect duration less than 24 h. As the first urine passed in the morning was sampled, it represented several hours of cortisol excretion. The higher the UCCR in well controlled dogs, the shorter the likely duration of effect, as longer periods of release from inhibition would presumably allow increasingly greater urinary excretion of corticoids. This theory was confirmed later by adrenal response testing some dogs with high UCCRs more than 12h after trilostane administration. One such dog with incomplete resolution of PDH signs was changed from once daily to twice daily trilostane administration, with an improved response. The other dogs had improved clinically, and so a change to twice daily dosing was not necessary. It is not at this time known what amount (duration and level) of exposure to elevated concentrations of glucocorticoids in a 24 h period is required to cause the signs of hyperadrenocorticism, or the reasons for individual variation in susceptibility to the adverse effects or ability to tolerate this exposure.

In one dog, the UCCR persisted well above the upper limit of normal despite good 24 h control as assessed clinically and by stimulation testing at the end of the dosing interval. A possible explanation for this discrepancy is that high concentrations of metabolites of trilostane or steroids that cross-reacted in the cortisol immunoassay were excreted in urine. Further studies are needed to evaluate this.

In another instance, a practitioner monitoring one patient after the trial ended, with the aid of a commercial laboratory, obtained UCCR results inconsistent with past and present clinical and other endocrine data. Enquiries indicated that the laboratory assayed corticoids by an immunofluorescence method. This might account for the discrepancy, as trilostane and/or its metabolites fluoresce in some media and can interfere with assays of this type.[4,28] An effect on immunofluorescence corticoid assays on urine but not plasma might be explained by preferential renal elimination of fluorescing metabolites and their presence in high concentration in this fluid. Subsequent testing in our laboratory (using immunosorbent radioimmunoassays) gave a lower and more concordant UCCR in this case.

Trilostane dose requirements and duration of effect

A wide range of trilostane doses was required to induce and maintain adequate adrenocortical suppression in these dogs. In people, marked individual variation occurs in absorption, metabolism and clearance of the drug[29] and is likely also to occur in dogs. Variable gastrointestinal absorption of trilostane is thought to be related in part to its poor water solubility.[30] In this study, the dog on the second highest dose of trilostane had a long history of severe inflammatory bowel disease, which may have hampered drug absorption. Variations in renal and hepatic function could account for further individual variation, as urine

and bile are both important routes for elimination of trilostane's metabolites.[31]

The trend for larger dogs to require a lower mg/kg dose than smaller dogs is consistent with differences in metabolic rate, and it might be more appropriate to dose dogs according to body surface area.

Based on our experience with this group of dogs, we recommend a starting dose of 10 mg/kg, with adjustment as necessary to achieve a cortisol response to tetracosactrin 3 to 8 h post-trilostane administration that indicates adequate, but not excessive, adrenal suppression. The therapeutic dose for most dogs is likely to be 16 to 19 mg/kg once daily. Some dogs may require this dose twice daily if there is inadequate duration of effect.

Many dogs had an initial 'sensitivity' to trilostane of rather short duration and the dose then had to be increased until a dose appropriate for continuing use was reached. The time required to achieve this varied between dogs, but the requirement seemed eventually to 'plateau', so that further increase was unnecessary. The increasing dose requirement may have been caused by induction of enzymes metabolising trilostane and its metabolites, up-regulation of inhibited enzymes, or ACTH over-ride of the blockade: most pituitary tumours retain some sensitivity to feedback inhibition by corticosteroids,[22] thus blocking corticosteroid production could release the pituitary tumour somewhat from negative-feedback and precipitate a rise in ACTH secretion, as noted during mitotane treatment in PDH dogs.[32]

Doses of trilostane required to control PDH here were higher than documented previously, though it is possible that in earlier studies[1,16] inadequate dosing led to poor efficacy. Previous investigators may not have persisted sufficiently with dose adjustments to pass through the 'sensitive' period and find the dose for long-term use, leading to perceptions that the initial improvement was unsustainable and that the drug was unsuitable for chronic use.

Later studies also reported using lower doses for PDH control but the target cortisol concentrations after stimulation testing in one of those studies[18,19] was higher (≤ 250 nmol/L) than used here. The other report[20] aimed for post-stimulation cortisols of 27 to 69 nmol/L, reflecting tighter control than in the present study, but it is not clear how many dogs achieved this; graphed data showed most dogs had post-tetracosactrin cortisol concentrations similar to those in the present study and the lower doses used to achieve this (4.1 to 15.6 mg/kg; median 6.1 mg/kg) cannot be easily explained.

The duration of effect of trilostane is likely to be influenced by the rate and degree of drug absorption from the gastrointestinal tract, rate of metabolism and clearance of drug, and the extent to which pituitary ACTH release causes pituitary over-ride of the partial enzyme blockade. These variables provide considerable scope for individual variation in duration of trilostane's effect, and may account for the requirement for 12-hourly dosing in some dogs.

Excessive adrenocortical suppression and mineralocorticoid effects

Theoretically, one advantage of trilostane is its rapidly reversible effect, attributable to its mode of action and short half-life. If hypocortisolaemia or mineralocorticoid deficiency causes problems, drug withdrawal usually results in recovery within 24 to 48 h. Precursors for steroid synthesis accumulate due to continued ACTH secretion in the face of enzyme blockade and steroid production could be amplified immediately after trilostane administration ceases. This might be useful when dogs treated for PDH have increased cortisol requirement during illness, anaesthesia or surgery. Ceasing medication would quickly restore cortisol production. Furthermore, trilostane dose reduction would permit partial restoration of adrenal function if this were deemed more appropriate.

A post-tetracosactrin cortisol concentration of ≤ 15 nmol/L was considered here to indicate excessive suppression. This is less than that used for mitotane because we assumed trilostane-treated dogs would be exposed to higher cortisol concentrations as trilostane's effects subsided later in the dosing interval. Although there is potential for glucocorticoid deficiency to develop in trilostane-treated dogs, this did not occur during the study period, although four dogs that continued to receive trilostane subsequently developed related signs. Because of the short duration of action of trilostane,[17] effects from overdosing would be expected to reverse rapidly on withdrawal of the drug. Nevertheless, in three of these four dogs iatrogenic hypoadrenocorticism persisted for weeks to months, though glucocorticoid supplementation was not required because baseline production was adequate once trilostane was withheld. This could be a long-term effect of exposure of adrenocortices to trilostane or its metabolites, or the accumulation of steroid precursor molecules. It is possible that the enzyme blockade, or metabolism and excretion of the drug, change over time. Alterations in adrenal gland size and structure have been reported[20] during trilostane treatment, probably as a consequence of chronic overstimulation and hyperfunction. It is possible that exhaustion of secretory capacity and/or premature cell death or damage occur in some dogs due to constant stimulation in the face of enzyme blockade.

Blood electrolyte concentrations were generally only tested here when the owner reported clinical signs compatible with hypoadrenocorticism. During the trial, no dogs showed abnormalities suggesting mineralocorticoid deficiency, including dogs given the highest doses. It was assumed that, if substantial electrolyte abnormalities were present in any treated dogs, they would have developed signs during the study period. One dog did later develop mineralocorticoid and glucocorticoid deficiency, after 22 months treatment. Apparently dogs do not readily develop mineralocorticoid deficiency, despite high trilostane doses and profound glucocorticoid suppression. It may be that they are exposed to sufficient mineralocorticoid hormone as the dose effect subsides to correct minor electrolyte abnormalities that occur during peak drug activity, or that in dogs trilostane does not generally affect mineralocorticoid concentration. This contrasts with human patients, in which trilostane is used to treat primary hyperaldosteronism[11-13] or diuretic-induced hypokalemia.[14] Glucocorticoid deficiency is not reported as a side-effect in these patients, but has occurred at much higher doses when treating human PDH.[8] It appears that species differences exist and warrant further investigation.

Other observations

A decision had to be made after 90 days about the response to treatment and whether to change to another treatment. The discrepancy between assessed clinical response and adrenal stimulation results was evident but had not at that time been recognised as being due to the dosing-testing interval. Although only 57% of dogs had ACTH stimulation tests indicating good endocrine control at 90 days, all were improved clinically and owners were sufficiently satisfied with the improvement to want to continue trilostane treatment. The investigators concurred with the owners in all cases.

Owners that ceased trilostane administration after the trial did so for reasons unrelated to PDH or the treatment. Excluding the five dogs that died, three others had illnesses unrelated to trilostane that required veterinary intervention and were resolved despite continued trilostane treatment.

In one patient two factors contributed to the failure to achieve complete endocrine control. Firstly, it was treated early in the series when, because of lack of experience, it took longer to titrate up to the correct dose. Secondly, it was euthanased with vertebral neoplasia at 170 days. At the time of its final stimulation test it was receiving about one-third of the dose other dogs were receiving at that stage of treatment. Given sufficient time, it is likely that adequate control would have been achieved, as there had been considerable clinical improvement.

Roles for trilostane

There are several possible roles for trilostane in the management of canine hyperadrenocorticism. Some will favour it as a first-line therapy in PDH because of its safety and rapidly reversible effect. It has a wider therapeutic range and causes fewer side-effects than mitotane. It does not require as close monitoring during induction as mitotane and may be a more appropriate choice for any client that is unlikely to observe their pet closely, or those having poor cognitive skills. Likewise, some veterinarians may prefer trilostane to reduce the need for vigilance early in treatment.

Trilostane has a place in the management of PDH-affected dogs that react adversely to other medical treatments. Patients with concurrent disease such as diabetes mellitus, in which a reduced food intake can have serious consequences, may be better treated with trilostane - it is less likely than other currently used medical treatments to cause inappetence or long-lasting iatrogenic hypoadrenocorticism and anorexia.

Trilostane may also be useful for short-term treatment of PDH-affected patients to improve general body condition and overall health prior to hypophysectomy or bilateral adrenalectomy.

Trilostane may prove valuable for managing canine hyperadrenocorticism caused by functional adrenal tumours. Although surgical treatment is usually the better option for many of these, trilostane may be suitable if owners do not agree to surgery, or if palliative treatment is required for patients with inoperable lesions.

If trilostane is to be used for treatment of PDH in client-owned animals, then the cost of the drug becomes an important consideration. Based on the mean dose rates used in our study and current wholesale prices and foreign exchange rates, the estimated annual cost of treating a dog with trilostane and monitoring it with 3-monthly veterinary visits and adrenal stimulation testing is around $2000 for a 5 kg dog, rising to $6900 for a 20 kg dog. This is twice (5 kg) or three times (20 kg) that for mitotane treatment. The cost disadvantage to using trilostane needs to be weighed against the benefits from other attributes of the drug.

Conclusions

Trilostane was found to be safe and effective in managing PDH in dogs. With experience in using the drug, the time needed to achieve control of PDH was similar to that required to induce and establish maintenance therapy with mitotane, which is regarded as the current standard treatment for canine PDH.

Dose rates required and duration of effect differed between dogs. A starting dose of 10 mg/kg is recommended, with upward or downward adjustment to achieve a cortisol response to tetracosactrin 3 to 8 h post-trilostane administration that indicates adequate adrenal suppression. The therapeutic dose for most dogs is 16 to 19 mg/kg once daily, though larger dogs tend to require a lower mg/kg dose than smaller dogs. In dogs in which there is inadequate duration of effect, twice daily dosing may be required. The most expedient means of judging adequate duration of effect is measurement of UCCR in a urine sample collected at home before dosing the dog (whether dosing is 12-hourly or 24-hourly). A high UCCR suggests escape from control of PDH in an interval shorter than the dosing interval, but a brief period of 'escape', indicated by mildly increased UCCR, is probably not important for overall control of the disease. It is not known for what proportion of the day it is necessary to maintain adrenal suppression to treat PDH satisfactorily. All endocrine tests should be interpreted in association with clinical response.

Trilostane will be very useful in dogs that tolerate other medical therapies poorly and/or those not able to have surgical treatment. It would be a better option for clients unable to monitor dogs satisfactorily during the potentially hazardous induction phase of mitotane treatment. Trilostane may also be useful for dogs with adrenal tumours causing hyperadrenocorticism, although this requires further investigation. The higher cost of trilostane therapy will be a disadvantage in many situations.

Acknowledgments

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(Accepted for publication 23 June 2003)

Common cranial nerve disorders in dogs and cats 2. CN V and CN VII

Lesions of the trigeminal and facial nerves are encountered in practice. In this article the author discusses diagnosis, treatment and prognosis of conditions involving their malfunction. Their common disorders are detailed in script supported by way of numerous diagrams and photographs. The functions of both nerves are listed as are the signs associated with their malfunction. While trigeminal lesions are seen less frequently they present with characteristic clinical signs and are therefore easier to identify; methods of differentiating sensory lesions from facial nerve lesions are described. CNS lesions involved in CN V deficiencies are presented and discussed. Idiopathic facial nerve paralysis is described and compared to cases secondary to otitis media/interna or involving some polyneuropathies.

Penderis J. *In Practice* 2003;25:256-263.

Pain associated with the sacroiliac joint region: a clinical study of 74 horses

The authors believe that sacroiliac (SI) joint pain is both misdiagnosed and true cases underdiagnosed. Following exclusion because of other causes of lameness or poor performance, horses showing clinical signs suggestive of SI pain were then subject to nuclear scintigraphic examination and infiltration of local anaesthetic around the SI region. Abnormal radiopharmaceutical uptake in the joint region, and/or a positive (reduced lameness) response to the local anaesthetic qualified horses for inclusion in the study. Dressage and showjumping horses appeared to be at particular risk. Affected horses were generally slightly older, taller and heavier than the general clinic population.

While Warmblood horses appeared to be in the majority, there was no correlation between conformation and the presence of SI joint region pain.

The authors believe the combination of comprehensive clinical assessment, nuclear scintigraphic examination and their described infiltration of local anaesthetic solution in the SI joint region may allow a definitive diagnosis.

Dyson S et al. *Equine Vet J* 2003;35:240-245.

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This article is being reprinted because of the poor quality reproduction of some of the pictures in the original printing.

Metaphyseal osteomyelitis in an immature Abyssinian cat

WA BRADLEY
Ku-ring-gai Veterinary Hospital, 290 Bobbin Head Road, North Turramurra, New South Wales 2074

Metaphyseal osteomyelitis is a rarely described condition in small animals, especially the cat. Infection, most commonly *Staphylococcus* sp, is considered to occur in the metaphyseal region of the immature animal due to vascular anomalies that predispose to the haematogenous seeding of bacteria in this area. There is also speculation that the characteristics of the bacteria that allow them to adhere to cartilage matrix, rather than vascular linings and erythrocytes, may provide an advantage for colonisation in the metaphysis, resulting in infection. This case describes the successful management of a case of distal radial metaphyseal osteomyelitis in an immature cat using surgical intervention and antibacterial therapy.
Aust Vet J 2003;81:608-611

Case report

A 6-month-old neutered male Abyssinian cat was presented with a week long history of a partial weight bearing lameness on the left foreleg. The cat had been on a balanced commercial ration and had no previous illness or trauma. At examination the cat was excitable and pyrexic (39.5°C). The only physical abnormality detected was pain on palpation of the left distal radial physeal region. There was no evidence of any wound in the area of pain. Radiographs of the region (Figure 1) demonstrated an area of lysis in the distal metaphyseal region of the left radius. The surrounding cortical bone was mildly sclerotic. There was no evidence of periosteal reaction or soft tissue swelling and the distal radial physis was patent. A tentative diagnosis of a developmental bone cyst was made. The cat was discharged and the owner advised to restrict exercise and return for repeat radiographs in 10 days. Ketoprofen 1 mg/kg once daily was dispensed for 5 days for analgesia.

Eleven days later the cat was bright and alert and non pyrexic (38.7°C). The animal was non-weight bearing on the left foreleg. The distal radial region was swollen and very painful on palpation. Radiographs (Figure 2) of the distal left foreleg showed the lytic area had increased in size and had a mixed radiological appearance. Several areas of lysis were seen with ossified material within the lesion. The surrounding cortices were thickened and the lytic area appeared to be extending proximally. The distal radial physis was patent and there was a smooth periosteal thickening extending to the diaphyseal region. No haematological or biochemical studies were undertaken.

The cat was anaesthetised routinely and the metaphyseal lesion was surgically explored with the aim of obtaining diagnostic material. After lateral reflection of the common digital extensor tendon, the lesion was entered using a Steinman pin. The lesion was curetted and the contents, reddish/brown, friable material, were submitted for histopathological examination. Care was taken to avoid damage to the distal radial physis. After curettage the lesion was thoroughly lavaged with sterile saline. An autogenous cancellous bone graft was harvested from the proximal left humerus and packed into the cavity created in the radial metaphysis. The incision was closed and the leg dressed. Postoperatively 0.015 mg/kg buprenorphine was administered for analgesia. The cat was commenced on oral clindamycin at a dose rate of 11 mg/kg twice daily the day following surgery and dressings were changed every 2 days post operatively until sutures were removed 10 days after surgery. The distal left foreleg remained markedly swollen for at least 5

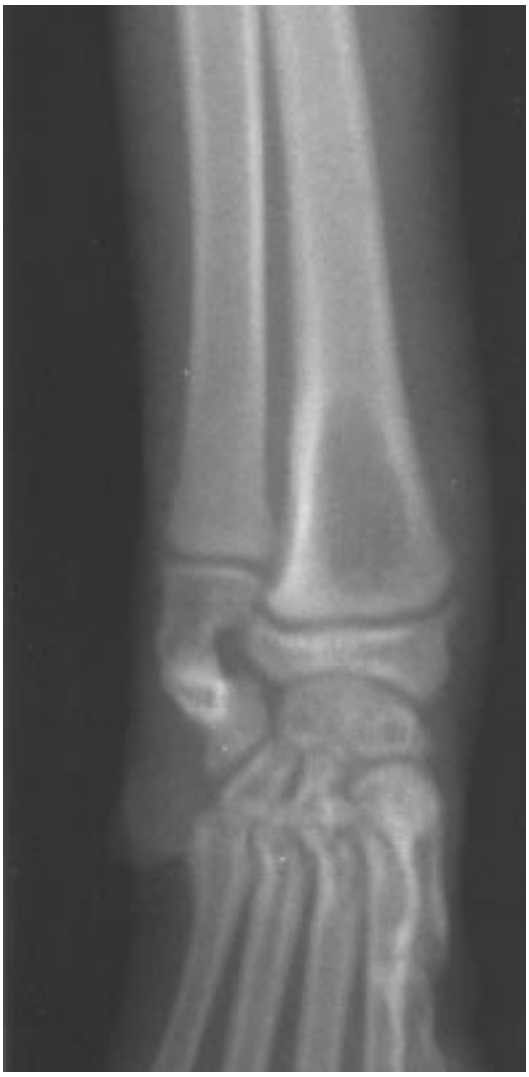

Figure 1. Craniocaudal view of the distal left forelimb of a 6-month-old Abyssinian cat on presentation (day 0), showing a lytic area in the distal radial metaphysis.

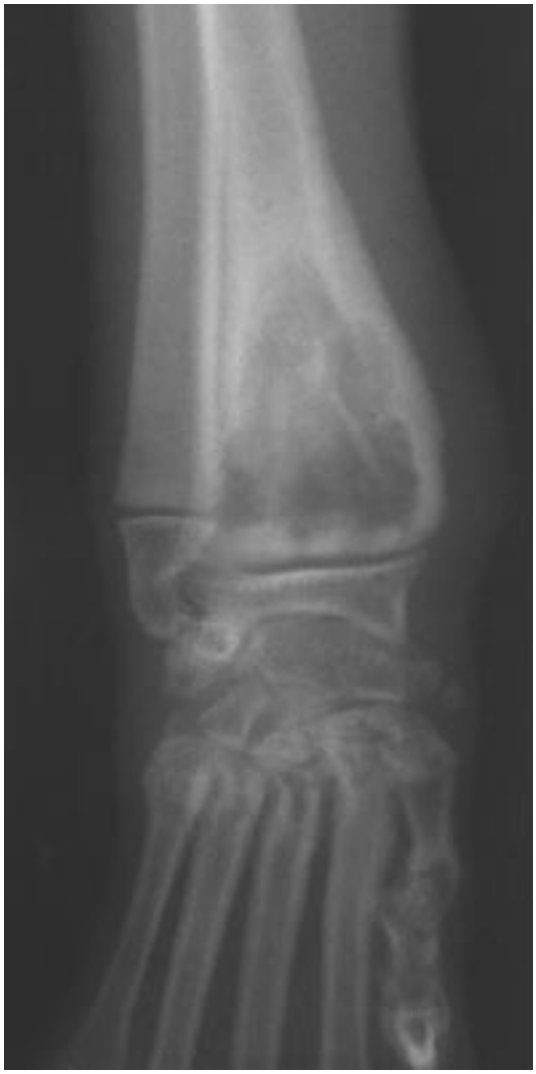

Figure 2. Craniocaudal view of the distal left forelimb 11 days after presentation, showing progression of the lesion seen at day 0, prior to treatment.

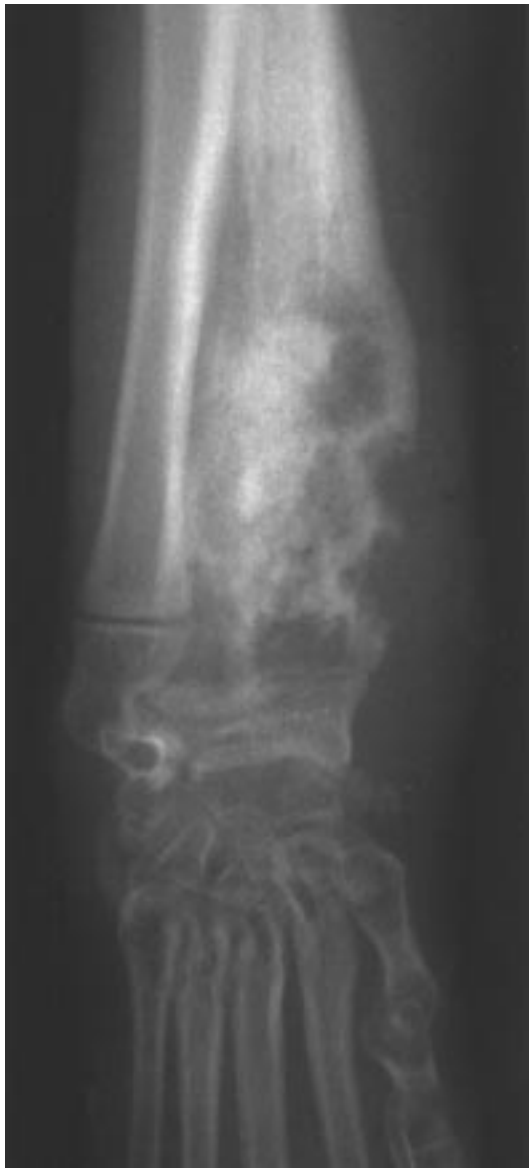

Figure 3. Craniocaudal view of the distal left forelimb 21 days after surgery, demonstrating some uptake of the grafted material but with lytic areas and derangement of distal radial cortices.

days postoperatively but the swelling had subsided by the time of suture removal. The leg was dressed in a support bandage that was changed weekly.

The biopsies showed active suppurative inflammation and necrosis surrounded by proliferating fibroblasts and areas of new bone formation. No bacteria were seen, but one small fragment of hair was seen within an inflammatory focus. Special stains failed to demonstrate any bacteria. Bacteriological cultures were not undertaken. A diagnosis of suppurative metaphyseal osteomyelitis was made on the basis of the radiological and histopathological findings.

Twenty-one days after surgery the leg was swollen in the radial metaphyseal region. A radiograph (Figure 3) demonstrated distal limb osteopenia. The surgical region showed a mixture of lytic and opaque areas, with very thin and disorganised cortices in the metaphyseal region. There was an increased opacity in the area of the grafted material and the diaphyseal periosteum was smoothly thickened in the proximal region of the lesion. The leg was redressed and clindamycin therapy continued.

One month after surgery the leg was normal size. At 6 weeks there was no evidence of pain on palpation of the region and a radiograph (Figure 4) demonstrated good ossification of the grafted material, with a thickened distal radius. The periosteum remained smoothly thickened. At this stage dressings were left off to encourage use of the limb. Approximately 10 weeks after surgery the cat was using the limb normally. There was no pain on palpation of the area of the original lesion. A final radiograph (Figure 5) demonstrated good ossification and the distal radial physis was patent. A trabecular pattern was present in the metaphyseal area and there was an area of lucency within the diaphysis extending distally, consistent with a continuation of the medullary cavity into the distal radial region. There was no evidence of deformity and comparative measurements of the left and right ulnae (olecranon to styloid process) and radii (midpoint proximal to midpoint distal radial physes) were the same. Clindamycin therapy was continued for a further 2 weeks then discontinued.

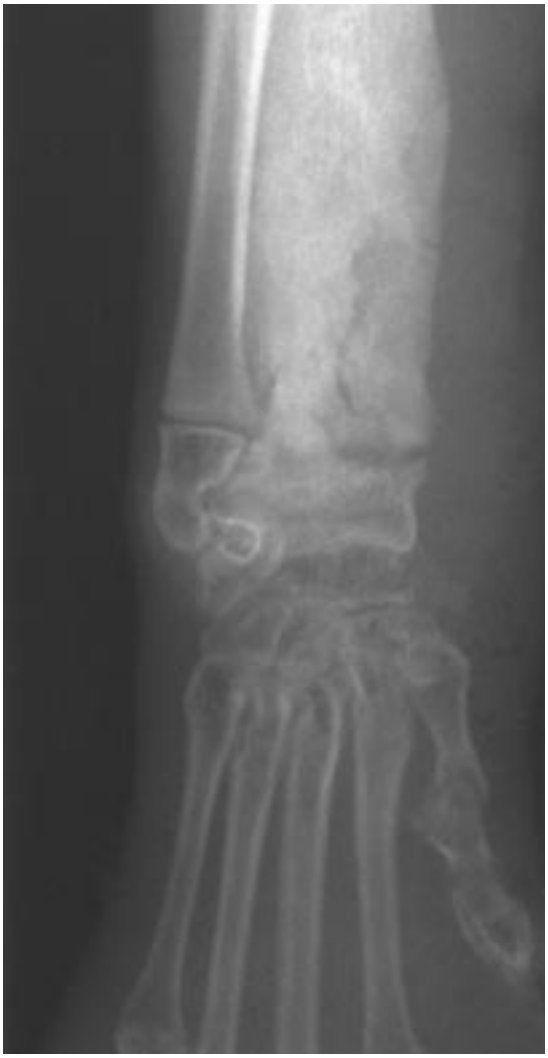

Figure 4. Craniocaudal view of the distal left forelimb 41 days after surgery, showing good uptake of the autogenous bone graft and continuing resolution of osteolysis. Metacarpal osteopenia is noted, probably associated with disuse of the limb.

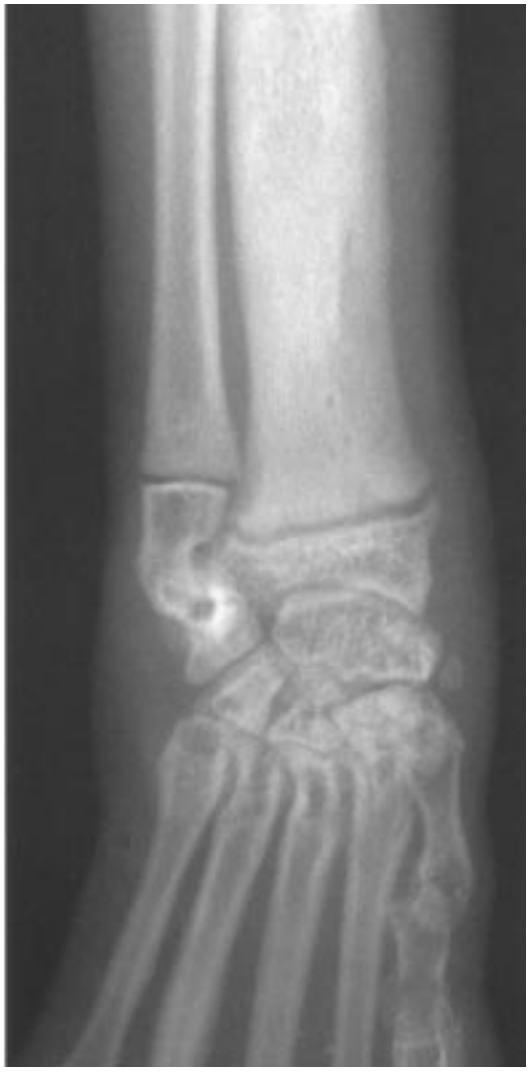

Figure 5. Craniocaudal view of the distal left forelimb 66 days after surgery, demonstrating good resolution of lysis and bone graft uptake.

Discussion

Osteomyelitis is defined as an acute or chronic inflammation of bone and associated soft tissue elements of marrow, endosteum, periosteum and vascular channels, usually caused by bacteria, and rarely by fungi and other microorganisms.[1] It can result from infection secondary to penetrating local trauma or from an haematogenous origin. Adult long bones are relatively resistant to haematogenous infections. There is a greater chance of infection in immature long bones, because haematological embolisation can occur in the microvasculature of growing bone.[2] Gilson and Schwartz[3] discuss the concept of bacteraemia resulting in preferential seeding of the metaphyseal region of immature long bones as a result of the vascular anatomy in young animals and children. They state that while there is a patent physis, metaphyseal vessels form capillary loops that expand into dilated venous sinusoids adjacent to the physis. The large increase in cross-sectional area of vasculature results in decreased local blood pressure and sluggish flow, leading to bacterial deposition. Septic thrombi reduce blood flow, reduce phagocytosis and decrease oxygen tension. These, plus bacterial enzymes, result in tissue necrosis. Once established, infection can spread via the Haversian and Volkmann canals to the periosteum and soft tissues.

Another theory, mentioned by Gibson and Schwartz,[3] suggests that growing capillary buds in the physis lack basement membrane and have actual gaps in their leading edge. This allows the escape of blood elements into the interstitium of the physis. During a bacteraemic phase, micro-organisms could escape into an area relatively inaccessible to phagocytes.

Johnson[4] disputes the initial sluggish blood flow theory as described by Gibson and Schwartz,[3] citing morphological studies which demonstrate that vascular loops connecting capillaries to venules do not exist. Another study is cited by Johnson[4] when considering the concept of vascular abnormality predisposing to the haematogenous seeding of bacteria in the metaphyseal area of young animals. It states that the endothelium of the capillaries invading the terminal hypertrophic chondrocytes (primary spongiosa) is discontinuous and allows the extravasation of erythocytes and bacteria. Lack of blood leukocytes and incompetence of the tissue-based phagocytes then allows the

development of haematogenous osteomyelitis in young animals. Compromise of host tissue defenses by micro-trauma or undefined mechanisms appears necessary.

In the case presented here, an initial diagnosis of a developmental bone cyst was made on the radiological appearance. The surgical procedure undertaken was aimed at harvesting diagnostic material and at effecting a resolution of the problem. Halliwell[5] advocates curettage of bone cysts and packing with autogenous bone grafts. Bone cysts contain a clear to serosanguineous material similar in composition to plasma. The material harvested from the cat described was considered more friable than plasma but showed no gross characteristics of suppuration. Histopathological examination of the material, however, was consistent with suppurative inflammation and antibacterial therapy was indicated. The finding of a small fragment of hair within an inflammatory focus suggested that the disease process may have been secondary to a traumatic episode, such as a cat bite. Alternatively, it may have been a coincidental finding associated with iatrogenic surgical contamination. There was no history or evidence of a traumatic or puncture wound at the initial examination and there was no evidence of scarring consistent with penetrating local trauma at the time of surgery. The presentation of this case was more consistent with an haematogenous infection. The presence of the smoothly thickened periosteal reaction indicated a gradual progression of the inflammatory disease and containment of any infection present.

Bone cysts are rarely seen in dogs[2] and a literature search failed to demonstrate any reports in cats. The radiographic sign of bone cysts is an expansile enlargement of the cortex.[5] A multilocular, sharply defined, intramedullary radiolucent defect can be seen. A short transitional zone occurs and no active periosteal bone or soft tissue proliferation is present. Halliwell[5] describes cysts as generally located towards the proximal or distal ends of a long bone, at or near the physeal plate. Cysts may also be divided by bony trabeculae or fibrous connective tissue.

The radiological appearance of osteomyelitis can be variable. Many signs, including irregular periosteal reactions, cortical lysis, increased medullary densities, soft tissue swelling, as well as involucrum and sequestrum production, are seen.[5] In the case presented here, the main feature was cortical lysis followed by progressive destruction of cortical bone with a mixed radiodensity. Dunn et al[6] described two cases of metaphyseal osteomyelitis, one in a 6-month-old Dalmatian and the other in a 13-week-old Border Collie. Both demonstrated diffuse areas of lysis in the metaphyseal regions of long bones and both demonstrated periosteal reactions, in one case moderately severe and in the other pronounced. Gilson and Schwartz[3] describe a 4-month-old Labrador with bilateral distal femoral metaphyseal osteomyelitis. The main radiological feature was lysis and there was no description of periosteal changes.

Johnson[4] states the cornerstones of osteomyelitis treatment include debridement, sequestrectomy, lavage, open wound drainage and fracture stabilisation (where applicable), administration of appropriate antimicrobial drugs and grafting of bone defects. He advocates that treatment protocols be planned according to individual patients' disease. Ideally culture and sensitivity, both aerobic and anaerobic, should have been undertaken on the case described, despite the absence of bacteria in histopathological specimens. In the two pups described by Dunn et al,[6] bacteria were not isolated from either pup's lesions. Antibacterial therapy was utilised, however, with both pups treated with amoxycillin/clavulinic acid and metronidazole. In the case reported by Gilson and Schwartz,[3] a coagulase negative *Staphylococcus* sp was isolated from corticocancellous biopsies of the left femoral metaphysis, and amoxycillin therapy was undertaken. They state that *Staphylococcus* sp are responsible for 60% to 90% of acute haematogenous osteomyelitis cases in children and speculate that the ability of these bacteria to adhere to the cartilage matrix, and not to vascular linings or erythrocytes, may provide an advantage in colony formation and the initiation of infection. The endothelial gaps in metaphyseal capillary buds, as discussed by Johnson,[4] will expose circulating bacteria to the cartilage matrix of the growing bone. In the case described here, antibacterial therapy was commenced despite the failure to demonstrate bacteria because the radiological and clinical appearance of the lesion was consistent with an acute haematogenous osteomyelitis.

Antibacterial therapy for acute haematogenous osteomyelitis requires therapeutic concentrations of antimicrobial drugs in the bone interstitial fluid space.[7] Despite previously held assumptions that antimicrobial drugs penetrate infected bone poorly, it is considered that osseous capillaries are not a significant barrier and that most antibacterials will readily transverse capillaries and are widely distributed in osseous interstitial fluid.[4,7] Clindamycin, a semi-synthetic derivative of lincomycin, is indicated in osteomyelitis caused by *Staphylococcus sp*[8] and was used in this case. It penetrates bone well and is effective in treating patients with experimentally induced staphylococcal osteomyelitis.[7] It can also be used against *Bacteroides fragilis* and other β-lactamase-positive organisms that are resistant to penicillins and first generation cephalosporins,[7] however many antimicrobial medications may be used.

Gilson and Schwartz[3] state that the prognosis for acute haematogenous osteomyelitis in man is good, with 80 to 100% cure rates provided the disease is diagnosed early and treated effectively. In this case removal of the suppurative material and filling the resulting deficit with an autogenous bone graft, together with the use of an antimicrobial until healing occurred, resulted in a favourable outcome.

Acknowledgment

The author would like to thank Dr Ann Crowley, for the histopathological report in this case.

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The use of botulinum toxin A for treatment of possible essential blepharospasm in a dog

A MEYER-LINDENBERG[a], KM WOHLFARTH[b] and EN SWITZER[a]

A 3-year-old Great Dane with bilateral possible essential blepharospasm resulting in ocular complications is described. Conservative treatment was not successful and the disease was treated with local injections of botulinum toxin A into the orbicularis oculi muscle. Blepharospasm disappeared completely 5 to 6 days after injection and did not reappear until 3 to 4 months later, at which time the injection was repeated. After several treatments over a period of more than 3 years no side effects have occured. Botulinum toxin A appeared to be effective in the treatment of essential blepharospasm in this dog.
Aust Vet J 2003;81:612-614

Blepharospasm is an involuntary contraction of the orbicularis oculi muscle.[1,2] There is a variety of causes of blepharospasm and the degree of severity can vary. In humans, mild, temporary forms occur in response to stress, excitation, exhaustion or bright light. These present as temporary, fibrillary twitching of the eyelids. Another form in humans is essential blepharospasm, an idiopathic disease thought to be caused by a supranuclear lesion.[3] The disease is rare and is classified as an extrapyramidal, focal dystonia.[1] It is characterised by bilateral, symmetric, involuntary, tonic muscle contractions, which later become clonic and can last for varying lengths of time.[4] In extreme cases, essential blepharospasm can cause temporary blindness by persistent closure of the eyelids.[2,4] Blepharospasm can also be secondary to irritation of the eye by foreign bodies, conjunctivitis, keratitis, uveitis, scleritis and also through diplophia and refraction errors.[3]

The neurotoxins of the gram-positive, rod-shaped bacterium Clostridium botulinum can be divided into eight immunologically different types (A, B, C1+C2, D, E, F, G), that have very similar modes of action. The most potent is botulinum toxin A. In humans botulinum toxin A has been the treatment of choice for focal dystonias, such as essential blepharospasm, since the early 1980`s.[1,5] It acts presynaptically at peripheral nerve endings, cleaving the protein SNAP-25 in the membrane of the acetylcholine vesicle.[6] Fusion with the presynaptic membrane is prevented, resulting in blockage of acetylcholine release.[7]

Following injection into muscle, dose-dependent paresis occurs that leads to functional denervation.[8] Through regeneration of the receptors, blockage appears to be reversible and muscle strength is completely recovered after 3 to 4 months.[9]

In the dog, blepharospasm mostly occurs as a result of irritation in connection with entropion, keratitis, conjunctivitis or foreign bodies.[10] The therapy for secondary blepharospasm consists of removal of the primary cause. In cases of primary entropion the treatment of choice is surgical correction.[11] In contrast, primary, idiopathic blepharospasm appears to be rare in dogs, with no reports in the available literature.

[a]Clinic of Small Animals, School of Veterinary Medicine Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany,
E-mail: andrea.meyer-lindenberg@tiho-hannover.de,
[b]Department of Neurology, University of Leipzig, Liebigstr. 22 a, D- 04103 Leipzig, Germany

The purpose of this paper is to report a case of possible essential blepharospasm in a dog and its treatment with botulinum toxin A.

Case report

In January 1998, a 3-year-old male Great Dane was presented at the Clinic of Small Animals, School of Veterinary Medicine, Hannover, with severe bilateral blepharospasm. The dog was fully vaccinated.

The Great Dane had shown a moderate bilateral ectropion since being acquired as a puppy. The blepharospasm had existed since the age of 8 months and was intensified by stress and bright light. Subsequently the dog developed severe hyperaemia of the conjunctiva, which the local veterinarian treated with a variety of ointments and eye drops (details unknown). The condition worsened. The veterinarian removed the nictitating membranes of both eyes, after which the eyes and the general condition of the dog worsened. As a result of permanently closed eyelids, the dog was nearly blind.

Secondary to blepharospasm, both eyes developed spastic entropion, which was worse in the right eye and led to severe corneal opacity. Conservative therapy continued to have no effect. One veterinarian performed a tarsorraphy on the right eye because of severe corneal ulceration and secondary oedema, however, the blepharospasm remained.

On presentation to our clinic, there was severe bilateral blepharospasm and the eyes were not visible (Figure 1a). The left eye showed enophthalmos as well as entropion of the lower lid. Manual separation of the eyelids revealed a severely hyperaemic conjunctiva with mucous secretion. At the base of the excised nictitating membrane, scar formation was visible. Entropion had resulted in superficial corneal ulceration with early superficial vascularisation, and anterior uveitis. The Schirmer-Tear-Test measured 25 mm after 1 minute.

Examination of the right eye necessitated loosening of the tarsorraphy. The eye was tightly closed because of blepharospasm. Separation of the eyelids revealed severe hyperaemia of the conjunctiva and severe corneal oedema with superficial and deep vascularisation. (Figure 1b). The Schirmer-Tear-Test measured 20 mm after 30 seconds. The fluorescein test showed a slight, diffuse colouration, which was chiefly central. No other parts of the eye could be viewed because of corneal opacity, but ultrasound examination showed no change in intraocular structures. Application of a topical anaesthetic did not relieve the blepharospasm. Electromyography (Viking^{TM}, Nicolet, Kleinostheim, Germany) of the orbicularis oculi muscle showed that complex high frequency discharges were present bilaterally. No electromyographic abnormalities were seen in other muscles.

The dog was hospitalised and anaesthetised. A 360° bulbar conjunctival graft was performed on the right eye and left in place for 3 weeks. To eliminate irritation by hair, the lower lids of both eyes, which mostly showed an entropion in the temporal area, were temporarily corrected via eyelid tacking

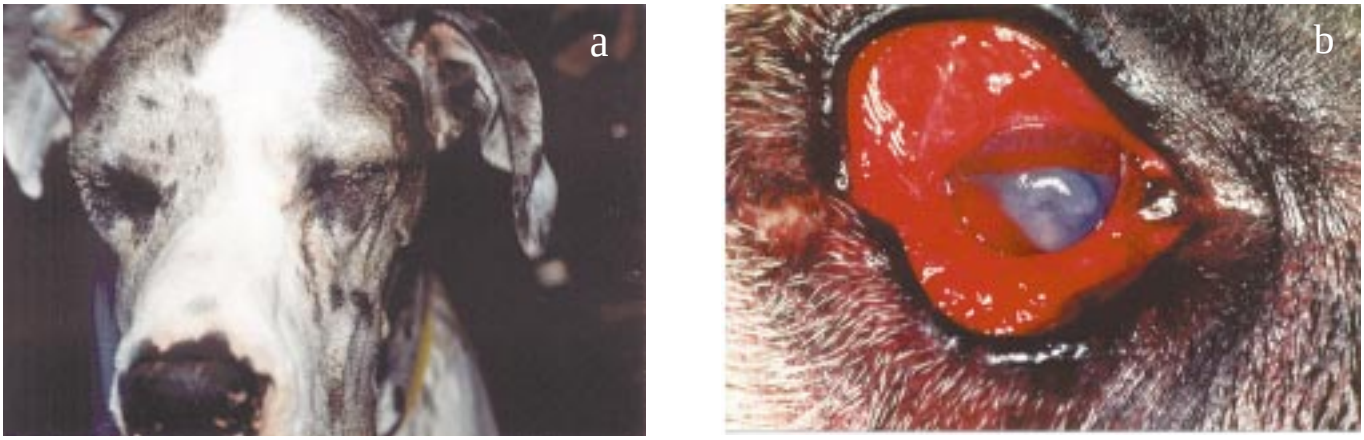

Figure 1. Photograph of a 3-year-old Great Dane on presentation. Both eyes showed severe blepharospasm and the eyes were not visible (a). After pulling the eyelids apart, the right eye (b) showed a severely hyperaemic conjunctiva and a severe oedema of the cornea with vascularisation, especially ventrally and dorsally. There was no nictitating membrane.

Figure 2. The same dog at presentation 14 days following local therapy with botulinum toxin A. The spasms had disappeared completely from both eyes. The position of the eyelids normalised despite bilateral ectropion.

with vertical mattress sutures. Topical therapy consisted of gentamicin ointment four times daily and atropine twice daily. The left eye was also treated with vitamin A ointment. As systemic therapy the dog received oral amoxicillin with clavulanic acid (12,5 mg/kg) and prednisolone (2 mg/kg) twice daily each. The dosage of the latter was gradually reduced. Despite therapy blepharospasm did not improve. Tetrazepam (Musarilä, Sanofi Winthrop GmbH, München, Germany) was given for several days without success. The outcome was the same for haloperidol (Haloperidol-ratiopharm®, Ratiopharm GmbH, Blaubeuren, Germany). Only treatment with primidone (Mylepsinum®, ICI-Pharma, Plankstadt, Germany) lead to slight, though unsatisfactory, improvement.

The owners consented to local therapy with botulinum toxin A (Dysport®, Ipsen Pharma, Ettlingen, Germany). The area around the eye was disinfected with iodine solution. Equal doses (33.3 MU) of botulinum toxin A were injected subcutaneously with a 25 gauge needle at six sites per eye in the area of the orbicularis oculi muscle. A total of 200 mouse units (MU, 2 mL botulinum toxin A) was used per eye. One MU is equivalent to the LD50 for mice of a given weight.[12] The dog was physically restrained, but not sedated, during treatment.

Three days after the injection, improvement of blepharospasm was evident, and the tacking of the lower eyelid was removed. After 6 days, the owners reported that the spasms had disappeared completely from both eyes. Gradually, enophthalmos diminished, and the position of the eyelids normalised, despite ectropion of the lower lids (Figure 2). The dogs general condition was good, and no further medication was applied. After about 1 month, another 100 MU at six sites per eye were injected because slight blepharospasm had returned. Each injection contained 16.6 MU. After this injection, the effect lasted for 3 months. Ectropion was corrected surgically with the modified Khunt-Szymanowski technique, after which the position of the eyelids was nearly normal, but blepharospasm continued to recur. The dog returned for further injections of botulinum toxin A, although the intervals between the injections became slightly longer (4 months). After more than 3 years of regular injections with botulinum toxin A no adverse effects, such as ptosis, conjunctivitis or changes in tearflow, have been detected.

Discussion

Essential blepharospasm as a form of an extrapyramidal focal dystonia is a relatively rare condition in man.[1] This appears to be true for dogs, as no reports of it exist in the available literature. The dog in this report appeared to have essential, primary blepharospasm, but the question remains whether it was a form of focal dystonia. The bilateral nature of the blepharospasm and the presence of myotonic discharges in the orbicularis oculi muscle supports this thesis, because in man it is characterised by bilateral, symmetric, involuntary, tonic muscle contractions.[4] In man, essential blepharospasm can cause temporary blindness through persistent closure of the eyelids.[2,4] The dog of the present case was also nearly blind because of severe blepharospasm. Other possible causes for blepharospasm were the presence of ectropion or the resected third eyelids. Correction of the ectropion did not resolve the blepharospasm, so this could be excluded as an underlying cause. There was no evidence of other possible causes of secondary blepharospasm, such as a foreign body or primary entropion.

Surgical treatment (for example neurectomy, myectomy) is not recommended for essential blepharospasm in humans.[1] Conservative therapy with tranquillisers and antiepileptics has also produced unsatisfactory results.[1,4] This was confirmed for

the dog in the present study, in which these drugs only led to slight improvement.

The treatment of choice for essential blepharospasm in humans is local application of botulinum toxin A.[1,5,13] Because of the favorable results in humans and the lack of improvement with conservative therapy, botulinum toxin A was chosen for treatment in the present case.

The therapeutic use of botulinum toxin A in the dog has not been described previously. Its toxic effects in this species have been described,[14] and the effects of botulinum toxin A on the voluntary nervous system, as well as on the autonomic nervous system in the dog, have been shown in several experimental studies.[15,16]

The onset and duration of effects in the present case were similar to those reported for human patients[9] - improvement of blepharospasm was first seen after 3 days, and the injections had to be repeated every 3 to 4 months because of recurring clinical signs.

The first dose of 200 MU per eye was higher than the average initial dose of DysportÔ (Botulinum toxin A, Ipsen Pharma, Etlingen, Germany) for humans (120 MU per eye) because it was feared that dogs might be less responsive to the toxin due to their natural resistance to botulism.[12,14] No adverse effects, such as ptosis, conjunctivitis or changes in tearflow were noticed in this case. These have occasionally been reported in humans.[13]

In conclusion we can state that although this is a single case report, and further studies with a larger number of patients will be necessary to verify our results, we have shown that botulinum toxin A has potential as an effective and safe therapy in essential blepharospasm of the dog. In addition, botulinum toxin A may be useful in the treatment of other conditions where temporary paralysis of specific muscles is required.

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BOOK REVIEW

***External Fixation in Small Animal Practice**. Kraus KH, Toombs JP and Ness MG. Blackwell Publishing, Oxford, 2003, 240 pages. Price £45.50. ISBN 0 632 05989 3.*

This practical publication details the applications of external fixation in small animal fracture management. The book is divided into two parts. Part One provides clinically useful information that is consolidated in Part Two with 130 pages of case examples.

Part One is constructed to reflect the clinical presentation and management of bone fracture in small animal practice. The basic principles of external fixation and important aspects of decision-making precedes a practical description of preoperatove care, fracture reduction, pin placement, the use of three second-generation fixation systems, the evaluation of postoperative radiographs, after-care, follow-up examinations and complications.

Part Two consists of numerous case examples. For each fracture, the history and fracture type is presented, along with surgical planning, the fracture repair, and subsequent follow-up examinations. All cases are supported by serial radiographs documenting any complications and subsequent fracture healing.

The aim of the book is to impart the authors' considerable clinical experience. It is more a practical guide than a definitive text. There are no references in the text and only a limited index. The book is most applicable to the small animal practice setting. The publication will help a new graduate avoid the early frustrations of external fixation, and the more experienced surgeon to integrate recent design improvements of fixation systems into everyday practice.

Multiple, high-quality, black and white photographs and radiographs accompany the text. The information is delivered in logical order that is easy to find. This publication would be a useful addition to any veterinary practice that is planning to use or currently uses external fixation in small animal fracture management.

SR Snelling

Dr Snelling is a Small Animal Surgical Registrar at the University of Melbourne's Veterinary Clinic and Hospital, Werribee, Victoria.

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REVIEW

Science and its application in assessing the welfare of laying hens in the egg industry

JL BARNETT[a] and PH HEMSWORTH[b]
Animal Welfare Centre, Animal Science Division, 600 Sneydes Road, Werribee, Victoria 3030

Objective To provide a rational framework for the scientific assessment of welfare and to use this framework to assess the welfare implications of issues relevant to the Australian egg industry.

Procedure A well-accepted approach to the assessment of animal welfare, based on assessing how well the animal is adapting, is described. This approach is used to consider the welfare implications of issues such as space, perches, nest boxes, dust baths, abrasive strips and non-cage housing systems.

Conclusions The role of science in the welfare debate is to provide biological facts and thus it is important to separate welfare and ethics. The welfare of an animal in response to a housing system or husbandry procedure can be assessed by evaluating how much has to be done by the animal in order to cope and the extent to which the coping attempts are succeeding. Using this approach there is evidence for improved welfare from increasing space in cages, based on reduced aggression, corticosterone concentrations and mortalities and increased production, and for incorporating perches, based on the reduction in injuries at depopulation. Similar evidence for the inclusion of dust baths and nest boxes is lacking. The data on abrasive strips are equivocal with recommendations from overseas for their inclusion, whereas some local data have shown an increase in mortality can occur. Similarly, the data on non-cage systems are equivocal. The data on bone strength suggest improved fitness in non-cage systems, the data on stress suggest fitness may be better, similar or worse in non-cage systems, and the limited data on immunology suggest fitness may be worse in non-cage systems than in conventional cages.
Aust Vet J 2003;81:615-624

There have been ongoing discussions by the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ; now called the Primary Industries Ministerial Council - PIMC) on options for a national approach to layer hen housing systems in Australia. A meeting of State Agriculture Ministers, in August 2000 resulted in a number of resolutions that have implications for housing of poultry. These included that all new cage systems must provide a floor space of 550 cm^2/hen, including the baffle, by 1 January 2001; that all cage systems that do not meet the 1995 standards are to be scrapped by 1 January 2008; research and development, based on furnished cages that include perches, nests, litter and abrasive strips, and non-cage alternatives such as barn and free-range be conducted in Australia by 2005, with the expectation that if the research is successful industry will implement such system(s). The 1995 standards for cages include a space allowance of 450 cm^2/bird, a slope on the floor of less than 8 degrees, a minimum of 40 cm height over 65% of the cage floor area and more than 35 cm at all points, and cage fronts that are full height and width. In addition to resolutions on cage dimensions, the Code of Practice is being revised and this may result in additional changes. To help put some of these resolutions in perspective this paper examines some of the literature on space and cage modifications and non-cage housing systems within the broader framework of the scientific assessment of welfare.

[a]Department of Primary Industries
[b]University of Melbourne, Institute of Land and Food Resources

The assessment of animal welfare

Our value systems for animals tend to operate at two levels.[1] Private attitudes, which may be difficult to enunciate, may be ascribed to belief, intuition, learning or experience. Communal value systems, which may arise from family, culture or some other social belief, are rarely upheld unanimously in the population, but may be supported by a sizeable proportion of it (for example, bull-fighting, particular practice of animal slaughter, and so on) and perhaps be reinforced by legislative control or codes of practice.

Some have argued that science and ethics cannot be separated in any discussion of animal welfare. For example, some authors have used the term 'animal welfare' to refer to an animal's quality of life.[2] Furthermore, they consider that our conception of animal welfare involves values as well as information, and others consider that a conventional definition of animal welfare does little more than establish the general area of discourse.[3] The widespread variation in science, philosophy and the general community in the definition of animal welfare has created considerable confusion and controversy that has hindered attempts to study animal welfare. Without a clear definition, welfare cannot be studied because it cannot be measured either directly or indirectly.

We believe that the most credible scientific definition of animal welfare is the following one: "The welfare of an individual is its state as regards its attempts to cope with its environment".[4] Using this definition, welfare risks can be assessed in terms of firstly, how much has to be done by the animal in order to cope with the environmental imposition and secondly, the extent to which the animal's coping attempts are succeeding. The rationale for this definition is considered in more detail later.

Therefore, with a satisfactory scientific definition of welfare, and this is presently open to considerable discussion within science, it should be the aim of science to provide an objective assessment of the welfare risks associated with the practice under question. Science therefore should aim to provide the facts on how well animals adapt to a housing or husbandry practice (that is, the welfare risks). Such information, together with the individual's value system, assists the individual in deciding whether or not a particular practice that imposes on an animal's welfare is acceptable (that is, whether or not the welfare risks are acceptable).

There is an ongoing debate about the role of science in the welfare debate and whether welfare involves both facts and ethics.[5] We suggest that an individual's opinion of an animal welfare issue is influenced by their values (for example, moral duties to the animals) together with their knowledge of how the issue in question imposes on the animals. Science is a descriptive discipline aimed at explanation and then prediction. Using science we try to discover and articulate natural laws and regularities that govern the behaviour and relationships of objects in the natural world.[6] Thus the role of science in addressing the welfare of domestic animals is to generate facts on how animals respond to the practice/conditions under question, while individuals will make value judgements to determine whether or not the practice is acceptable to them. A continuing difficulty confronting scientists studying animal welfare has been the definition of animal welfare. Although some may not agree, a solution to this impasse is that the role of science is to provide facts on how well animals cope with their environment. Such a consideration includes the issues of emotions, natural behaviours in natural settings and preferences. The issue of whether or not animals require environments that provide more than those that address their 'basic' biological requirements (for example, pleasure) is another level of discussion that needs to occur. Nevertheless, if we can develop a consensus that those conditions that create biological dysfunction are the most serious for animals, then we can probably reach some agreement that it is these issues that are the most important to be promptly addressed. Notwithstanding the possible conservative nature of this approach, it will allow significant progress to be made in improving animal welfare.

There are five broad approaches used by scientists in studying animal welfare:

- the 'feelings-based' approach,
- the 'animal-choices' approach,
- the 'nature of the species' approach,
- the ' freedoms for animals' approach, and
- the 'functioning-based' approach or the 'homeostasis-based' approach.

These five approaches will be briefly considered here.

Feelings

This approach defines animal welfare in terms of emotions and thus it emphasises reductions in negative emotions such as pain and fear, and increases in positive emotions such as comfort and pleasure.[2]

The modern notion of emotions in both the animal behaviour and psychology literature highlights the linkage between visceral or bodily arousal and cognitive processes.[7,8] Any discrepancy or any interruption of expectations or of intended actions, produces undifferentiated visceral (autonomic) arousal and the associated sensation of the emotion, whether positive or negative, depends on the cognitive evaluation of this discrepancy or conflict between the state of the world and the expectations of the individual. While it is accepted that humans have a great variety of emotions, animal behaviourists generally consider that animals are restricted to a few basic emotions such as anger, fear, joy and happiness. This is predicated on the view that animals probably only have emotions to deal with certain kinds of survival problems, for which there is some strong evolutionary benefit. For example, while we might expect animals to show fear because of the adaptive value of being frightened in a dangerous situation, there is no reason to expect animals for example, to show pity to other species because there would be no clear adaptive advantage if they did.[7]

The difficulties in studying emotions as though they were objective states of bodily arousal is well recognised in the literature.[9] Whereas each emotion may reflect a different pattern of arousal, the visceral response to many emotions is reasonably uniform in animals. Most animals react physiologically in essentially the same way whether the arousal is sexual, fear provoking or if there is the anticipation of play or food. It is obviously a major challenge to study and understand emotions in animals, however there are some examples in the literature that indicate that it is possible to assess the strength of emotions in animals in intuitively negative and positive emotionally arousing situations. Behavioural and physiological correlates of fear of humans by pigs demonstrate our ability to quantify the level of fear towards a specific stimulus in pigs.[10] Some of the motor patterns and neural changes presumably associated with emotions in humans and animals appear to be highly specific.

Brain lesion studies and studies involving electrical stimulation of the brain indicate that particular neural circuits such as components of the limbic system appear to mediate or control emotions.[11] For example, rats learn to press a lever when the reward (reinforcement) was a brief burst of electrical stimulation of the septal area of the limbic system. Such studies indicate the potential to associate positive and negative emotions with specific behavioural and neural changes. Defining emotions to further develop the feelings-based assessment of welfare is likely to occur in the next 10 years and will provide a major contribution to the welfare debate.

Animal choices or preferences

Animals have functional systems controlling, for example, body temperature, nutritional state and social interactions. By investigating these functional systems and the associated motivational mechanisms, there is opportunity to identify the resources or stimuli in the environment that are required by or are important to animals, and thereby learn something about an animal's needs.[1] Some of these motivational systems can be regulated by physiological consequences (such as consumption of food), whereas others require the display of a particular behaviour (such as rooting behaviour in pigs). Driven by the view that animal choices may indicate the existence of important underlying needs, there has been and continues to be considerable interest in studying the preferences of animals for resources, such as space, flooring and a parturition or nest site. The preferences of animals for resources can be studied by allowing the animals to choose between resources with preference being measured by either the time the animal spends with the resources or the resource that is selected. The simplest preference study involves allowing the animal to make a choice between two situations in which the resource is varied. For example, it was found that laying hens preferred a spacious cage to a confined cage and that neither time of day nor strain of bird was influential in this choice.[12] Observing animals in complex environments that provide a range of activities will also provide details of the animal's preference for habitats and resources.[1]

In an attempt to measure the strength of an animal's choice, scientists have incorporated tasks in which the animal has to expend energy or take risks in gaining access to an alternative resource. For example, operant conditioning techniques, in which an animal learns to perform a response, such as lever pressing, to gain access to an alternative resource, have been used to measure the value that the animal puts on the resource. Pre-parturient sows worked harder on the basis of lever lifting to gain food than access to straw.[13] Consumer demand theory has been used with preference testing to put a value on the animal's choice:[14] The strength of motivation ('need') for a resource can be measured through the animal's willingness to consume ('work' for) the resource as the 'price' of the resource increases. Thus, by measuring consumption at increasing prices, needs can be classified as necessities where the animal works harder to maintain consumption (called an inelastic demand function) or luxuries where the animal does not maintain consumption by working harder (elastic demand function). Using this approach needs can be ranked in terms of their demand functions.

Preference or choice testing has been criticised on several grounds and further research, not only on methodological issues, but also on understanding the principles underpinning the animal's decision is required.[15] For example, one of the most serious challenges to this approach is that an animal's short term choice may reflect its proximate (immediate) needs, which are likely to vary markedly over time, rather than the animal's ultimate needs or those necessary for survival, growth and reproduction.[15] For example, since an animal's choice between feed and space will be markedly affected by short term changes in hunger, the choice of space is more likely immediately after feeding rather than before.

In attempting to determine what animals need it is worthwhile to consider a need as being indicative of having a deficiency, often manifested as a homeostatic maladjustment. A need can therefore be defined as a requirement, which is fundamental in the biology of an animal, to obtain a particular resource or respond to a particular environmental or bodily stimulus.[1] Hence, some needs are for food, water or heat, but others are for a certain behaviour such as grooming, exercising or nest building to occur. When an animal has an unsatisfied need, its motivational state will usually elicit behavioural and physiological responses that remedy that need, so the individual will be able to cope with its environment. If a need cannot be satisfied, the consequence in either the short term or the long term will be poor welfare.

A problem associated with the use of the word 'need', especially in legislation, is that the deficiencies involved range from the rapidly life-threatening to those that are relatively harmless in the short term. Most of what is strongly avoided is harmful and most of what is strongly preferred is beneficial. However, some of what is wished for is not necessary, in the sense of essential for life, so the references to 'fundamental to the biology of the animal' and to 'deficiency' in the definition and understanding of 'need' are valuable.

Nature of the species

The principle underlying this approach is that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways.[2] This approach is reflected in the much quoted proposal that has been incorporated into the 'five freedoms' requirement for animals: animals should have the 'freedom to perform natural behaviour'.[16] However, of all the approaches to assess welfare, the nature of the species approach has least scientific credibility because it fails to define both 'natural' and the welfare risks if such 'natural' conditions are not provided. Until these attributes are rigorously defined for both the 'nature' approach to welfare assessment and elements of the five freedoms, such approaches may be used to reflect an ethical position but are not open to scientific scrutiny.

The view that animals should perform their full 'repertoire' of behaviour was common in early welfare research, but there are a number of shortcomings as a criterion for animal welfare.[17] 'Wild' behaviour often represents an animal's efforts to survive in a life and death struggle and therefore many of these responses are adaptations to cope with extreme adverse situations. Such situations clearly reduce animal welfare and are thus situations from which domestic animals should be spared. Furthermore, mortality is generally higher in wild populations than domestic ones. For example, piglet mortality in the wild boar (*Sus scrofa*) often exceeds 25%,[18] a situation that clearly would be unacceptable in commercially raised pigs. Thus the 'natural behaviours' that are desirable or undesirable in terms of animal welfare require definition together with the rationale for their inclusion or exclusion. To date there are no such agreed definitions or rationales.

The notion that we can improve animal welfare by respecting the 'nature' of animals is intuitively appealing.[2] However modern domestic animals are the product of thousands of generations of selective breeding and consequently the behaviour and physiology of domestic animals have been modified during domestication.[19] While the behaviour of domestic animals in wild or semi-wild conditions is often similar to their wild relatives, there are differences in the behavioural responses and thresholds to a number of stimuli such as sexual stimuli, novel stimuli, humans and environmental conditions.[1,20]

The Five Freedoms

The starting point for the Five Freedoms was the UK Report of the Brambell Committee,[21] which concluded, amongst other things, that all intensively housed animals should be provided with sufficient space to be able to stand up, lie down, turn around, groom themselves and stretch. With developments over subsequent years, such behavioural requirements became known as the Five Freedoms. The UK Farm Animal Welfare Council proposed in 1992 that the welfare of animals can be protected by recognising the Five Freedoms:[22]

- Freedom from hunger and thirst,
- Freedom from discomfort,
- Freedom from pain, injury and disease,
- Freedom to express normal behaviour,
- Freedom from fear and distress.

While most would agree with the ethical basis of this general approach, it requires a number of definitions, which are often not provided by proponents. For example, freedoms 2 and 4 clearly require definition. Discomfort, particularly in relation to consequences for welfare, requires definition, and as discussed in the previous section on the 'nature of the species' approach, the normal behaviours that are both desirable and undesirable, from a welfare perspective, require definition. Similarly, the levels of hunger, fear and stress that may impinge on animal welfare require clarification. Until these definitions and clarifications are made and widely agreed, as indicated in the previous section, the five freedoms approach to welfare assessment may be used to reflect an ethical position, but is not open to scientific scrutiny.

Homeostasis

The definition of animal welfare that underpins this approach is "The welfare of an individual is its state as regards its attempts to cope with its environment".[4] In this definition, the "state as regards attempts to cope" refers to both how much has to be done by the animal in order to cope with the environment and the extent to which the animal's coping attempts are succeeding. Attempts to cope include the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses. The extent to which coping attempts are succeeding refers to the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, health and freedom from injury. Therefore, using such a definition, the risks to the welfare of an animal by an environmental challenge can be assessed at two levels: firstly the magnitude of the behavioural and physiological responses and secondly the biological or fitness costs of these responses.[1,23,24]

A subtle but important component of this approach, therefore, is that welfare is considered within the concept of biological fitness.[1,25,26] This concept of biological fitness generally applies to natural populations and refers to 'fitter' animals having a greater genetic contribution to subsequent generations;[27] this is based on their abilities to successfully survive, grow and reproduce. While the last attribute may not always apply to individual farm animals since reproduction is either controlled or absent for many farm animals, the ability to grow, survive and reproduce could be considered measurements of 'fitness' within the limits of the management system. Most production systems in agriculture have breeding and growing components and these can generate considerable data on reproductive success of individuals. For example, conception rates and mortality, morbidity and growth of offspring can be used as a measure of 'fitness'. Similarly, reproductive performance of domestic species has been linked with welfare.[28,29]

An attribute of the 'homeostasis' approach that affords this approach credibility within scientific circles is that it contains some widely accepted criteria of poor welfare. Furthermore, there are some excellent examples of the value of this 'homeostasis' approach in assessing animal welfare.[24] For example, handling studies on both young and adult pigs have shown that fearful pigs have a sustained elevation of plasma free corticosteroid concentrations; the consequences of this chronic stress response in these fearful animals include depressions in growth and reproductive performance.[30-32]

A counter argument is that this example involves extreme effects and our current knowledge may not allow detection of more subtle or less serious risks to welfare. The example of fearful pigs clearly demonstrates the consequences of animals failing to cope with an environmental change: such biological changes and biological costs for the animal clearly enable the interpretation with some considerable degree of confidence that the welfare of these animals is seriously compromised. Nevertheless, less serious challenges should be reflected in biological changes, admittedly of lower magnitude, with consequent effects on fitness variables such as growth, reproduction, injury and health. Short term challenges can also be studied with this approach. The behavioural and physiological responses of cattle to two branding procedures were studied to assess the relative aversiveness of the procedures,[33] while behavioural and physiological responses together with growth performance were utilised to assess the welfare implications of a husbandry procedure regularly imposed (daily injections) on pigs.[25]

Repetitive and stereotyped behaviours are part of the biological response of animals to a long term challenge and it is appropriate to consider stereotypies within the homeostasis approach to welfare assessment. Stereotypic behaviour can be defined as those behaviours that consist of morphologically identical movements that are regularly repeated, have no obvious function, or are unusual in the context of their performance.[34] Examples of these behaviours are chain pulling, bar biting, sham chewing, head weaving and excessive drinking.

There has and continues to be considerable controversy on the causation and function of stereotypies in farm animals. A brief review of some examples from the literature demonstrates this controversy. Excessive chain manipulation by sows is a stereotypy seen in gestating sows housed on tethers and it has been shown that food restriction contributes to the development of this stereotypy.[35] The authors have postulated that the appetitive behaviour of foraging may persist and develop into a stereotypy in these sows because these appetitive sequences are

positively reinforcing and there is also insufficient negative feedback from the consummatory behaviour (feeding) and its functional components (food). Unavoidable fear or stress and barren and restrictive environments have also been implicated in the development of other stereotypies. Examples are body-rocking in mentally handicapped patients when distressed and where the occurrence of stereotypies increases with increasing confinement.[36] It has also been proposed that some forms of stereotypies reduce responses to aversion by affecting the animal's perception of the situation.[37] Thus it is clear that different forms of stereotypies may have different causes, such as frustration, stress and lack of control and stimulation, however our understanding of the motivational basis of stereotypies is poor.

A similar controversy exists in relation to the function of stereotypies. Based on early evidence of associations between stereotypies and physiological signs of coping such as reduced corticosteroid concentrations, reduced adrenal gland weights and reduced ulceration, there is a view that stereotypies may be a coping response. However more recent studies and re-interpretation of some of the early evidence, questions this general coping hypothesis for at least some forms of stereotypic behaviours.[36,38] Furthermore, whereas some evidence exists to indicate that stereotypies may be coping mechanisms in the short term, it is unknown whether they exert benefits in the long term. Irrespective of the function of stereotypies, the existence of a stereotypy is indicative at the least of a past problem for the animal in coping with its conditions. Stereotypies that result in physical damage to or illness in the animal (such as the development of lesions in stall-housed sows that persistently rub their tail roots from side to side against stall fittings or wind-sucking in horses where persistent wind-sucking can lead to colic) have obvious and immediate implications for the welfare of farm animals. Thus stereotypies should not be used alone to assess risks to animal welfare: they should be used together with other biological responses and consequent effects on biological fitness.

There are two contentious issues that we recognise in using the homeostasis approach to scientifically assess animal welfare. One involves the definition of animal welfare and the other is whether the homeostasis approach for the assessment of animal welfare adequately includes feelings. The homeostasis approach utilises the definition of animal welfare proposed by Broom[4] and is based on the premise that maladaptation generates animal welfare problems. Adaptation is considered, at the individual animal level, to involve behavioural and physiological responses that assist the individual to cope with its environmental conditions.[1] There may be some disagreement within science on the appropriateness of this definition. For example, some argue that animal welfare only concerns animal feelings.[39] Nevertheless, as discussed earlier, we and others believe that Broom's definition is more widely accepted both within science and by the wider community. Without a consensus on a definition of animal welfare there can be no consensus on the scientific assessment of animal welfare. In relation to the issue of feelings, Broom has emphasised the evolutionary advantage of feelings.[40] As others have proposed,[40,41] we believe that feelings are part of the body's regulatory system and function to either remove animals from harmful situations or attract animals to beneficial situations.

Another criticism of the homeostasis approach is that it is conservative and, because of methodological limitations, a lack of difference using this approach may not mean that welfare is unaffected. Nevertheless, its conservatism should be seen as a strength, because, if differences are found, they are likely to be of importance to the animal. This approach has been successfully used to demonstrate changes in welfare due to physical and social environments and in situations that affect an animal emotion, namely fear.[24] There is a wide acceptance of this approach when it demonstrates risks to welfare, for example, due to overcrowding or chronic fear responses. However, the lack of responses, particularly in situations to which humans have some degree of antipathy, such as confinement housing systems, is often seen as indicative of inadequate methodology. That is, if the data do not align with some individuals' perceptions, common responses are to criticise the methodology or change the definition of welfare. There is no argument that better methodology is required. However, it is not logical to accept or deny the same methodology on the basis of its fit with human perceptions. Similarly, changing the definition of welfare from something that can both be defined and measured, to include words such as 'suffering' or 'ethical values' that cannot be assessed using the scientific method, because of a lack of definition, cannot be seen as a forward step in the welfare debate. Indeed, at the present state of knowledge, to attempt to measure suffering as an entity could be considered a misdirection of limited resources, both in terms of monies and in terms of improving animal welfare. It could lead to similar open-ended arguments that occur in the definition of welfare and could provide yet another excuse for making only limited gains for animals' welfare. Peter Medawar suggests "there is no limit upon the ability of science to answer the kind of questions that science can answer".[42] The question of animal suffering and ethical values are holistic entities not open to scientific investigation.

Conclusions on welfare assessment

With our present knowledge, the 'homeostasis' approach appears to offer science the best assessment of the welfare of animals. As a research tool, this approach involves comparing housing systems or husbandry procedures and risks to welfare are assessed on the basis of relative changes in biological (behavioural and physiological) responses and corresponding decreases in fitness. Assessing motivation using preference testing has the potential to measure the animal's important underlying needs, and thus provides a valuable addition to the homeostasis approach in studying animal welfare.

In the future, there are obvious opportunities to integrate the 'feelings' approach within the 'homeostasis' approach. If we accept that emotions in animals are important adaptive responses that assist survival, it is an easy step to recognise that the visceral or bodily arousal, the cognitive processes and the associated sensation of the emotion are part of the animal's biological response to the challenge. Indeed, emotions may have some adaptive advantage such as acting as a reinforcer,[40] which makes it more likely that the individual will learn to carry out the adaptive action. Further indication of the adaptive function of emotions is that they can modulate memory formation in several ways.[43] Studies principally on laboratory rodents have shown that a fear-provoking stressor, presumably via its effects on hormones in the sympathetic-adrenomedullary axis and hypothalamic-pituitary-adrenal axis, may play an important role in memory formation and recall.[44] Some of these effects can be viewed as having adaptive value in helping the animal to search, scrutinise and remember threatening stimuli or situations.

Along similar lines, it has been proposed that feelings or emotions are involved in monitoring the effectiveness of regulatory actions, being positive when the regulation is successful (homeostasis is achieved) and negative when it is not.[45] Similarly an emotion, such as pleasure or anxiety, has been considered as a functional state of the animal induced by specific signals which rapidly organise response systems (approach or avoidance) relevant to broad categories of relevant stimuli.[46] Interestingly, this general view has been extended by suggesting that the animal's tolerance or sensitivity to rewarding and aversive stimuli may be closely related to the state of the animal in terms of welfare.[46] In fact, the authors have proposed that, together with neurobiological knowledge, an increased insight into the welfare of the animal can be gained by measuring the anticipatory behaviour of the animal for rewards in a Pavlovian conditioning paradigm: animals deprived of essential stimuli react more readily not only to stimuli that they are deprived of but also rewarding and aversive stimuli in general. Such philosophical discussions accompanied by experimental validation will assist in further developing the concept of welfare. These attempts to conceptualise animal welfare will lead to further development and refinement of the methodology to study animal welfare. This limited discussion on integrating the two research approaches, the feelings and homeostasis approaches, demonstrates not only how the concept of welfare has and will continue to develop, but that increased agreement amongst scientists on the concept of welfare will lead to greater consensus on ways to study animal welfare. There is a wide acceptance of the scientific method in problem solving and its ability to contribute to our understanding of the factors that contribute to welfare. It would be unfortunate, in relation to improving animal welfare, if agreement cannot be reached on a single definition of animal welfare. There would appear to be no benefits in having a scientific definition and another that includes aspects that cannot be resolved by the scientific method. Public perceptions are not ignored in the welfare debate. They are a quite rightly a significant driver in raising questions, but from a scientific perspective they are not part of the answer. With our present knowledge, the most scientifically credible approach to welfare assessment involves measuring the magnitude of the biological responses to the challenge and also the consequences of these behavioural and physiological responses on the animal's ability to grow, reproduce and remain healthy. Information on the animal's preferences for resources should provide valuable information complementing this approach. This is the approach that is utilised in this review.

Implications for poultry

There has been considerable research in recent years on developing furnished cages or modifying conventional cages as a replacement for conventional cages.[47-53] Of particular interest has been the incorporation of perches (to improve bone strength), solid sides (to improve feather condition), abrasive strips (to reduce claw length and subsequent injuries), nest boxes (to provide for nesting behaviour) and sand-baths (to provide for dust-bathing behaviour).

Space allowance in cages

The literature on the effects of space allowance indicates that in general, within a range of 300 to 650 cm^2 per caged laying hen, increasing the area per bird increased egg production, food consumption and weight gain and decreased mortality.[54-56] An obviously important explanation is the reduced feeding space that is generally associated with an area reduction in cages of generally constant depth.[55] Another explanation is that crowding may lead to increased corticosterone concentrations, which in turn may adversely affect both production efficiency and health. An 11% increase in plasma corticosterone concentrations in caged hens was reported when space allowance was decreased from 460 to 350 cm^2 per bird.[57] However, it has been shown that birds would not consistently work for additional space.[58] While these data, particularly those on production and mortality, would suggest that additional space is of benefit to birds, the precise area is difficult to define. The recent ARMCANZ recommendation is for 550 cm^2 per hen.

Perches

A major problem that arises from keeping hens in cages is the problem of broken bones that occurs as a result of handling and transport.[59,60] Some of these problems occur as a result of cage design and certainly improved door design (such as S-shaped full width doors[61,62]) should improve access and reduce the risk of bone breakages when removing birds from cages. However, it would be preferable if bone strength was improved so that the risk of broken bones is reduced. Whereas the provision of perches in cages improves bone strength,[63-65] there can be detrimental consequences on production. In cages with perches there were increased cracked eggs[66] and reduced egg mass output.[67] However, this can be influenced by both perch design and the age of pullets when exposed to perches.[68] Whereas there appears to be clear advantages to the strength of some bones by the provision of perches, attention is required on the position, size and shape of the perch.[47,67] Also the effects of perches on non-load bearing bones is unclear and these bones may be adversely affected by, or derive no benefit from, perches; there are reports of deformation of the sternum due to perches.[69] Access to perches during rearing decreased cannibalism during the laying period.[70]

From a scientific perspective of welfare there is good argument, on the basis of the potentially improved bone strength and its positive consequences for health, for the inclusion of perches within cages. However, depending on the shape and location of the perch, there can be a production cost in terms of increased cracked or dirty eggs which is an economic cost.[49] Integrating these two pieces of information is a political decision not a scientific one.

Dust baths

In addition to providing a substrate for birds to dustbathe, another reason for the inclusion of dust baths is to reduce the use of the nest box as a dust bath. Nevertheless, the welfare benefits of dust baths are far from clear. Studies have shown that hens do not make any great effort to obtain access to litter or sand,[58,71] although they prefer litter to wire mesh.[72] It has been suggested birds are not highly motivated to dustbathe[73] and it has been shown that birds' motivation to gain access to litter is highly variable.[74] Nevertheless, it has been suggested that dust baths are essential to maintain feather integrity and for welfare.[75] In terms of fitness variables, experiments with young chickens indicate a risk of pathological feather pecking when straw or wood-shavings are used as a substrate,[76] although rearing with access to sand or peat reduced subsequent feather pecking and that access to straw, as an environmental enrichment, during the layer phase also reduced feather pecking.[77] A

positive effect of hay, both during rearing and the laying period in reducing feather pecking, has also been shown.[78] Nevertheless, the implications of these rearing experiments for the provision of dust baths in cages to improve welfare is unclear.

Nest boxes

The lack of a nest site in conventional cages has been considered as the biggest welfare problem in this system of housing.[79] The importance of the nest box is based on evidence of preference tests, evidence of frustration in the absence of a nest and the strong motivation of hens to use a nest.[80-83] Hens prefer litter to a wire floor,[84-86] although it is not clear if litter is an important resource for hens.[78] Evidence of frustration includes restlessness, stereotyped escape behaviour and prolonged searching behaviour prior to oviposition and directing nest building towards an apparently inappropriate substrate such as wire.[80,87] The strong motivation of hens to nest is based on their willingness to 'work' to gain access to a nest site, once they have learned to use it; they will travel through air blasts and push weighted doors[88] or access a closed dust bath.[82] There has been consideration of whether animals can be frustrated or experience a sense of deprivation by not having certain resources they have never experienced.[87] For nesting, no differences were found in the motivation of birds to use a nest between birds previously experienced or inexperienced with a nest. However, naive birds did not recognise a visual stimulus with some features of a nest, although it must be recognised that the birds in this study were unable to physically interact with the 'nest'.[89] There is a suggestion that birds may synchronise their behaviours within cages and this may have welfare implications if nest sites are limited.[90] It has also been shown that there is considerable variation in choice of nest-site but not nesting motivation.[91]

Several aspects of nest design have been examined and current commercial nests incorporate some of these features such as the use of artificial floors to increase attractiveness, sloped (roll-away) floors to improve egg quality, nest excluders to improve nest hygiene[92] and enclosed nests to reduce 'stress-associated' egg abnormalities.[93] There is no evidence that nests affect fitness, notwithstanding that developmental problems with the use of next boxes including roosting in the nest boxes, laying eggs outside of nest boxes, the higher incidence of cracked eggs and using the nesting material for a dust bath can or have been solved.

Abrasive strips

One of the criticisms of keeping birds in cages is the excessive length that claws can reach by the end of the laying period because hens in conventional cages are not able to wear down their claws as effectively as birds kept in non-cage systems. Floor layers spend time scratching litter or soil and this behaviour wears the claws and keeps them blunt. However, in cages, claw length of the middle toe can reach over 40 mm[94-96] and in some strains the claws can become long, twisted and cracked and have a pronounced curl. A method by which claws can be kept short and blunt is to fit 8 mm wide strips of abrasive tape onto the egg guard; birds' claws scrape against this tape while they are feeding. In cages that had the strips, birds had significantly shorter claws than control hens throughout the laying period, the length of the claws of the middle digits did not exceed the length of those in pullets or birds kept on litter floors and there were fewer broken or twisted claws.[52] An abrasive paint may improve the durability of the abrasive.[97]

In Australia, an experiment with abrasive strips in layer cages has indicated that abrasive strips were effective in reducing claw length.[98] In this study the authors found that the angle and size of the egg baffle in different manufacturers' cages affected the reduction in claw length, although the abrasive strip was considered of benefit in all cage types they examined. More recently the effect of abrasive strips and abrasive paint in layer cages on claw length, claw sharpness, foot condition, feather cover, body scratches and mortality of hens was compared.[99] Abrasive paint was found to be more effective as a claw shortener than abrasive strips, based on length of claws and sharpness. However, hen mortality from prolapse and cannibalism combined was significantly higher in cages fitted with abrasives, in one of two experiments (6.3% for paint, 5.9% for strips and 1.6% for control treatments in experiment 1, respectively and 0% for paint, 0.8% for strips and 0%, respectively in experiment 2). Differences between the studies included strain of bird (Hyline Gold and Hyline Brown in experiments 1 and 2, respectively) and space allowance (545 vs 690 cm^2 per hen). It was speculated that when birds are frightened or competing for a position at the feeder, they might abrade their vent region on the paint or strip and this may encourage vent pecking. The increased light levels in the Australian study may account for the increase in cannibalism and prolapse.[99]

Non-cage systems

With the exception of the studies by Tauson and colleagues, which are replicated experiments,[48,50,100] most of the other studies on non-cage systems, such as barn and aviary, have either minimal or no replication[101-105] and thus it is difficult to draw rigorous conclusions from them and considerable caution is recommended when using these data.[106] Nevertheless, there is considerable support for these non-cage systems, in part on the basis of the increased behavioural repertoire they permit[104,107] and lower levels of fear in the tonic immobility test.[108] A number of non-cage systems, including details of economics, advantages and disadvantages, have been described.[109] Nevertheless, some of the components of the system have been systematically studied. For example, it is generally agreed that bone strength is improved in non-cage systems,[65,94,110,111] although it has been identified that all systems with perches result in keel bone deformation.[102,112] Other aspects that have been or are being studied are spacing between perches[113] (their data suggest that birds are less successful negotiating distances greater than 1.0 m), space allowances for different behaviours[114] (the frequency of walking and ground pecking was reduced as space allowance decreased)[115] and rearing conditions (low density rearing resulted in less feather pecking prior to the laying phase).[108] While it has been shown that hens prefer to congregate with familiar than unfamiliar birds, although the unfamiliar birds become familiar with experience,[115] the relevance of this to welfare and housing design is unknown.

While there is a general lack of information on non-cage housing of poultry, such as barn and aviary systems that are in commercial use, there is some possibly relevant information from deep litter pen and yard systems. These systems, which are probably more typical of backyard keeping of a few birds for home egg consumption, may provide litter on the floor, considerably more space than in cages, nest boxes and perches. Based on the literature on corticosterone concentrations in cages and

non-cage systems (floor pens and yards), there is no unequivocal evidence that level of confinement per se has any consequences for the welfare of the laying hen although the data are equivocal. For example, corticosterone concentrations were similar in cages and outside range pens (7430 cm^2/bird),[116] while corticosterone concentrations in floor pens can be higher,[117-120] lower[121] or not different[122] than in some cages. In part these differences appear to depend on the space allowance and/or group size of birds in cages; the literature has been reviewed.[123] Immunological data suggest that birds in floor pens may be relatively immunocompromised, although this experiment was not designed to compare immunological status between systems and from this viewpoint there were some confounding factors.[124]

Conclusions

The issue of poultry housing is likely to remain a controversial topic until some fundamental issues, such as achieving an agreement on methodologies used to assess welfare and developing techniques to better measure welfare, such as measuring emotions, are addressed. Nevertheless, using the available methodologies within the framework of the homeostasis approach to welfare assessment provides evidence that there is a welfare benefit in conventional cages from both more space, on the basis of reduced mortalities, and incorporating perches, based on a 'fitness' measure of the likely fewer 'injuries' due to improved bone strength. However, there is no similar evidence for the incorporation of nest boxes or dust baths. Studies on solid sides and abrasives also indicate that overseas research may not be directly applicable to Australian conditions and that environmental factors (such as light or temperature) may affect behaviour, physiology or mortality. Therefore, it is important that these housing features are evaluated under local conditions before making any recommendations. Furthermore, the interactions between the items of furniture that together make up furnished cages also warrant examination as the findings from a furnished cage may be different from the reported literature on individual items of furniture. Notwithstanding the relative lack of data in non-cage systems, the data on bone strength suggest improved fitness in non-cage systems, the data on stress suggest fitness may be better, similar or worse in non-cage systems, and the limited data on immunology suggest fitness may be worse in non-cage systems than in conventional cages.

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BOOK REVIEW

Small Animal Cardiology. *Nelson OL. Butterworth-Heinemann, Elsevier Science, Marrickville, 2003, 213 pages. Price AUD$81.40. ISBN 0 7506 7298 6.*

This compact (11 x 15 cm) book designed for veterinary undergraduates, veterinary technicians and practising veterinarians is part of the eleven book 'Practical Veterinarian' series. Composed of twelve chapters arranged in a logical order, the initial three chapters address basic cardiovascular physiology, cardiovascular examination and diagnostic methods. Succeeding chapters discuss various cardiovascular conditions.

The text is easy to read and boxes and tables throughout the chapters allow information to be quickly accessed. Pathophysiology-based treatment plans presented in table form are most useful.

There are numerous black, white and 'shades of grey' diagrams throughout the book. They are mostly copied from other cardiology texts and many are poorly reproduced. Lack of colour and the need to reduce them to fit the size of the book detract a great deal from their usefulness. Likewise, attempts to reproduce radiographs and ECG tracings have resulted in illustrations that are not clear and look scruffy.

The information presented is accurate and mistakes are confined to typographical errors and incorrectly labelled diagrams. A US-produced text, it discusses only US distribution of heart worm disease and does not mention moxidectin as an effective prophylactic.

This book meets the aim described in the preface, "Not meant to replace the reference books, the guides in our series complement the larger books by serving as an introduction to each topic… or as a quick review…". It does indeed provide easy to read information. The downfalls of this book are its size and price. The need to reduce diagrams has detracted from its usefulness and the reader is constantly needing to turn the page! Although a reasonable production, I would rather spend my recent graduate dollars on a larger more detailed cardiology text or a more general medicine text that contains equivalent cardiology information in addition to information on other body systems.

SM Lillis

Dr Lillis is an Intern in Small Animal Medicine and Surgery at the University of Melbourne.

Serum protein electrophoretic pattern in young and adult camels

ZI CHAUDHARY[a], J IQBAL and J RASHID
Veterinary Section, Abu Dhabi Municipality P.O. BOX 10829, Abu Dhabi, United Arab Emirates.

Objective To compare the electrophoretic pattern of serum proteins in clinically healthy adult camels (between 3 and 8 years of age) and camel calves (less than 3 months of age).

Design Laboratory analysis of serum from healthy camels.

Procedure Blood was collected from 30 healthy adult camels and 30 camel calves and the serum separated. Total protein of each serum sample was estimated by automated chemistry analyser. The proteins were fractionated by automated electrophoresis on agarose gel.

Results Serum proteins migrated on the agarose gel as one albumin, two α (α_1 and α_2-globulins), two β (β_1 and β_2-globulins) and one γ-globulin fractions. In adult camels the mean concentration of total protein, albumin α_1, α_2, β_1, β_2 and γ-globulins was 56.8 ±1.5, 30.7 ± 0.8, 2.4 ± 0.1, 3.2 ± 0.1, 9.7 ± 0.3, 3.4 ± 0.2 and 8.6 ± 0.3 g/L, respectively. These values in calves were 49.7 ± 1.8, 23.7 ± 0.8, 3.2 ± 0.2, 3.1 ± 0.2, 14.2 ± 0.2, 4.0 ± 0.2 and 4.1 ± 0.2 g/L, respectively.

Conclusion The concentration of total proteins, albumin and γ-globulins was higher ($P < 0.05$) in the adult camels than in camel calves. The concentrations of β_1 globulins was higher ($P < 0.05$) in calves as compared to adult camels.
Aust Vet J 2003;81:625-626

More than 200 plasma proteins have been described and estimated in humans and animals and comprise about 5 to 7% (50 to 70 g/L) of plasma.[1] Many of these plasma proteins change markedly in disease conditions and with age.[2] The major site for their synthesis is the liver but also the immune system consisting of monocytes/macrophages, lymphoid and plasma cells.

Determination of the serum protein electrophoretic profile (SPE) is an important diagnostic aid in clinical biochemistry. Although a specific diagnosis can seldom be made with the SPE, a particular SPE can be associated with certain types of disease process and in this way provide the rationale for further definitive studies of the patient.[1]

Because the proteins of an individual or of a species are synthesised under genetic control, it is to be expected that variations in proteins would occur between individuals and between species.[3] The serum proteins have been studied intensively in many animal species,[3,4] yet little work has been done to study the SPE of camels. This study was carried out to determine the normal electrophoretic pattern of different serum proteins of young and adult dromedary camels (*Camelus dromadarius*).

Materials and methods

Two different age groups of clinically healthy camels in the United Arab Emirates were included in this study. The 30 adult camels were between 3 and 8 years of age of either sex (none of the females was pregnant). The 30 camel calves were less than 3 months of age.

Blood samples (5 mL) were collected from the animals into silicone-coated glass tubes. The tubes were centrifuged at 1500 *g* for 5 min to separate the serum. Serum isolated from each sample was run on KoneLab60i biochemistry analyser (Thermo Clinical Labsystems, Finland) to estimate total serum proteins. The analyser used photometric measurement of total protein by formation of a complex with cupric ions in alkaline solution. This method was preferred over refractometer because of greater sensitivity.[2] The serum proteins of these samples were fractionated, stained and quantified using an automated electrophoresis on agarose gel system (Helena Laboratories, France). The data were analysed by using the computer software programme "InStat, version 3". The SEM was calculated and t-test was used to compare the means of adult camels and camel calves. The t-test assumes that the differences are sampled from a Gaussian distribution. This assumption was tested using the method of Kolmogorov and Smirnov.[5] The P value was > 0.10. Therefore data passed the normality test with $P > 0.05$ and it was concluded that there were Gaussian distributions for the values.

Results

The mean concentrations of total serum proteins as recorded by chemistry analyser were 56.8 ± 1.5 and 49.6 ± 1.7 g/L, respectively, in adult and young camels (Table 1). Serum concentration of total protein was high ($P < 0.05$) in adult camels as compared with calves.

Electrophoresis in agarose gel identified one albumin, two α globulin (α_1 and α_2), two β globulin (β_1 and β_2) and one γ-globulin fractions. Since there were no standard sera available the identification and migratory pattern of serum proteins was compared with bovine and llama.[1,6] Albumin was the most prominent and most homogenous serum protein on agarose gel and migrated farthest. The mean concentration and range of albumin in adults and young camel calves is shown in Table 1. The concentration of albumin was significantly higher ($P < 0.05$) in adult camels than in camel calves.

The α fraction consisted of α_1 and α_2 globulins. The mean concentration and ranges of α_1 and α_2 globulins are presented in Table 1. No significant difference ($P < 0.05$) in the concentration of α_1 and α_2 were detected between both groups. Like most domestic animals β globulins in camels migrated as a fast (β_1) and a slower (β_2) fraction. The mean concentration and ranges of β globulins are shown in Table 1. The serum concentration of β_1 globulins was higher ($P < 0.05$) in camel calves as compared with adult camels. The γ globulin fraction of camels was observed as one broad based peak. The concentration of γ globulins was higher ($P < 0.05$) in adult camels than in camel calves (Table 1).

[a]Address for correspondence - Email: zafarchaudhary@netscape.net

Table 1. Concentration of serum proteins of adult and young camels

Component	Concentration (g/L)			
	Adult camels (n = 30)		Camel calves (n = 30)	
	Mean (SEM)	Range	Mean (SEM)	Range
Total protein	$56.8^{A} \pm 1.5$	50.0 - 64.1	$49.7^{B} \pm 1.8$	45.0 - 53.0
Albumin	$30.6^{A} \pm 0.8$	27.0 - 35.1	$23.7^{B} \pm 0.8$	22.0 - 26.0
A/G ratio	1.2 ± 0.0	1.1 - 1.3	0.9 ± 0.04	0.8 - 0.1
Alpha 1	2.4 ± 0.1	1.8 - 2.8	3.2 ± 0.2	2.8 - 3.6
Alpha 2	3.2 ± 0.1	2.4 - 4.1	3.1 ± 0.2	2.9 - 3.5
Beta 1	$9.7^{A} \pm 0.3$	8.5 - 11.2	$14.2^{B} \pm 0.2$	13.6 - 14.6
Beta 2	3.4 ± 0.2	3.0 - 4.3	4.0 ± 0.2	3.7 - 4.4
Gamma	$8.6^{A} \pm 0.3$	4.0 - 10.5	$4.1^{B} \pm 0.2$	3.7 - 4.4

Values in the same row with different superscripts are significantly (P < 0.05) different.

Discussion

The mean concentration of total serum proteins recorded in both groups of camels are in agreement with the earlier reports.[7,8] However, in one study[9] a higher total serum protein concentration of 75.30 ± 1.30 g/L has been reported in non-pregnant dromedary camels but this apparent difference may have been due to the small number of camels (n = 7) in that study.

Electrophoresis of the serum samples produced six peaks comprising albumin, α_1 and α_2, β_1 and β_2 and γ-globulins. The α-globulins include lipoproteins, which are synthesised in the liver.[1] The β-globulin fraction contains complement, haemopexin, transferrin, and C-reactive protein. In this study, the γ-globulin fraction was recorded as one broad peak, whereas in most other domestic animals this peak is observed as two fractions, a fast migrating γ_1 and a slower γ_2-globulin. IgMs, and IgEs are found primarily in the γ_1 fraction and IgGs are associated with the γ_2 fraction.[2]

There are very few reports published regarding serum protein profiles of camelids. Serum protein concentrations in alpacas have been studied in South America,[10,11] and serum protein electrophoresis has been performed on North American llamas.[12,13] Means of total protein, albumin, α, β and γ globulins concentration in mature male and female llamas (n = 7) have been reported as 63.0, 35.0, 7.0, 10.0, and 11.0 g/L, respectively.[6] While there is a small difference in serum protein concentrations of llamas and camelids, they have similar electrophoretic patterns.

Species differences have been observed in electrophoretograms of domestic animals including cat, cattle, dog, horse and sheep. For example in ruminants, cattle have five peaks representing albumin, α_1, α_2, β_1, β_2, and γ globulins.[4] The electrophoretogram of camels more closely resembles that of sheep than cattle, although there is a difference in concentration of the serum proteins.

The results of this study indicate that there was a significant difference (P < 0.05) between adult and young camels in the mean concentration of total protein, albumin, β_1 globulins and γ globulins. Total protein, albumin and γ globulins were significantly higher (P < 0.05) and β_1 globulins were significantly lower (P < 0.05) in adult camels as compared to camel calves. The difference between the two groups may be due to physiological factors because the concentration of total proteins and albumin increases with age due to progressive increase in globulins. Lower concentrations of total protein and albumin in the calves is due to liver output[1] and low concentration of γ globulins is due to the immaturity of the lymphoid system.[6] It has been reported that the neonates of llamas have low total protein and relatively low globulin, and this concentration remains low until production of globulins by the maturing immune system.[14] The β globulins increase considerably during pregnancy and after parturition due to an increase in C-reactive proteins.[2] The increase of β_1 globulins in calves appears to be the result of suckling.

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Toxoplasmosis in Indo-Pacific humpbacked dolphins (*Sousa chinensis)*, from Queensland

R O BOWATER[a], J NORTON[a], S JOHNSON[a], B HILL[b], P O'DONOGHUE[c] and H PRIOR[d]

Objective To describe the clinical signs, gross pathology, serology, bacteriology, histopathology, electron microscopy and immunohistochemistry findings associated with toxoplasmosis in four Indo-Pacific humpbacked dolphins (*Sousa chinensis)* that stranded in Queensland in 2000 and 2001.

Design Clinical assessment, gross necropsy, and laboratory examinations.

Procedure Necropsies were performed on four *S chinensis* to determine cause of death. Laboratory tests including serology, bacteriology, histopathology and transmission electron microscopy were done on the four dolphins. Immunohistochemistry was done on the brain, heart, liver, lung, spleen and adrenal gland from various dolphins to detect *Toxoplasma gondii* antigens.

Results Necropsies showed all of four *S chinensis* that stranded in Queensland in 2000 and 2001 had evidence of predatory shark attack and three were extremely emaciated. Histopathological examinations showed all four dolphins had toxoplasmosis with tissue cysts resembling *T gondii* in the brain. Tachyzoite stages of *T gondii* were detected in the lungs, heart, liver, spleen and adrenal gland, variously of all four dolphins. Electron microscopy studies and immunohistochemistry confirmed the tissues cysts were those of *T gondii*. All four dolphins also had intercurrent disease including pneumonia, three had peritonitis and one had pancreatitis.

Conclusion Four *S chinensis* necropsied in Queensland in 2000 and 2001 were found to be infected with toxoplasmosis. It is uncertain how these dolphins became infected and further studies are needed to determine how *S chinensis* acquire toxoplasmosis. All four dolphins stranded after periods of heavy rainfall, and coastal freshwater runoff may be a risk factor for *T gondii* infection in *S chinensis*. This disease should be of concern to wildlife managers since *S chinensis* is a rare species and its numbers appear to be declining.
Aust Vet J 2003;81:627-632

Toxoplasma gondii is a protozoan parasite of worldwide distribution that infects homeothermic animals including primates, marsupials, birds, rodents, other mammals and humans.[1,2] In Australia *T gondii* is widespread and has been described from numerous intermediate wildlife hosts including birds such as the southern cassowary, crimson rosella, satin bowerbird, domestic pigeon, regent parrot; marsupials such as the koala, common wombat, brown antechinus, kowari, mulgara, and agile wallaby; rodents such as the domestic house mouse and brown rat; and other mammals such as the dingo, domestic dog and cat, sheep and horse.[3] Toxoplasmosis is rarely reported in marine animals but has been recorded in a west Indian manatee,[4] a California sea lion,[5] seals,[6,7] Atlantic bottle-nosed dolphins and a spinner dolphin.[8,9]

In 2000 and 2001, five Indo-Pacific humpbacked dolphins (*Sousa chinensis)*, stranded in Townsville, and one stranded in Gladstone. This paper describes toxoplasmosis in four of these dolphins, three from Townsville and one from Gladstone. We describe the findings from necropsy, histological, immunohistochemical and electron microscopic examinations.

Materials and methods

Source material

In February 2000, an Indo-Pacific humpbacked dolphin stranded alive in Townsville, Australia (dolphin 1) and was transported by officers of the Queensland Parks and Wildlife Service (QPWS) to a local marine aquarium for rehabilitation, but died 3 days later. In September 2000, another *S chinensis* was found dead on the bank of a river 40 km south of Townsville (dolphin 2). The dolphin was reported by members of the public to be rolling from side to side in the water and bumping into boats near a boat ramp, just before its death. In June 2001, a third *S chinensis* stranded live on the foreshores of Townsville, but died soon after stranding (dolphin 3). In July 2001, a fourth *S chinensis* was found dead in Gladstone harbour (dolphin 4).

Clinical pathology

Clinical tests were only possible on dolphin 1 that stranded alive, because all other dolphins stranded dead, or died soon after stranding. Blood was taken from the tail vein of dolphin 1 for a complete blood count and a serum biochemical profile.

Clinical therapy

Treatment of dolphin 1 included oral antimicrobial therapy with Clavulox palatable tablets (Pfizer, Animal Health Australia) administered via orogastric tubing on day 1. Baytril 150 mg antibacterial tablets (Bayer Australia Ltd) and fluids were thereafter given orally, twice daily. Despite 3 days of such therapy, the dolphin died.

Necropsy procedure

All three dolphins that stranded in the Townsville region were necropsied by veterinary pathologists at Oonoonba Veterinary Laboratory, Townsville, Queensland. Necropsy of the dolphin that stranded in Galdstone harbour (dolphin 4) was done by QPWS officers and tissue specimens were collected and forwarded to Rockhampton Veterinary Laboratory for histological examination.

[a]Oonoonba Veterinary Laboratory, Queensland Department of Primary Industries, Animal and Plant Health Service, PO Box 1085, Townsville, Queensland 4810
[b]Rockhampton Veterinary Laboratory, Queensland Department of Primary Industries, Animal and Plant Health Service, PO Box 6014, Rockhampton Mail Centre, Queensland 4702
[c]Department of Microbiology and Parasitology, School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, Queensland 4072
[d]Yeerongpilly Veterinary Laboratory, Queensland Department of Primary Industries, Animal and Plant Health Service, 665 Fairfield Road, Yeerongpilly, Queensland 4105

Histological preparations and serology

Tissue specimens collected from all four dolphins were fixed in 10% buffered neutral formalin for 48 h, trimmed and processed routinely for histology.[10] Sections were cut at 5 µm and stained routinely with haematoxylin and eosin for light microscopy. Serum collected from dolphin 1 was tested for *T gondii* antibody, using an indirect haemagglutination test kit (Laboratoires Fumouze Division Diagnostics, Le Malesherbes, France).

Bacteriology

Various samples of organs and tissues were taken from dolphins 1, 2 and 3 for bacterial isolation. Samples included the brain, kidney, lung, liver and pericardial fluid from dolphin 1; lung, pancreas and small intestinal contents from dolphin 2; kidney, liver, peritoneal and pleural fluids from dolphin 3.

Transmission electron microscopy

Selected sections of paraffin-embedded brain tissue from all four dolphins were examined by transmission electron microscopy after processing by standard methods.[11]

Immunohistochemistry

Immunohistochemistry for detection of *T gondii* antigens was performed on selected sections of brain (dolphins 1 and 4), liver (dolphins 2 and 4), heart (dolphins 2 and 4), lung (dolphin 4), spleen and adrenal gland (dolphin 3) using a commercial avidin-biotin immuno-peroxidase staining technique (Vectastain Kit; Vector Laboratories Inc, Burlingame, CA).[12] The primary antibody used was *T gondii* polyclonal antiserum raised in goats (Dako Corp, Carpinteria, CA). The secondary antibody used was an anti-goat serum raised in rabbits (Vector Laboratories Inc). Brain and liver from mice experimentally infected with *T gondii* were used as positive controls while brains from mice that had been experimentally infected with *Neospora caninum* were used as negative controls.

Results

The locations where the four *S chinensis* stranded in northern Queensland in 2000 and 2001 are illustrated in Figure 1.

Clinical findings and gross necropsy

Lesions detected at necropsy included superficial, nonpenetrating skin lacerations and wounds consistent with predatory shark attack. All three dolphins that stranded in the Townsville region (dolphins 1 to 3) were extremely emaciated, showing prominence of the skull and spine. Dolphin 4, which stranded at Gladstone was in good bodily condition. Other common findings included wart-like skin lesions at the genital opening (dolphins 1, 2 and 4), and the presence of several well-demarcated rounded ulcers (0.5 to 1.0 cm diameter) on the dorsal surface of the tongue and soft palate (dolphins 1, 3 and 4). Other significant clinical and gross necropsy findings for each dolphin are presented below.

Dolphin 1

The dolphin was an adult female 245 cm in length and weighed 117 kg. It was dehydrated, weak and ataxic, unable to lift its rostrum above the water line to breathe, and unable to right itself in the water, continually rolling to either side. The right eye showed corneal opacity and the teeth were moderately worn. At necropsy, both lungs were emphysematous with disseminated small caseous nodules more numerous in the caudal third of both lung lobes. Fibrin tags adhered to the pleura of the right lung and the bronchial lymph nodes were enlarged. The pancreas was swollen and palpably hard in several areas, and there was torsion of the omentum at the site of attachment to the proximal duodenum. The mesenteric lymph nodes throughout the abdominal cavity were enlarged.

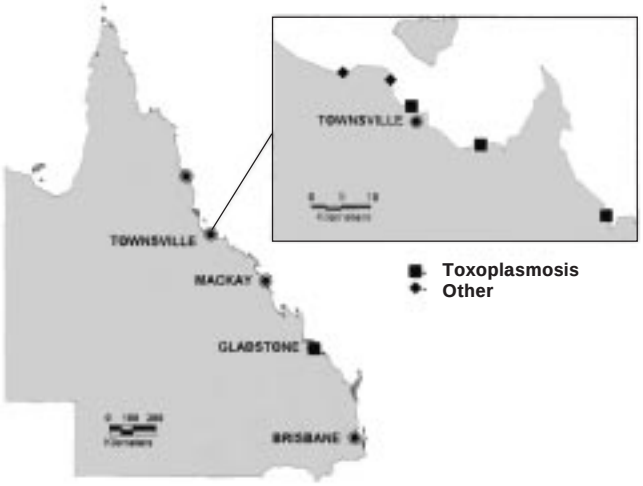

Figure 1. The location of four *Sousa chinensis* with toxoplasmosis that stranded in northern Queensland in 2000 and 2001.

Dolphin 2

The dolphin was an adult female, 247cm in length. At necropsy, there were fibrin tags adhering to the pleura of both lungs. The mesenteric lymph nodes were enlarged, and the mucosal surfaces of the pyloric region and bile duct were reddened. The stomach was distended and contained remnants of fish bones.

Dolphin 3

The dolphin was an adult female, 232cm in length. There was a small straight scar 1 cm long located mid-ventrally, approximately 60 cm dorsal to the genital opening that connected by a sinus tract to the abdominal cavity. The mesenteric lymph nodes were enlarged and there was haemorrhagic fibrinous peritonitis. Fibrin adhered to the lining of the abdominal wall where the sinus tract opened into the abdominal cavity, and also to the mesentery, serosa of the stomach, pancreas, liver, right hemi-diaphragm and pleura of the right lung. A stingray barb, 15.2 cm long was found lodged in the liver, surrounded by fibrous tissue, purulent exudate and fibrin. The stingray spine was identified as belonging to *Dasyatis* sp (P Last, personal communication).

Dolphin 4

This dolphin was a male, 180 cm in length in good body condition. Gross necropsy findings were reported as unremarkable by QPWS officers.

Serology

Dolphin 1 showed significant haematological findings including neutrophilia (19.83 x 10^9/L). Serum fibrinogen ranged from 3.6 g/L to 4.0 g/L over 3 days and was within normal limits. Creatinine kinase rose from 232 U/L to 1526 U/L and lactic acid dehydrogenase from 1575 to 2365 U/L over 3 days.

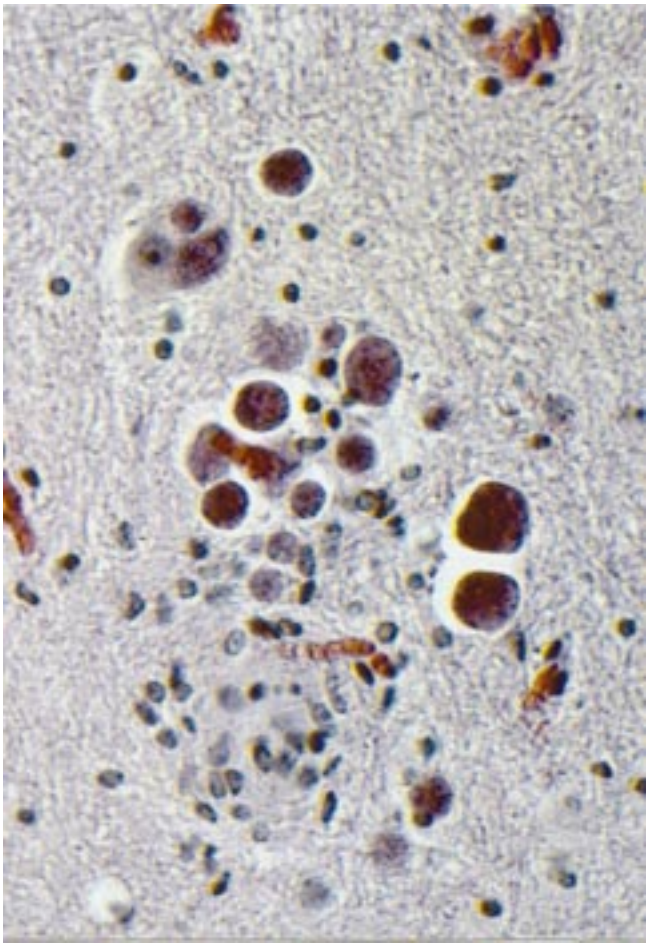

Figure 2. Light micrograph showing numerous *Toxoplasma gondii* cysts in the brain of *Sousa chinensis*, associated with gliosis. Haematoxylin and eosin x 300.

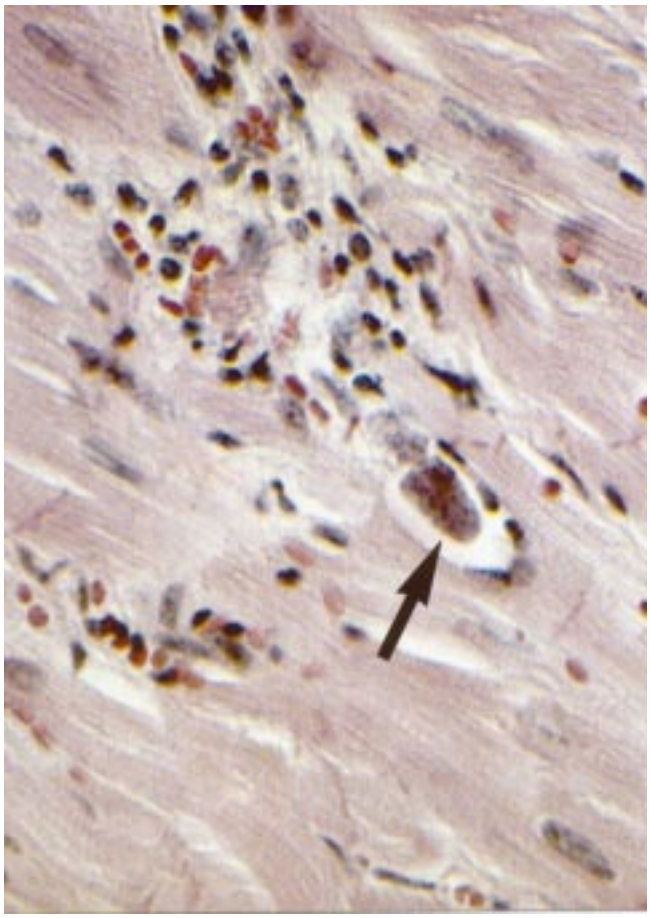

Figure 3. Light micrograph of the heart of *Sousa chinensis*, showing tachyzoites (arrow) of *Toxoplasma gondii* within the cardiac muscle. Note the adjacent focus of mononuclear inflammatory cells. H and E x 300.

Serum from dolphin 1 tested positive for *T gondii* antibody at a dilution of 1 in 2560.

Bacteriology

A pure growth of *Pseudomonas aeruginosa* was isolated from the lungs of dolphins 1 and 2. A number of bacteria were isolated, variously from the kidney, liver, peritoneal and pleural fluid of dolphin 3 including *Clostridium perfringens*, *Edwardsiella tarda*, *E hoshinae*, *Pseudomonas* sp, *P aeruginosa*, *Vibrio alginolyticus* and *V harveyi*. The bacteria isolated from dolphin 3 were considered secondary invaders and will not be considered further.

Histopathology

Significant histopathological findings from all four dolphins are shown in Table 1. The most striking lesions were seen in the brains, where there were large numbers of protozoan cysts, ranging in size from 6 to 30 µm, scattered throughout the neuropil but mainly in the brain stem and cerebrum with few cysts found in the cerebellum (Figure 2). Many cysts appeared to be fragmenting. Intense infiltrates of mononuclear inflammatory cells, consisting mainly of macrophages and lymphocytes, were often associated with these cysts. There were numerous microglial nodules throughout the brain tissue but these were not always associated with discernible cysts. Perivascular cuffs of mononuclear inflammatory cells were also evident. Multifocal areas of necrosis were commonly observed and these were similarly infiltrated with mononuclear inflammatory cells.

Organisms consistent with tachyzoite stages of *T gondii* were also detected histologically in various tissues including the parenchyma of the liver (dolphin 2), the myocardium (dolphins 2 and 4) (Figure 3), the spleen and adrenal gland (dolphin 3).

Other histological findings included purulent interstitial and bronchopneumonia (dolphin 1), fibrinous bronchopneumonia (dolphin 2), diffuse fibrinous bronchopneumonia and choroiditis (dolphin 3), and diffuse interstitial pneumonia (dolphin 4).

Immunohistochemistry

Immunoperoxidase staining performed on selected sections of brain, liver, heart, lung, spleen and adrenal gland from the various dolphins all tested positive and confirmed that suspected tachyzoites present in these organs were those of *T gondii* (Table 2). The immunoperoxidase staining performed on the brain and liver of mice experimentally infected with *T gondii* used as positive controls tested positive, whereas brains of mice experimentally infected with *Neospora caninum* tested negative.

Transmission electron microscopy

Examination of the electron micrographs of brain tissue in all cases showed numerous polyzoic cysts within the neural tissue (Figure 4). The cysts were identical in ultrastructure to those of the cyst-forming apicomplexan parasite *T gondii*.[1] The cysts were round in cross-section ranging from 20 to 30 μm in diameter. They were located intracellularly within enlarged host cells, but the host cell type could not be discerned. The cysts were bounded by a thin membranous wall (up to 40 nm thick) supported by a granular ground substance. The cyst wall did not contain any protrusions and was surrounded on the external surface by host cell mitochondria and endoplasmic reticulum. The cysts were aseptate and contained up to 70 bradyzoites in cross-section. The bradyzoites were elongate and measured up to 5.2 μm in length by 1.7 μm in width. They contained a prominent apical complex consisting of an anterior conoid, polar ring, 8 to 10 large rhoptries and 20 to 50 smaller micronemes. The bradyzoites were bounded by a pellicle consisting of two membranes, the inner membrane being discontinuous at the anterior and posterior polar rings and the lateral micropore. The membranes were supported by 22 subpellicular microtubules evident in cross-section. The nucleus was located in the posterior half of the cell body and was closely associated with a mitochondrion and Golgi body. The bradyzoites contained variable amounts of endoplasmic reticulum, some dense granules and occasionally some polysaccharide granules.

Table 1. Significant histopathological findings from four *Sousa chinensis* with toxoplasmosis (dolphins 1, 2, 3 and 4) that were necropsied in Queensland in 2000 and 2001

Organ	Histopathology	Dolphin			
		1	2	3	4
Brain	Non-suppurative encephalitis, gliosis, focal necrosis & *T gondii* cysts present	+	+	+	+
Heart	Occasional foci of necrosis and myocarditis	-	+[a]	+	+[a]
Liver	Focal disseminated perivascular inflammation and hepatocyte necrosis	+	+[a]	-	+[a]
Peritoneal cavity	Peritonitis	+	-	+	+
Pancreas	Pancreatitis	+	-	-	-
Lungs	Pneumonia	+	+	+	+[a]
Tongue	Ulceration	+	-	+	+

[a] *T gondii* tachyzoites present
+ present
- absent

Table 2. Results of *T gondii* immunohistochemistry from organs of four *Sousa chinensis* (dolphins 1, 2, 3 and 4) with toxoplasmosis necropsied in Queensland in 2000 and 2001.

Organ	Dolphin			
	1	2	3	4
Brain	+	NT	NT	+
Heart	NT	+	NT	+
Liver	NT	+	NT	+
Lung	NT	NT	NT	+
Spleen	NT	NT	+	NT
Adrenal gland	NT	NT	+	NT

+ = positive
NT = not tested

Discussion

Our findings from histopathological examination of tissues and electron microscopy are similar to other reported cases of toxoplasmosis in marine mammals, in that *T gondii* tissue cysts were detected in the brain, and *T gondii* tachyzoites, in association with focal necrosis, were detected in various other body organs.[4-9] The electron microscopical findings were consistent with tissue cysts of *T gondii* and allowed differentiation from other cyst-forming coccidia including *Sarcocystis*, *Frenkelia*, *Besnoitia*, *Hammondia* and *Neospora*. The cysts could be differentiated from those of *Sarcocystis*, *Frenkelia* and *Besnoitia* variously by the presence of a thin primary cyst wall, the absence of a secondary cyst wall, the absence of septae and the small size of the zoites. They differed from those of *Hammondia* and *Neospora* in the small size of the membranous cyst wall and the reduced organelle content of the zoites, especially the number of rhoptries. In each of our four cases we observed large numbers of tissue cysts in the brain, and tachyzoites were observed in at least one other organ including the heart, adrenal gland, spleen and liver. The presence of numerous tissue cysts in the brain in all four dolphins suggested the possibility that these resulted from the reactivation of a chronic infection through the debilitating effect of intercurrent disease. All of the dolphins had intercurrent disease, including pneumonia (all four dolphins), pancreatitis (dolphin 1) and peritonitis (dolphins 1, 3 and 4).

Four *S chinensis* that stranded in northern Queensland in 2000 and 2001 had encephalitis attributable to infection with *T gondii*. Clinical examination was possible in only one of these and it showed clinical signs of ataxia prior to death. The importance of toxoplasmosis in Indo-Pacific humpbacked dolphins is uncertain. This study suggests that the disease should be of concern to wildlife managers since the species is listed as rare under the Nature Conservation Act 1994. Their numbers appear to be declining, but relatively little is known to date about population sizes, ecology and biology of *S chinensis*.[13,17,18]

The manner in which these dolphins became infected with *T gondii* is uncertain. Toxoplasmosis is a parasitic disease that infects only homeothermic animals.[1,2] Cats are the only known definitive hosts,[1] and usually become infected by ingestion of intermediate hosts that are infected with tissue cysts of *T gondii*.[1,2] Infection in intermediate hosts is by ingestion of food, soil or water that is contaminated with oocysts of *T gondii*.[1,14-16] Intermediate hosts can also be infected vertically by transplacental transmission of tachyzoites or by ingestion of other intermediate host containing *T gondii* tissue cysts.[1, 2]

Recent studies in the Townsville region have shown that *S chinensis* feed on a variety of marine and freshwater fish and benthic invertebrates (G Parra, personal communication). A similar diet of common estuarine and reef fish, and occasionally cephalopods and crustaceans has been found for *S chinensis* in South Africa and Hong Kong.[17] There is no evidence to date

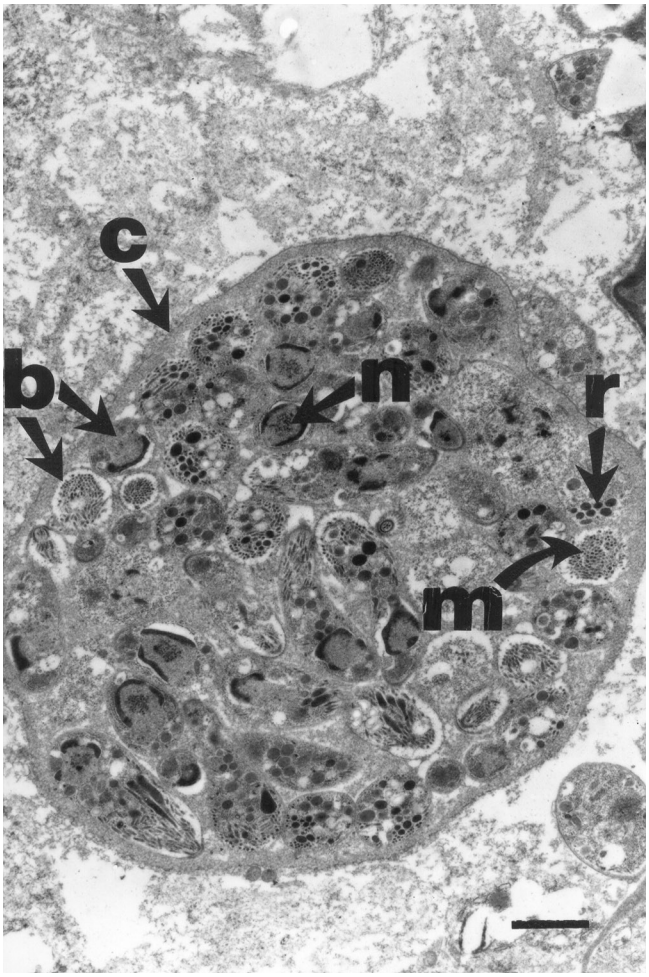

Figure 4. Transmission electron micrograph of an aseptate cyst of *Toxoplasma gondii* in the brain of *Sousa chinensis*. The cyst is contained by a membranous cyst wall (c). Within the cyst are numerous bradyzoites (b) in cross section. The bradyzoites are identifiable by their organelles including subterminal nuclei (n), apical rhoptries (r) and apical micronemes (m). Bar = 1.43 μm.

that *T gondii* infects poikilothermic animals such as fish or invertebrates.[1] However it is possible that molluscs or crustaceans may mechanically concentrate *T gondii* oocysts, acting as vectors for infecting marine mammals such as *S chinensis.* Lindsay et al recently demonstrated that *T gondii* oocysts can be removed from seawater by eastern oysters (*Crassostrea virginica*) and retain their infectivity. [19] Furthermore if *S chinensis* occasionally fed upon unusual prey such as wounded or dead seabirds or rodents that were infected with *T gondii* tissue cysts, then they may incidentally acquire toxoplasmosis.

In Australia *S chinensis* have a broad geographic distribution yet exhibit some territoriality within inshore coastal areas and do not migrate vast distances throughout their natural range.[18] Recent studies on the ecology and migration patterns of *S chinensis* in the Townsville region have shown that they frequent inshore creeks, rivers and coastal areas that are highly urbanised (G Parra, personal communication). Infection with *T gondii* may thus be acquired locally under specific circumstances. The ingestion during feeding, of water or sediment contaminated with *T gondii* oocysts is one possible route of infection. It is possible that coastal waters become contaminated with oocysts of *T gondii* from sewage discharge or floods that carry sediments contaminated with them into creeks and rivers. It may not be coincidental that three infected dolphins stranded in the Townsville region within a year of heavy rainfall and flooding. Miller et al recently reported that coastal freshwater runoff is a risk factor for *T gondii* infection in southern sea otters (*Enhydra lutris nereis*) in southern California. [20] Coastal freshwater runoff may similarly be a risk factor for *T gondii* infection in *S chinensis.*

S chinensis may also acquire toxoplasmosis by direct ingestion of infected feline faeces discarded from recreational vessels or from feral cats. In Townsville and Gladstone, feral cats inhabit the rock walls associated with the ports and marinas and domestic cats are kept as pets on board boats and recreational yachts. Domestic cats are also commonly kept as pets in households throughout Townsville and Gladstone, and cat faeces may be discarded into sewers. Although the proportion of infected cats excreting oocysts is generally not high in a given population (less than 2% in most countries),[1] a cat may shed millions of oocysts in its faeces and they can remain viable at 15 to 35°C for up to 1 year.[1] Townsville has a tropical climate where the average sea surface water temperatures vary from 24.6°C in winter to 29.1°C in summer (Australian Oceanographic Data Centre). Townsville sea surface water temperatures favour the long term viability of *T gondii* oocysts in coastal waters. One of the dolphins that had toxoplasmosis was from Gladstone harbour and was thought to be one of a pod of four to six animals regularly seen at the entrance to Auckland creek and the adjacent marina.

A pilot survey will be conducted on the eastern coast of Queensland to assess the significance of this disease in marine mammals that inhabit marinas and urban coastal areas. This could have implications for the future management of *S chinensis*, especially pertaining to disposal of domestic cat faeces in marinas and sewage systems in Australia, and for the control of feral cat and rodent populations in coastal regions, especially in marinas and ports.

Acknowledgments

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Idiopathic mucosal lesions of the arytenoid cartilages of 21 Thoroughbred yearlings: 1997- 2001

Abnormalities of the upper respiratory tract of Thoroughbred yearlings are commonly identified during pre- or post-sale endoscopic examination. Some may progress to the detriment of subsequent performance resulting in financial penalty for any purchaser. Published studies of sales examinations make no mention of mucosal lesions of the arytenoid cartilage.

This paper reports results from the post-sale, upper airway endoscopic examinations of 3312 Thoroughbred yearlings during a 5 year period and follows the discovery by the authors of mucosal ulcers and small granulomas on the axial surface of one or both arytenoid cartilages at post-sale endoscopic examination. Mucosal lesions were seen in 0.63% of the yearlings evaluated and represent the commonest documented abnormal condition of the upper portion of the respiratory tract. At subsequent examination 15 horses had healed without complications. Two horses with bilateral ulceration developed a granuloma at each site, while another developed a granuloma which led to arytenoid chondropathy. One horse was not available for follow up.

The authors conclude that pre-sale endoscopic examination of Thoroughbred yearlings should include careful study of the arytenoid cartilage, particularly at the rostral margin of the vocal process. Medical therapy should be considered and progress monitored. Lesions discovered post-sale warrant notification of the owner and sales company.

Kelly G et al. *Equine Vet J* 2003;35:276-281.

Medetomidine-ketamine anaesthesia induction followed by medetomidine-propofol in ponies: infusion rates and cardiopulmonary side effects

Volatile anaesthetic agents are commonly employed in equine surgical cases especially in procedures exceeding 1.5 h duration. A major deficiency of these agents is that they are cardiopulmonary depressants and may result in a high fatality rate, particularly when anaesthesia is maintained for 3-4 h or more. While several alternatives to inhalational anaesthesia in the horse have been evaluated, the only injectable anaesthetic suitable for long-duration procedures is propofol which requires combination with various α_2 adrenoreceptor agonists to produce satisfactory depth of anaesthesia. However this regimen presents problems including hypoxaemia, hypercapnia and relatively high costs.

Various propofol/medetomidine combinations of total IV anaesthesia have been used successfully, with good quality of recovery. However inductions were inconsistent and unsatisfactory in some cases.

In this study the anaesthesia of six ponies was induced with medetomidine-ketamine and then maintained with medetomidine-propofol.

Induction is reported as excellent and cardiovascular function remained stable. Recovery to standing after 4 h of anaesthesia averaged 31.1 min and was achieved after one or two attempts.

The authors propose this IV regimen as a possible alternative to inhalation anaesthesia with potential to reduce anaesthesia-related fatality rates in horses.

Bettschart-Wolfensberger R et al. *Equine Vet J* 2003;3:308-313.

SHORT CONTRIBUTIONS

Iridovirus-associated mortality in farmed Murray cod (*Maccullochella peelii peelii*)

MJ LANCASTER
MM WILLIAMSON
CJ SCHROEN

Victorian Institute of Animal Science,
475 Mickleham Road,
Attwood, Vic 3049

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Iridoviruses are large double-stranded DNA viruses which have been isolated from insects, fish, amphibians and reptiles.[1] Severe systemic infections characterised histologically by large cytoplasmic basophilic inclusions and ultrastructurally by aggregates of icosahedral virions have been described in many wild, farmed and ornamental fish species.[1,2] In Australia, Epizootic Haematopoietic Necrosis Virus (EHNV) has caused high morbidity and mortality rates in redfin perch (*Perca fluviatilis*)[3] and low morbidity but high mortality rates in rainbow trout (*Oncorhynchus mykiss*).[4] This short communication describes high mortality rates associated with an iridovirus-like agent, apparently not EHNV, in two age groups of farmed Murray cod.

Some 9,000 of 10,000 (90%) 4 to 6 cm fingerling Murray cod died over a 3 to 4 week period in February 2003, when water temperatures peaked at 26 to 27°C. Approximately 140 of 560 (25%) 10 to 15 cm fingerlings died during the same time period. Inappetence was the first sign noted, followed by lethargy and death within 4 to 7 days. Older fish remained clinically normal.

Significant gross changes were not detected in fingerlings from either group. Histological examination revealed apoptotic cells in renal haematopoietic tissue, gill capillaries, gill interstitial tissue and spleen. Cells with a large basophilic cytoplasmic inclusion were seen in the spleen, renal haematopoietic tissue, gill connective tissue (Figure 1) and heart. Areas of focal necrosis and inflammatory cell accumulation associated with bacteria were present in the liver and spleen of several dead fish. An indirect immunoperoxidase test incorporating rabbit polyclonal anti-EHNV antiserum stained EHNV inclusions in redfin perch tissue used as a positive control, but did not stain inclusions in any cod tissue. Significant bacteria were not cultured aerobically from livers or spleens.

Splenic inclusions detected histologically were examined by electron microscopy. Formalin-fixed splenic tissue was reprocessed from wax blocks, post-fixed in 2% osmium tetroxide, dehydrated and embedded in araldite resin. Ultrathin sections were cut, mounted on formvar-coated copper grids and stained with uranyl acetate and lead citrate. Many clusters of non-enveloped icosahedral virus particles were identified in cytoplasmic remnants of splenic cells (Figure 2). The minimum diameter of 25 virions ranged from 132 to 165 nm, mean 148 +/- 8 nm.

This viral morphology and associated tissue changes are characteristic of the group of systemic piscine, amphibian and reptilian iridoviruses.[1,5,6] Viral diameters fitted the reported size range of 153 +/-10 nm.[1] Other intracytoplasmic icosahedral viruses reported in fish are much smaller: birnaviruses at 60 nm[7] and nodaviruses at 25-30 nm.[8] Three iridoviruses have been reported from wild or farmed fish in Australia: lymphocystis virus,[9] EHNV,[3] and Bohle virus. Lymphocystis virus infection results in massive hypertrophy of fibroblastic cells, not evident

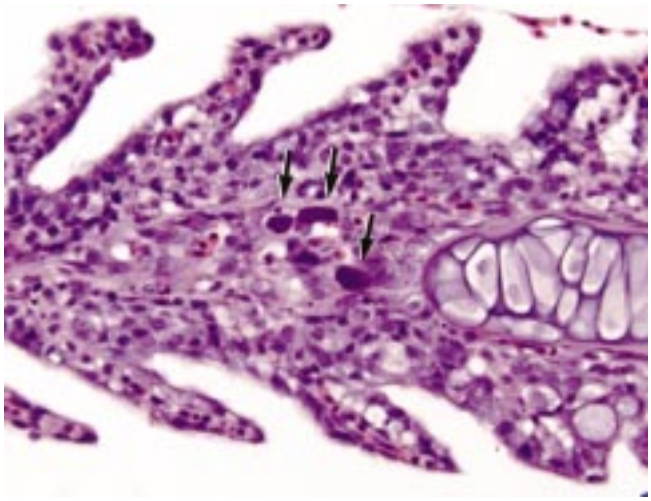

Figure 1. Histological section of gill tissue with several intracytoplasmic inclusions (arrows). Haematoxylin and Eosin x 400.

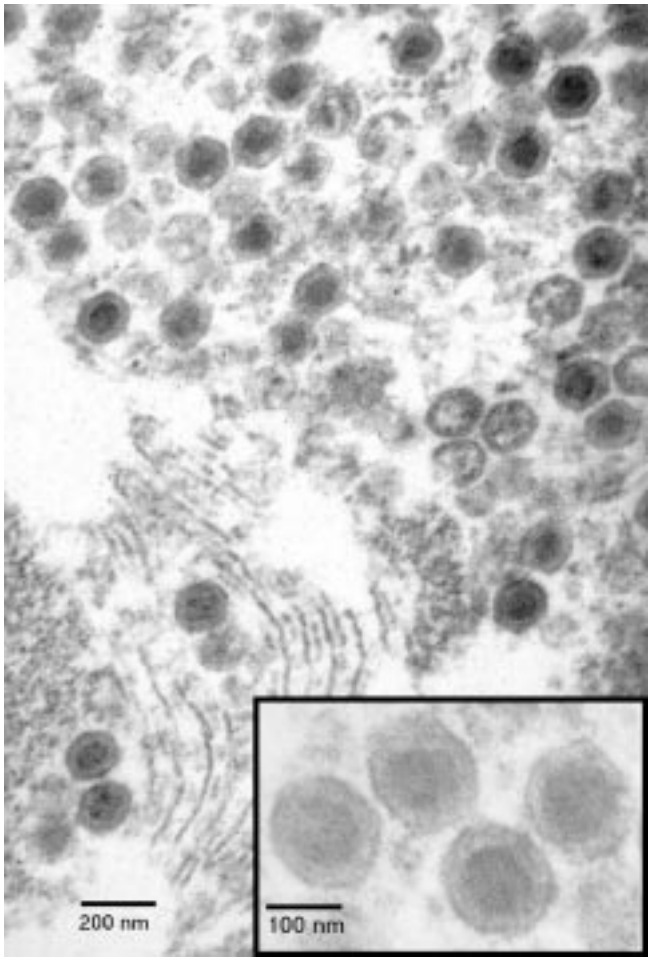

Figure 2. Electron micrograph of icosahedral virions within a splenic cell.

in these cod, and its virions are large (225 +/- 6 nm).[9] Experimental infection of barramundi (*Lates calcarifer*) with Bohle virus, originally isolated from the ornate burrowing frog (*Limnodynastes ornatus*) in Queensland,[10] resulted in 100% mortality,[11] but reports of experimental infections of Murray cod with Bohle virus were not found. Juvenile Murray cod have been infected with EHNV by bath and intraperitoneal inoculation.[12] Only the latter route produced clinical disease, and pancreatic necrosis was the only consistent histological feature.

Polyclonal antibodies raised against EHNV detect the systemic fish and amphibian iridoviruses.[1] EHNV shares antigens with the Bohle iridovirus,[13] European Sheatfish Virus and European Catfish Virus.[14] Similarities with the frog viruses of Europe has resulted in these viruses being grouped together in the *Ranavirus* genus of the family *Iridoviridae*.[1] Our negative results from the immunoperoxidase test suggests that this virus is not EHNV and may not be a member of the genus *Ranavirus*.

The source of the virus in this outbreak was most likely other infected fish (or an infected amphibian), although mechanical transfer via water birds, for instance, cannot be ruled out. The cod were farmed in several tanks in a single recirculatory system with water from a chlorinated domestic supply. The fish originated from three different locations in south-eastern Australia. Although reports of natural disease outbreaks in Murray cod associated with intracytoplasmic viral inclusions were not found, this species may harbour an iridovirus that requires special circumstances to cause disease. Larger cod fingerlings were introduced to the farm 2 to 3 weeks before mortality commenced in the smaller fingerlings. Minced frozen rainbow trout fillets were fed to the smaller fingerlings during January. Rainbow trout have been demonstrated to carry EHNV, and this virus is resistant to freezing. A few goldfish (*Carassius auratus)* from a separate dam on the farm were fed to broodstock cod in late 2002. Two types of iridovirus have been reported in this species, albeit not in Australia, and their virions were 180 nm in diameter.[1] Bohle virus has not been reported in Victoria. Typical iridovirus inclusion bodies have been detected in ornamental fish imported into Victoria (MJL unpublished observations) and other parts of Australia[15] but a link with these species was not established.

In conclusion, an iridovirus, apparently not EHNV, may cause significant mortality in farmed Murray cod. A more definitive conclusion awaits isolation of the virus and its inoculation into uninfected Murray cod. The source of this virus was not established.

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Suspected *Phalaris paradoxa* (paradoxa grass) poisoning in horses

CA BOURKE — NSW Agriculture, Orange Agricultural Institute, Forest Road, Orange, New South Wales 2800

SM COLEGATE — CSIRO Livestock Industries, Plant Toxins Research Group, Private Bag 24, Geelong, Victoria 3220

S SLATTERY — Rural Lands Protection Board, PO Box 18, Narrabri, New South Wales 2390

RN ORAM — CSIRO Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory 2601

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C	Coerulescine
HPLC	High pressure liquid chromatography
HPLCesiMS	Electrospray ionisation mass spectrometry
HPLC/UV/esi MS	Photodiode array ultra violet absorption
M+ + H	Mass to charge ratios of molecular ion adducts
MC	Methoxylated coerulescine
MP	Methoxylated phalarine
M2Me-THBC	Methoxylated 2 methyl tetrahydrobe tacarboline
2Me-THBC	2 Methyl tetrahydrobetacarboline
6MeO2Me-THBC	6 Methoxy 2 methyl tetrahydrobeta-carboline
P	Phalarine
TLC	Thin layer chromatography

Phalaris poisoning has been reported internationally in association with the ingestion of numerous *Phalaris* species by sheep and cattle, most notably *P aquatica* (phalaris grass) and *P arundinacea* (reed canary grass).[1] These outbreaks of poisoning have involved either a motor neurone dysfunction called 'staggers',[2] death due to a peracute brain dysfunction called polioencephalomalacia-like sudden death,[3] or death due to a peracute heart dysfunction called cardiac sudden death.[3]

To date none of these phalaris poisoning syndromes have occurred in sheep or cattle grazing pastures dominated by either *P paradoxa* (paradoxa grass) or *P coerulescens* (blue canary grass). Likewise, none of the phalaris species so far associated with poisoning in sheep and cattle have poisoned horses. Outbreaks of 'staggers' have been associated with the presence in phalaris of methylated tryptamine and related beta-carboline alkaloids.[2] The toxins responsible for PE-like sudden death and cardiac sudden death remain uncertain, although evidence has been presented to suggest a possible causal role for N-methyl tyramine in the latter syndrome.[4]

In 1999 Colegate et al[5] reported sudden deaths in horses grazing *P coerulescens* dominant pastures. The cause of death appeared to be heart failure and the possible causal agents identified in the suspect grass material included tetrahydro-beta-carboline, oxindole, and furanobisindole alkaloids. In 1992 and again in 2001, C Bunce and P Gough (personal communication) independently of each other noted sudden and unexplained deaths of horses in the Moree district of New South Wales. On each occasion the horses were grazing winter-spring fallow cultivation paddocks, where the available pasture was a mixture of fodder oats (*Avena sativa*), black oats (*A fatua*) and a *Phalaris* grass, identified locally as *P paradoxa*. This syndrome appeared to be very similar to that described by Colegate et al [5] for *P coerulescens*.

In early spring of 1999 we investigated the sudden death of three of five horses at Narrabri, New South Wales. The animals were grazing a 12 ha fallow wheat paddock. At the time of the deaths approximately 80% of the feed on offer was a *Phalaris* grass, subsequently identified by one of us (RO) as *P paradoxa*, and the remainder a mixture of black oats (*A fatua*) and wild turnip (probably a *Brassica* or *Rapistrum* sp). The paddock had been ploughed the previous month and subsequent rain had produced a good germination of phalaris, oats and wild turnip. Approximately 10 days before the deaths the unploughed headland along three sides of the paddock had received one boom spray width of herbicide (glyphosate).

On 10 September two horses were found dead; they had been apparently normal the day before. Hoof prints in the moist earth indicated that they had been galloping, before skidding and falling down dead. On 11 September the remaining horses were moved out of the paddock, but in the process one of the horses became very excited, galloped for about 400 m, suddenly stopped, fell down, rose to its feet again, fell down, struggled vigorously for a few moments and died. The dead animals were aged between 9 and about 20 years. At the time of the deaths the phalaris on the previously ploughed ground was growing well, but was only 10 cm or less in height. Phalaris growing on the unploughed headlands was more advanced and some plants were setting seed. The poisoning event outlined above is consistent with that described for horses grazing *P coerulescens* dominant pastures in Victoria and South Australia.[5] In the *P coerulescens* report the dead horses were subjected to post-mortem examination and subsequent laboratory studies, including histopathology. However, in the present case they were not. Consequently some may decide the sudden deaths could have had a non-phalaris related cause. Previous observations of *P paradoxa* related horse deaths at Moree would support a phalaris association, as would the improbability that unrelated incidental events, for example aortic rupture, snake bite, and lightening strike, could affect three of five horses in the same paddock at about the same time.

P paradoxa is native to the Mediterranean region, Canary Islands and Madiera.[6] It is a winter-spring annual that has become naturalised in North America, South America, and throughout the Australian mainland.[7] Paradoxa grass prefers fertile clay soils, such as those encountered in the North Western Plains of New South Wales. It is a serious weed of cereal crops that is difficult to control by cultivation alone, necessitating treatment with selective herbicides. Large quantities of seed are produced in late spring, some of which is shed from the panicles. The remainder falls in clusters of seven spikelets; the central one is bisexual and forms a seed, but the

others are male. Near the base of the panicle the six outer spikelets are short, stiff, club-shaped and sterile. Taxonomically, *P paradoxa* is more closely related to *P coerulescens* than to any other *Phalaris* species.[6] *P paradoxa* can sometimes be confused with *P minor*, another winter growing annual that often invades wheat crops on fertile soils.

To further support this association between horse deaths and *P paradoxa*, and the apparent similarity to horse deaths from *P coerulescens*, phytoalkaloidal profiling was carried out on phalaris plant material. Herbage samples from five accessions of *P paradoxa*, two of *P coerulescens* and one of *P aquatica* (unknown cultivar), were soaked in dilute acid (0.1 m mol / L HCl) for 16 h. The extracts obtained were treated with solid phase cation exchange resins and analysed using TLC and HPLC as previously described.[8] A major refinement on previous studies was that the effluent from the HPLC separation was monitored using HPLCesiMS in addition to the photodiode array UV absorption previously described.[8] The mass spectrometric refinement provided mass identity confirmation of the eluted peaks.

Phalaris species and cultivars can be difficult to differentiate at certain times in their growth cycle, for example the early germination phase. Consequently one of us (RO), an international expert in the botanical identification and propagation of this genus, confirmed initial plant identifications by propagating the material under glasshouse conditions until differentiation and identification were unambiguous. In addition to the samples of *P paradoxa* from naturalised populations at Moree and Marsden NSW, there were two accessions from the Mediterranean area (S179 from Crete and CPI 14073 from Italy) and a non-descript variety (GH 2/1) of unknown source. The sample from Crete (S179) had more leaf development than the others and was later flowering, whilst the sample from Moree had more stem and was earlier flowering.

TLC comparison of the *P paradoxa* extracts showed a qualitative similarity in the alkaloidal components from all samples. Quantitative comparisons were not attempted because we were not trying to ascertain pan-seasonal, climatic, or individual plant population, toxin variations. The major alkaloidal components were phalarine, coerulescine and 2-methyltetrahydro-β-carboline (2Me-THBC). In contrast to the other samples, the relative amount of 2Me-THBC in the sample CPI 14073 (Italian heritage) was predominant, with only trace amounts of phalarine and coerulescine. The major difference between the *P paradoxa* samples and an extract from *P coerulescens* collected at the site of horse deaths in Darraweit, Victoria, was the presence of 6-methoxy-2-methyltetrahydro-β-carboline (6MeO2Me-THBC), instead of 2Me-THBC, in the *P coerulescens* sample. However, another sample of *P coerulescens*, collected at Hamilton, Victoria, demonstrated the reverse, that is, a similar phytoalkaloidal profile to the *P paradoxa* samples, thus confirming previous observations of the regional differences in the relative amounts of methoxylated and non-methoxylated alkaloids in *P coerulescens* samples.[5] The alkaloidal profiles of the *P paradoxa* and *P coerulescens* samples were quite different to that of the sample of *P aquatica* analysed at the same time, which contained only trace amounts of phalarine and coerulescine, but large amounts of the expected methylated tryptamines.

The HPLC/UV/esiMS analysis supported the tentative TLC analysis of the samples. The analysis of the mass to charge ratios of the molecular ion adducts (M^++ H) clearly confirmed the presence of phalarine (P; M^++ H, 405) coerulescine (C; M^++ H, 203) and 2Me-THBC (M^++ H, 187) in the *P paradoxa* samples and the *P coerulescens* samples *ex* Hamilton and Darraweit. The HPLC/UV/esiMS analysis of the CPI 14073 paradoxa sample confirmed the TLC-indicated predominance of 2Me-THBC over trace levels of phalarine and coerulescine in contrast to the other *P paradoxa* samples and the *P coerulescens* sample *ex* Hamilton where the order of decreasing ion response was phalarine > coerulescine > 2Me-THBC. These observations also confirmed the qualitative increase in the relative amounts of methoxylated alkaloids (MP; M^++ H, 435 : MC; M^++ H, 233 : M2Me-THBC; M^++ H, 217) in the *P coerulescens* sample sourced from Darraweit in Victoria such that the order of decreasing ion response was MC > MP > C > P > M2Me-THBC > 2Me-THBC. In contrast to the *P paradoxa* and *P coerulescens* samples, the sample of *P aquatica* showed only trace amounts of the furanobisindoles (P, MP) and oxindoles (C, MC) relative to the predominant dimethyltryptamine (M^++ H, 189).

The field associations of *P coerulescens* and *P paradoxa* with cases of sudden death in horses were supported by the similarity of the phytoalkaloidal profiles for these two species. It has been postulated[5] that these toxins may be the furanobisindole alkaloid phalarine and or the oxindole alkaloid coerulescine but a causal relationship between ingestion of alkaloids and horse deaths has yet to be established. Cultivars of *P aquatica* have only trace amounts of these alkaloids and have not yet been associated with the sudden death of horses. Conversely the 5-methoxy dimethyltryptamine related alkaloids that commonly occur in *P aquatica* have been responsible for outbreaks of staggers in ruminants but never in horses. There are physiological differences in gastrointestinal function between ruminants and horses and this may account for the apparent sensitivity of horses to the toxins in *P coerulescens* and *P paradoxa* but insensitivity to those in *P aquatica.* Horses for example have the ability to detoxify 5-methoxy dimethyltryptamine and convert it into the less active and more rapidly excretable compound bufotenine (5-hydroxy dimethyltryptamine). This has been demonstated in horses in the United States by administering 5-methoxy dimethyltryptamine orally and then finding bufotenine but no 5-methoxy dimethyltryptamine in post treatment urine samples (RA Sams et al, personal communication). It has also been indirectly demonstrated in Australia by the finding that horses grazing *P aquatica* pastures during late winter and early spring can excrete bufotenine in their urine.[9]

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Efficacy of the prophylactic use of thiamine and pyridoxine in sheep during an outbreak of *Phalaris aquatica* 'Polioencephalomalacia-like sudden death' poisoning

CA BOURKE — NSW Agriculture, Orange Agricultural Institute, Forest Road, Orange, New South Wales 2800

D RENDELL — 170 Mount Baimbridge Road, Hamilton, Victoria 3300

SM COLEGATE — CSIRO Livestock Industries Plant Toxins Research Group, Private Bag 24, Geelong, Victoria 3220

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Phalaris aquatica 'PE-like sudden death' poisoning in sheep has been recognised in the literature since 1992[1,2] but has presumably occurred since phalaris pastures were first sown in Australia. The clinical signs of *Phalaris* PE-like sudden death are very similar to those described in outbreaks of PE. However, their rate of development is much faster, the period between the introduction of animals to a poisonous pasture and death is much shorter, and the number of animals sometimes affected much larger, than that experienced with conventional thiamine-depletion PE. Consequently although we choose to use the description 'PE-like' for this syndrome we acknowledge that it is a convenient name rather than a definitive one, and that the toxin responsible for, and the pathogenesis of, this syndrome remain unknown.

It has been postulated that *Phalaris* PE-like sudden death may involve either a unique rapidly acting thiamine (vitamin B1) antagonist,[1] or a pyridoxine (vitamin B6) antagonist[2] such as that present in *Albizia versicolor*,[3-5] which causes a condition in sheep that shares a close clinical similarity with *Phalaris* PE-like sudden death. Sheep flocks grazing on *P aquatica* cultivar Holdfast pastures on a number of farms in the Hamilton district of western Victoria, during autumn and early winter of 2002, suffered losses due to *Phalaris* PE-like sudden death. When *P aquatica* pastures become poisonous in this way, they only remain so for several weeks, therefore it is the usual practice of many farmers to test suspect paddocks during an outbreak period with a small group of 'sentinel' sheep, before allowing the main flock onto the paddock. We took advantage of this practice, whilst investigating a cluster of cases of *Phalaris* PE-like sudden death at Hamilton between April and July 2002, to assess the efficacy of thiamine and pyridoxine in protecting sheep against this form of phalaris poisoning.

Based upon an assumption that many sheep may have the ability to quickly adapt to the sudden death toxin it was hypothesised that the prophylactic administration of thiamine or pyridoxine might prevent anticipated high mortality rates long enough for sheep to adapt and thereby make use of otherwise unusable pastures. A precedent for such an approach can be found in the work of Gummow et al, [5] where they successfully used the oral administration of pyridoxine to protect sheep against the poisonous effects of the ingestion of pods from *A versicolor*. If a protective effect could be demonstrated for thiamine or pyridoxine it would also assist in a more focused analytical chemistry approach to the isolation and identification of the unknown toxin.

Either thiamine or pyridoxine was administered to groups of sentinel sheep in five separate trials conducted during the Hamilton outbreak (Table 1). Merino ewes of 45 to 60 kg body weight were used. Within each trial the available sheep were randomly distributed among groups. All the treated sheep were dosed about 1 h before access to the phalaris pasture; most received a second dose 24 h later (Table 1). All sheep were observed regularly for the first 48 h. Thiamine and pyridoxine were supplied as their hydrochlorides. For intramuscular injection of thiamine a solution containing 125 mg/mL (Vitajek^{R}, Roche) was used. For oral administration both thiamine and pyridoxine were freshly prepared as aqueous suspensions containing the required dose per sheep in 60 mL. (Prepared from dry powder [Roche], or from crushed pyridoxine tablets [Blackmore] for trial 1.) For the purpose of these trials any animal that died within 48 h of being moved onto a phalaris pasture was presumed to have died of *Phalaris* PE-like sudden death.

The results are presented in Table 1. The intramuscular administration of thiamine in trial 1 failed to demonstrate any prophylactic protective effect for that compound by that route at that dose rate. From the combined results for the oral, repeated dose treatments (trials 1 to 4), it can be seen that there were 8 deaths from 75 (10.6%) untreated sheep, 8 deaths from 75 (10.6%) thiamine treated sheep, but only 2 deaths from 71 (2.8%) pyridoxine treated sheep. This result in favour of a positive pyridoxine effect was statistically significant ($P < 0.05$). However, in the final trial, comparing no treatment with an oral pyridoxine treatment, 4 of 116 animals from the latter group died, whereas none of the 184 untreated animals did. It was concluded therefore that neither thiamine nor pyridoxine afforded any significant protection against *Phalaris* PE-like sudden death under the circumstances of this study. From the results of the final trial, conducted between 2 and 4 July, 34

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Table 1. Sheep treatments and subsequent deaths, over all treatment trials, in groups placed on potentially toxic *P aquatica* pastures at Hamilton, Victoria, during 30 May to 4 July 2002.

		Treatment				PE-like deaths (No.)	
Trial No.	n	Substance	Route	Dose (g)	No. doses[a]	0 - 24 h	24 - 48 h
1	15	Nil				4	0
	15	Thiamine	IM	1.0	1	3	1
	15	Thiamine	PO	1.0	2	1	0
	11	Pyridoxine	PO	1.0	2	1	0
2	20	Nil				0	2
	20	Thiamine	PO	2.0	2	2	1
	20	Pyridoxine	PO	2.0	2	0	0
3	20	Nil				0	0
	20	Thiamine	PO	2.0	2	2	1
	20	Pyridoxine	PO	2.0	2	1	0
4	20	Nil				1	1
	20	Thiamine	PO	2.0	2	1	0
	20	Pyridoxine	PO	2.0	2	0	0
5	184	Nil				0	0
	116	Pyridoxine	PO	5.0	1	4	0

[a]First dose was given 1 h before access to phalaris pasture, second dose 24 h later.
IM, intramuscular. PO, per os.

days after the start of trial 1 and 10 weeks after the first outbreak in this district this year, it was concluded that the period of pasture toxicity was coming to an end. Consequently no further trials were conducted.

The results of the present study do not support the hypotheses of thiamine or pyridoxine antagonists being involved in the pathogenesis of this poisoning syndrome. However because cause of death in these trials was presumptive it still remains possible that the apparent protective effect observed with the pyridoxine treatment up until the last trial was real. Consequently further trials using a pyridoxine pre-treatment and a complete pathological investigation of any sheep that die would still be worthwhile. This study utilised conventional clinical veterinary advice for the selection and application of dose levels with no attempt to quantify resultant systemic levels of the agents administered. Whether a greater protective effect could have been achieved by a method that maintained higher systemic levels of either agent remains unknown. Likewise, whether such antagonists are involved, but that the rate of progression of the condition is so fast and overwhelming that affected animals cannot respond to the prophylactic administration of thiamine or pyridoxine also remains unknown.

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