Australian Society for Veterinary Pathology

VETERINARY PATHOLOGY REPORT

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SECRETARY'S REPORT

"Veterinary Pathology Report" now has the status of a publication (ISSN 1445-1999). It will appear in the ISSN International Register and grace the shelves of the National Library of Australia!

2001 ANNUAL ASVP CONFERENCE

Tony Ross, Coordinator of the ASVP QA Committee, reported that the first slides of the Histotechnique and Histopathology QA Programme have been circulated and results complied. Tony and his colleagues are to be congratulated on this important initiative.

The meeting resolved that the executive should proceed with the establishment of an ASVP website. It is proposed that this website will contain a membership list and details of the executive, an ASVP membership application form, a register of Australian veterinary pathology laboratories and job advertisements. Further sections will be protected by a password and restricted to ASVP members only - any news for circulation and the Veterinary Pathology Report.

A contract has been given to an experienced IT technician at the Institute of Medical & Veterinary Science (Adelaide) to construct the website and it is hoped that in the not too distant future we will have an e-Vet Path Report!

The Registry of Domestic Animal Pathology is now under review. The meeting was of the opinion that the Registry will have more chance of support from industry if it can be demonstrated that the material it contains can be readily accessed by veterinary pathologists around Australia. Roger Kelly will be part of this process by presenting the next "travelling slide session".

The meeting expressed the view that the ASVP should monitor training opportunities for new pathologists, particularly in view of the demographics of our discipline, the decline of many State veterinary laboratories, and the inadequate funding of veterinary schools.

It has been a tradition at these conferences to present an award to relatively new members of the pathology discipline or non-veterinarians engaged in some aspect of investigative veterinary pathology. The ASVP executive has decided on a joint presentation this year - to **Karyn Orzeszko**, University of Melbourne, for her presentation on the genesis of canine prostatic neoplasm and **Mark Krockenberger**, Sydney University, for his talk on cryptococcosis. Congratulations to both and a $50 prize will be awarded.

Karyn has a B.Appl Sci from RMIT and has extensive experience here and overseas as a laboratory histologist. In brief, her studies (funded by a grant from the Canine Research Foundation) indicate that most canine prostatic tumours probably arise from prostatic urothelium.

Mark is a Sydney veterinary graduate (1993) who incorporated a BSc (Vet) on the digestive physiology of the southern elephant seal (1991) into his course. After graduation, he worked in large and small animal practices in NSW, Vic and the UK, before embarking on a PhD on cryptococcosis in koalas and lecturing to undergraduates in veterinary pathology.

Many thanks to all those who contributed to the success of this meeting, as invited speakers, case presenters or active audience participants.

The 2002 ASVP meeting will be held in Adelaide on May 4 and 5 at the Robson Theatre, Institute of Medical and Veterinary Science (IMVS). The meeting also approved the suggestion that the theme for this conference should be "Forensic Pathology", an appropriate subject for the State that gave you the infamous Snowtown "Bodies in the Barrels" case! It is, therefore, not too early to start thinking of your forensic case presentation.

JOHN FINNIE

TREASURER'S REPORT

Carried forward	12,849.74
Income	
Members fees[1]	5,907.00
Interest	115.72
Other income[2]	298.32
AGM registrations	5,992.00
TOTAL INCOME	12,313.04
Expenses	
AVA administration services[3]	1,095.81
AGM costs[4]	11,505.78
Bank fees	170.70
Misc expenses[5]	273.14
TOTAL EXPENSES	13.045.43
CLOSING BALANCE	**12,117.35**

ROBERT RAHALEY, HONORARY TREASURER

[1] Membership fees include arrears fees paid by members. Actual potential annual income for the Society with current membership and fee structure is $4,795.

[2] Includes conference sponsorship and a $48.32 adjustment to the accounts.

[3] AVA SA administers membership fee collection and collation/postage of the Vet Path Report.

[4] AGM costs include some costs from 2000 AGM in Fremantle.

[5] Includes postage, Financial Institute Duty and Debits Tax.

UNDERSTANDING OF TSEs

Deborah Middleton

BIOLOGY OF TSEs

The first Transmissible Spongiform Encephalopathy recognised was scrapie - known for over two hundred years and deduced by Icelandic farmers to be infectious. Transmission of the disease was demonstrated in 1934 by inoculation from sheep to sheep and in 1961 scrapie was transmitted from sheep to mice. The mouse and similar laboratory animal models have been critical to much of the research into this group of disorders.

Nature of the infectious agent

This has been the source of much investigation and continuing controversy and three views have been propounded.

1. a small virus
2. a small informational molecule + host protein (virion)
3. a self replicating protein

The self-replicating protein is currently a strongly argued hypothesis although it is important to note that there are senior research groups still favouring the virino hypothesis.

Prion Protein

It is accepted that a protein is central to the transmission and pathogenesis of TSEs. In the 1980's, a 27-30kd protein specific for infected tissues was identified via **proteinase extraction** of host tissues. This protein was termed **PrP** for protease resistant protein. The PrP gene is present and active in both normal and infected animals and produces the same number of transcripts of a 33-35 kd cell membrane associated protein. The function of the protein is unknown and PrP knockout mice grow and reproduce normally as well as being resistant to TSEs. In normal animals, the protein is termed PrPc and has about 40% α-helical and 10% β-sheet content. It is a soluble protein and is sensitive to proteinase K. It has a 3 to 6 hr half-life.

In infected animals, the protein is identical in amino acid sequence to PrPc but has undergone a conformational change to a 40% β-sheet content and 25% α-helical content. The protein is termed PrPsc, is insoluble and is partially resistant to proteinase K degradation - proteinase K removes only 67aa leaving a 27-30kd protein residue. Turnover is very slow if at all and it can aggregate to form amyloid.

Proteinase extraction is still central to many modern tests for TSE diagnosis.

Pathogenesis

Most investigative work has been done with scrape agent. Peripheral challenge either through the diet or through the skin (IP or IV) is followed by proliferation in lymphoid tissue. Arrival in lymphoid tissue may be via mobile dendritic cells but the precise method is not known. This is an important phase that precedes neuroinvasion and the presence of agent in lymphoid tissues is central to concerns about agent spread following organ transplantation and blood transfusions.

Follicular dendritic cells have a critical role in the lymphoid phase of the disease. The normal role of FDCs is to trap and retain native antigen in the form of immune complexes for presentation to B cells. PrPsc has been found to accumulate in the germinal centres of animals with TSEs. It appears as an extracellular material between the cellular processes of FDCs as well as in the lysosomes of macrophages. It has been shown that interference with the function of FDC blocks agent replication in the spleen and delays neuroinvasion.

The presence of PrP on the surface of lymphocytes in blood may be irrelevant to the development of neuroinvasion and direct spread to peripheral nerves is more likely. The mechanism of transfer of infectious agent into nervous tissue is unknown. Germinal centres of lymphoid tissues are poorly innervated and it may be that B cells or macrophages play a role in transferring agent to more highly innervated parts of the tissue.

The species barrier

It has long been recognised that TSEs exhibit a longer incubation period on first passage into a new species than after the disease has stabilised in a new species. This may be thought of as a natural resistance to infection and is called the “species barrier”. It is a complex phenomenon that is a consequence of many factors including the nature of the host and infecting prion proteins and the strain of infecting agent. The importance of the species barrier is unpredictable and the size of the barrier between, for example cattle and people has not been quantified for obvious reasons. However, it is likely that it will be larger than the barrier from cattle to cattle (normally referred to as 1 or non-existent). Another important issue is the barrier from cattle to mice as this affects interpretation of infectivity studies in tissues. It has been estimated that mice are at least 1000 times less sensitive as an assay model than cattle.

Animal TSEs

CWD occurs in mule deer and Rocky Mountain Elk in Wyoming and Colorado.

Scrapie: Along with CWD scrapie is the only TSE known to be associated with lateral transmission in the field. TME was responsible for several outbreaks in Wisconsin in the USA. The origin was not determined but there was no history of exposure to sheep tissues, only fallen cattle. FSE and EUE have been strain typed as BSE.

Human TSEs

Kuru - in many ways the best known of the human TSEs from its association with ritual cannibalism with the Fore tribes of New Guinea.

CJD - sporadic or iatrogenic associated with use of neurosurgical instruments, electrodes, dura mater and corneal grafts, and cadaver sourced hGH, GSS and FFI.

vCJD - in 1995 a neurological syndrome similar to spCJD but with significant differences was identified in younger people. It had a long clinical phase with presentation as ataxia, peripheral sensory disturbances, behavioural changes and other psychiatric symptoms. It was characterised by EEG changes not typical of spCJD and had different neuropathology. There was extensive involvement of the cerebellum consistent with ataxia as a presenting sign and large florid amyloid plaques.

Human PrP gene

The prion protein is found on human chromosome 20 and there are at least two polymorphisms that can impact on expression of TSEs.

Codon 129: ATG to GTG
Meth to Val

Methionine/valine heterozygosity and valine homozygosity lead to a later age of onset and longer clinical disease.

Codon 178: Aspartic acid to asparagine influences the kind of TSE to which the person is susceptible.

All vCJD cases studied so far (>80) have been homozygous for methionine at codon 129. Other genotypes may be seen later in the epidemic as was noted in the hGH disease event. It is difficult to assess the ultimate size of the outbreak of vCJD in people as the range of incubation periods is as yet unknown and the number of people exposed to an infectious dose may never be determined.

Ovine PrP gene

Much work has been done on the genetic susceptibility of sheep to scrapie. Several variant alleles of the ovine PrP gene have been found leading to many possible combinations of genotypes.

The most important of these are polymorphisms at codons 136 (alanine/valine), 154 (arginine/histidine) and 171 (glutamine/arginine/histidine). The situation is made more complex as the allele combinations are distributed differently in different breeds.

Ovine PrP gene susceptibility

The illustrated allelic combinations demonstrate increasing susceptibility to scrapie. Knowledge of sheep genotype is particularly valuable in ram selection and the incidence of clinical scrapie can be controlled by selecting for resistant genotypes. ARR confers a low risk in all breeds and has a dominant influence.

In Cheviots, AHQ also confers a low risk.
In Suffolks, VRQ is rare in any case.

Bovine PrP gene

Similar polymorphisms have not been identified in cattle.

Agent strains

This has been referred to previously when the species barrier was mentioned. It is a critical concept to understand when thinking about TSEs and it provided the scientific link between BSE, FSE, EUR and vCJD. Many agent strains have been recognised in scrapie and these are indistinguishable in the source species the sheep. Only one strain has been recognised in BSE and this has remained the same throughout the outbreak. It is not similar to any recognised scrapie strain although this does not preclude its origin in scrapie. It is known that passage of scrapie through different species can lead to permanent changes in its biologic properties. In a sense, agent strains are a reproducible laboratory artefact. They describe the characterisation of TSE isolates largely on the basis of their behaviour in genetically defined strains of laboratory mice.

Incubation period: enclosed in mice by the Sinc gene (the same as the PrP gene) which has two alleles s7 (leucine 108, threonine 189) and p& (phenylalanine 108, valine 189). The effects of s7 and p7 on incubation period depend on the strain of agent.

Mice commonly used in strain typing studies are RIII and C57BI (s7,s7), VM (p7,p7) and an s7,p7 heterozygote.

Lesion profile: a semiquantitive representation of the distribution of vacuolar changes in 9 grey matter regions and 2 white matter regions of the mouse brain. The system was developed to streamline the process of examining numerous brain sections.

vCJD: BSE
The aetiological connection between vCJD and BSE was provided by the “Queniborough cluster”. The cluster was first recognised in 1998 following 3 deaths from vCJD in the space of 12 weeks in a small village in Leicestershire. Now 5 deaths have occurred and the village is colloquially referred to as the “the village of the damned”. Exhaustive epidemiological studies have made a connection between butchering practices supplying meat to the village that would have lead to contamination of meat for human consumption with brain tissue from cattle. The critical period when the method of head splitting was carried out was 1980 and 1991.

TSE diagnosis - clinically affected animals.

Histopathology - does not guarantee a diagnosis in all clinically suspect cases especially if the material is autolyzed or some normal vacuolation gives rise to inconclusive results.

SAF - misses about 20% to 25% of cases but is useful if the tissue is autolyzed.

Immunohistochemistry - approaches immunoblotting in sensitivity especially when appropriate antigen retrieval processes are carried out and amplification steps employed. Antibodies are not specific to PrPsc but fixation and processing tend to make PrPc unavailable for staining. Therefore the techniques essentially detect PrPsc.

Immunoblotting - The "gold standard" test. It produces three gel brands in the final picture. There has been considerable discussion on the significance of the mw of these bands in determining the nature of the infecting agent (BSE vs scrapie) but there is no clear importance at this stage that can be attached to their appearance.

Rapid post mortem tests

TSE diagnosis - preclinical

This has focussed on detection of PrPsc which is a **problem with BSE**. BSE has only been detected in the brain and spinal cord of naturally infected cattle, and in the distant ileum (early) and bone marrow (late) in experimentally infected cattle. None of these lend themselves to peripheral sampling methods.

There is considerable interest in the use of peripheral **lymphoid tissues** for detection of PrPsc in scrapie and in vCJD. It is found in the tonsils and third eyelid tissue of sheep that subsequently developed scrapie and in the appendix of a patient that developed vCJD later. It has also been used in tonsillar biopsy to confirm a suspect vCJD patient. However, it is known that some scrapie strains do not replicate in lymphoid tissue of sheep and third eyelid tissue (sampling does not require anaesthesia) atrophies in older animals.

Effort is going into improved **concentration methods** for detection of PrPsc in for example urine, CSF and milk. Prion infectivity is packaged so a huge sample size is needed to test these sample times - hence the need for improved concentration methods.

ICE test - competitive immunoassay with signal picked up by capillary electrophoresis. The test was developed for use on buffy coat and is thus applicable to scrapie but probably not BSE. It is likely to be used as a flock test rather than a test for individual animals and a lot of work has yet to be done to translate it into a useful field diagnostic test.

Epiphenomena

The best hope may be for recognition of an epiphenomenon around which a diagnostic tests can be based eg urine protein excretion of a non-infectious protease resistant PrP isoform.

Diagnosis of BSE vs scrapie

There is as yet no test for differential diagnosis of cattle and sheep TSEs in both species apart from strain typing studies in mice. Some hope was expressed in examination of **glycosylation bands** on immunoblots - the unglycosylated band is at the foot of the gel, two monoglycosylated bands occur together and the diglycosylated form is at the top. The size of the bands overlaps with BSE and some sheep scrapie that were strain typed as non-BSE so the significance of the observations is unclear at this stage.

Strain typing in mice is still the only way of characterising the agents but is too long, expensive, uses animals and has a low throughput.

BSE in sheep

This is an important issue. Several experiments are underway to determine whether BSE is present in sheep in the field in UK. This is important particularly as experimental BSE in sheep behaves like scrapie in that it has a lymphoid proliferation phase. Should it also laterally transmit like scrapie it may then provide a continuing source of infection to the human population. A particular problem is that BSE in sheep may not be diagnosed as different from scrapie - experimental disease could not on first appearance be definitely excluded from being scrapie.

DEBORAH MIDDLETON
SENIOR VETERINARY SCIENTIST/DIAGNOSTICIAN
DIAGNOSIS & EPIDEMIOLOGY UNIT
AUSTRALIAN ANIMAL HEALTH LABORATORY

ASVP VETERINARY HISTOPATHOLOGY QUALITY ASSURANCE PROGRAM

Following a resolution at the 2000 ASVP annual general meeting, a one year trial histopathology QA program began in March 2001. It is a quarterly assessment of the diagnostic skills of the histopathologist and the slide preparation skills of the histotechnologist. Tony Ross is the coordinator and the funds are from the National Registry of Domestic Animal Pathology.

Participation is free. Responses can be individual or the collective view of all pathologists at the lab. Assessors will change for each round and individual results are confidential. In March, June, September and December 2001, duplicate unstained slides from 2 cases are sent to participating laboratories. Both sets of slides are stained. One set is retained and the other sent to the coordinator for histotechnical evaluation. Histopathology reports should include: description, morphological diagnosis, aetiological diagnosis and discussion of further tests and follow up. Responses using the participant's code are emailed or faxed to the coordinator who collates them and passes them to the assessor. Individual and collated results for reports and slides are sent to participants.

The program meets the needs of vet labs and NATA for participation in external QA programs. Almost all vet labs in Australia are enrolled with: 12 public sector, 8 private sector, 5 university labs plus 5 independent pathologists participating. A total of 76 pathologists are involved. A review of the trial will be conducted in early 2002. It is probable that a proposal will be put to ASVP for continuation as a user pays QA program. Results of the first round were released at the May 2001 ASVP AGM in Melbourne.

For further information, contact Tony Ross, NSW Agriculture, ph 02 4640 6312, fax 02 4640 6400, email tony.ross@agric.nsw.gov.au.

WORKING PARTY ON PROFESSIONAL STANDARDS

At the 2000 AGM, a report of the Working Party on Professional Standards was accepted by the meeting and several resulting issues were discussed. The meeting then carried a motion that:

- **The ASVP strongly endorses Proficiency Testing and Continuing Education for veterinary pathologists in Australia.**
- **The ASVP propose an ongoing and evolving scheme for the above.**
- **If the decision from SCAHLS is not forthcoming in 3 months, a trial program is instituted with funding from the previously endorsed SCAHLS National Business Plan.**

This report is to advise on progress since that time.

1. There has been no further interchange between members of the working party and therefore no additions or modifications to the report tabled at the last AGM. No submissions from ASVP membership were received by the Chairman.

2. SCAHLS met in July 2000 and agreed that the NRDAP had an important role in the maintenance of pathology standards in Australia and that provision be made in the submission to Animal Health Australia for funding support. This request for support was subsequently rejected.

3. SCAHLS met again in December 2000 (by teleconference) and agreed that a proposal for a new SCARM agreement to fund the NRDAP (with the option of two levels of funding) be prepared. The paper was prepared and circulated to SCAHLS members but to date the issue remains unresolved.

4. A submission to AHA from AFFA, which included a request for support, resulted in AHA agreeing to the allocation of $5,000 to fund a review of NRDAP. The review is to be chaired by Terry Nichols but the team has not yet been announced.

5. A trial Mystery Slide scheme, proposed by Tony Ross at the 2000 AGM, was conducted during April 2001. Sections from a mammalian and an avian case were circulating to participating laboratories to test both histoprocessing skills and histopathological interpretation skills.

RB RICHARDS
CHAIRMAN, WORKING GROUP ON PROFESSIONAL STANDARDS

NATIONAL REGISTRY OF DOMESTIC ANIMAL PATHOLOGY

Most activities of the National Registry continued during 2000 despite the continuing absence of a Registrar. Professor Roger Kelly of the University of Queensland delivered the 2000 continuing education courses in each state and territory. The courses reviewed emerging diseases of pigs including Menangle virus, Nipah virus, Porcine respiratory and reproductive syndrome and Porcine circovirus syndromes (dermatitis and nephropathy syndrome, postweaning multisystemic wasting syndrome). In addition, diseases of the liver were reviewed with participants taking advantage of Dr Kelly's international expertise in this area.

In 2001 the courses will again be delivered by Dr Kelly. The themes will be vesicular diseases of domestic animals and arboviral diseases of domestic and wild animals.

The slide-of-the-month quality assurance coordination continued under the guidance of Dr Rod Reece.

A refunding bid with 2 options was put to the subcommittee on Animal Health Lab Standards in December 2000 for decision and recommendation to Animal Health Committee - no decision at May 2001. An earlier approach to Animal Health Australia resulted in AHA commissioning a review of the Registry. This will be chaired by Dr Terry Nichols of AFFA and is expected to be completed by October 2001.

Outcomes for each option are listed below:

Activities	Option 1	Option 2
Slide-of-the month	✓	✓
Annual training course	✓	✓
Quarterly proficiency testing	✓	✓
Collection renovation	✓	✖
Tutoring for pathologists	✓	✖
Electronic access	✓	✖
Second opinion service	✓	✖

Note: Proficiency testing will operate on a "user pays" basis; the cost to administer the scheme will be minimal.

OPTION 1

The estimated cost of full activities is:

Year	2001/02	2002/03	2003/04
Half salary	$34,000	$35,000	$36,050
On costs (20%)	$6,800	$7,000	$7,200
Travel/accommodation	$6,500	$6,750	$7,000
Histoprocessing	$2,000	$2,200	$2,400
Photography/IT	$2,000	$2,000	$2,000
Postage	$1,000	$1,000	$1,000
	$52,300	$53,950	$55,650

OPTION 2

The estimated cost of basic activities is:

Year	2001/02	2002/03	2003/04
Consultant	$10,000	$10,500	$11,000
Travel/accommodation	$6,500	$6,750	$7,000
	$16,500	$17,250	$18,000

The Registry Management Committee strongly recommends Option 1.

Renovation of the slide collection - in the form of restaining and cover slipping has begun and is expected to take 2 years to complete.

An exciting new initiative managed by the Registry began in March this year. A national quality assurance scheme for veterinary histopathology has begun. See separate report for details.

Collection enhancement, updating software, second opinion services and tutoring are no longer offered. However, pathologists are still welcome to visit the Registry for self tuition.

A NEW NAME

The Registry name suggests a static collection locked in a lab somewhere. It does not reflect the active delivery of continuing education, QA and other services. The management committee is soliciting suggestions for a new name for the Registry. How about PathQual, Auspath or OzePath? All offerings welcome.

Finally, the committee and ASVP executive would like to thank John Glastonbury for support and years of service on the Registry Committee. John has resigned as he is moving out of the Regional Veterinary Laboratory at Menangle to a field staff training position based at Wagga Wagga.

For further information contact Tony Ross ph 02 4640 6312, fax 02 4640 6400, email tony.ross@agric.nsw.gov.au.

MEMBERSHIP RECORDS

AUSTRALIAN SOCIETY OF VETERINARY PATHOLOGISTS

In order to update our membership records, particularly regarding email addresses, it would be appreciated if members could email to our administrative assistant, Barbara Gill, at avasa@ava.com.au, the following:

- Name
- Address
- Telephone number(s)
- Email

Myocarditis due to *Haemophilus somnus* in yearling cattle

Patrick Staples, Regional Veterinary Laboratory, Orange

300 yearling cattle were being strip grazed on lush clover-based pasture. Nine animals died over a four week period. The animals were found dead on routine inspections made every three to four days. There was no evidence of healthy animals bloating. One sick, pyrexic steer recovered with antibiotic treatment. One moribund steer was autopsied with samples submitted to the laboratory. At autopsy myocardial haemorrhages were noted. Heart, skeletal muscle, kidney and liver were examined histopathologically revealing the following lesions; severe, acute, suppurative and necrotising myocarditis with thrombosis and small gram negative bacilli present intravascularly; skeletal muscle contained a focal, acute necrotising myositis, associated with bacterial colonies; occasional renal bacterial emboli were present. Culture of liver and heart yielded mixed bacterial growths with *Haemophilus somnus* predominating. The meningoencephalitis, pleuropneumonia and myocarditis forms of the *Haemophilus somnus* disease complex occur most commonly in feedlot calves from 6 to 12 months of age. The myocardial form of the disease seen in this case may have been predisposed by the management system - strip grazing at high stocking rate.

Spindle cell tumour in a 4 year old Shitzu male

Alan Kessel, Rural Veterinary Centre, Camden

The animal was admitted to the clinic with an 8mm subcutaneous mass at the lateral abdomen and two previous masses had been removed from this area, the most recent 10 days ago. A previous histopathology report diagnosed a spindle cell tumour, possibly a schwannoma. Surgical excision of the mass revealed mostly reactive scar tissue, but there was a microscopic dense cluster of moderately pleomorphic spindle cells at one border of the section. Within 6 weeks several masses had regrown at the site and were forwarded for histopathology.

The larger dorsal midline mass (approximately 2.5 cm x 1.5 cm) and the smaller ventral mass (approximately 1 cm diameter) are of similar morphology. The masses are well circumscribed but non-encapsulated multinodular dense collections of neoplastic cells. The cells are arranged as closely packed sheets divided at irregular intervals by slim strands of fibrovascular tissue; thicker strands of this tissue divide the mass into grossly visible nodules. The neoplastic cells fall morphologically into two types. The predominant cell type contains an oval to irregular vesicular nucleus with a small prominent nucleolus. Cell outlines are indistinct, so that some cells appear to be multinucleate or even syncytial. The cytoplasm is generally vacuolated and may also appear eosinophilic and fibrillar - in most 400 x field there are isolated cells with more extensive eosinophilic cytoplasm and a spindle shape.

These cells are in some instances multinucleated and have the appearance of reactive proliferating skeletal muscle fibres. In other cells there is little cytoplasm but their prominent rowing of nuclei. At the edge of the nodules this cell type is somewhat infiltrative and has a classic spindle appearance. The mitotic rate averages 6/400 x field. In the other cell line the nucleus is smaller and the chromatin markedly condensed; cytoplasm is clear and the nuclei appear to be surrounded by a clear zone. These cells are diffusely infiltrated throughout the other neoplastic cells (which predominate); however, there are regular collections of 20-30 of these clear cells. There is the very occasional cell that combines aspects of both cell lines. In one area of fibrous tissue separating two nodules of neoplastic cells are two small capillaries that contain a dense collection of neoplastic cells in their lumens. All lateral margins appear clear of tumour cells and the deep thin fascial margin is clear.

Results of Immunohistochemistry:

Negative:	keratin, S100, neurofilament
Intermediate:	smooth muscle actin
Positive:	vimentin
	Desmin (also weak cytoplasmic positive in adnexa and epidermis)
	Myoglobin - good positive (also weak nuclear staining in epidermis and adnexa)

Thus it is a spindle cell tumour (positive vimentin), of muscle origin (desmin and myoglobin).

Please note that these tumours are rare. Most arise in tissue devoid of striated muscle fibres - the origin is believed to be a pluripotential mesenchymal cell. Most are reported in young animals (average age 2-3 years) and about one third are associated with the bladder wall. Often they appear to be congenital (especially those in the heart).

True rhabdomyosarcomas are very aggressive usually, and often metastasise to skeletal muscle. Local lymph nodes and lung are other common sites.

NB: This animal was lost to follow up - didn't even pay the bill.

Scids in an Arab foal

Alan Kessel, Rural Veterinary Centre, Camden

The clinic received a tracheal wash from this animal when it was one month old. The animal had presented to the referring veterinarian with an apparent respiratory condition that was partially responsive to antibiotics. The wash contained large numbers of neutrophils and a pure growth of serratia liquefasciens was obtained. The next sample obtained were second opinion slides of tissue taken during a very limited post mortem when the foal was 3 months of age. Histologically there were scattered areas of atelectasis with a purulent bronchopneumonia. On closer examination of the lung sections there were large numbers of large basophilic intranuclear inclusions in the epithelial cells that line the bronchi and some of the bronchioles strongly suggestive of adenovirus infection.

It is highly likely that this animal is suffering from Severe Combined Immunodeficiency -SCID - a recessively inherited disease in Arabs that results in almost complete atresia of lymphoid organs and thus severe immunodeficiency. These animals succumb often to what would otherwise be minor infections. Adenovirus infection here strongly suggests this diagnosis - the only time I have seen it is in cases of SCIDS. As well you should find a persistent peripheral lymphopaenia and minimal amounts of immunoglobulin in the blood. There is a test to detect carriers - this is of importance as if this animal is a SCID foal then both the sire and dam are carriers and this is an important fact to ascertain for future breeding of these animals.

The referring veterinarian was contacted and a more complete history obtained - the foal was a pure bred Arab, and on two previous complete blood counts the animal had been markedly lymphopaenic (0.1 x 10/9/1, and 0.2 x 10/9/1). The sire has travelled overseas, but the veterinarian has urged the owner of the dam to have the animal assessed.

Necrotic enteritis and peritonitis in farmed juvenile barramundi

John Humphrey, Berrimah Veterinary Laboratories (BVL), NT Department of Primary Industry and Fisheries

A syndrome of peritonitis and necrotic enteritis is described as a cause of ongoing, low grade mortalities and hatchery-reared juvenile barramundi reared in nursery and grow-out stages.

History and Clinical Signs:

The syndrome occurs initially in nursery fish of approximately 40-50mm and continues in fish up to 300-350mm following transfer to sea cages for grow-out.

Initially, fish show mild abdominal distension. Subsequently, marked distension of the abdomen occurs and fish swim lethargically at or near the surface. In early stages, some fish continue to feed, but ultimately affected fish cease feeding, die and then undergo rapid putrefaction.

A prevalence of approximately 0.005% to 0.2% on any one day is recorded. The syndrome continues over weeks to months. Prevalence appears to increase following environmental stress and decrease with restricted feeding.

Gross Pathology:

Affected fish present with distended abdomens and on occasions, show localised or generalised hyperaemia with ecchymotic haemorrhages in the abdominal integument. On palpation, the abdominal contents are soft and fluctuant. Discoloured flocculant fluid is readily aspirated from the abdominal cavity.

A temporal picture of initial necrotic enteritis with peritonitis, ultimately resulting in partial or complete loss of intestinal structure, exuberant necrosis of the visceral mass and intense abdominal suppuration is evident on examination of opened abdominal cavities. Copious amounts of putrid, fibrinopurulent, fatty or oily exudate are present within the abdominal cavity. Often, fibrinous adhesions bind the visceral mass and extend to the peritoneal surface of the abdominal wall. The hepato-pancreas and abdominal fat show moderate to severe necrosis. On careful dissection, the stomach, pyloric caeca and intestines may show severe necrosis and in some cases perforations of the intestine are present. In all but the earliest cases, the tissues of the visceral mass are friable and tear readily.

In some cases, fish have fresh ingesta in the stomach and similar material may be seen in the abdominal cavity.

The internal surfaces of the swim bladder and the underlying kidney are generally spared from the degenerative and necrotising processes occurring in the abdominal cavity.

Histopathology:

Histopathological changes primarily relate to massive necrosis of visceral tissues including the mucosae of the stomach, intestine, pyloric caeca, necrosis of the hepato-pancreas, an intense inflammatory cellular infiltration of the peritoneal surfaces of the visceral organs, mesentery and abdominal wall and extensive fibrinous deposits.

Massive bacterial colonisation and proliferation in affected tissues is invariably present. Gram negative rods predominate but other Gram positive and Gram negative bacteria may be present. In many cases, bacterial proliferation is evident primarily in the lumen of the intestine and the pyloric caeca.

Associated changes include granular degeneration of abdominal muscle, pericarditis and myocarditis, haemorrhage and oedema.

Tissues in advanced cases have an appearance of massive autolysis and putrefaction, even though the fish may have been sacrificed for examination. Other tissues are well preserved. In earlier cases, severe, localised or generalised necrosis of the mucosa of the intestine accompanied by bacterial proliferation in the lumen is evident.

Compared to healthy fish, extra-hepatic exocrine pancreatic tissue is difficult or impossible to detect in the abdominal fat or mesentery of affected fish. Similarly, within the hepato-pancreas, exocrine pancreatic tissue is sparse, atrophic and generally devoid of secretory granules compared to healthy individuals. In some cases, a local cellular inflammatory response is centred on these atrophic pancreatic acini.

Bacteriological Examination:

Large numbers of bacteria of mixed morphology, representing both Gram negative and Gram positive organisms are present on Gram staining of peritoneal fluid. The predominant bacterial types are Gram negative rods, with lower numbers of *Clostridium*-like species present.

Cultural examination of the abdominal fluid usually results in a mixed bacterial flora. In the majority of cases examined, *Photobacterium damsela* and *Vibrio harveyi* are isolated as the predominant organisms, except for advanced cases where massive suppuration in the abdominal cavity predominates.

Streptococcus iniae as not been isolated from the diseased fish.

Eperythrozoon suis in pigs

Lorna Melville, Berrimah Veterinary Laboratories (BVL), NT Department of Primary Industry and Fisheries

In late February a Darwin piggery reported pale, yellow pigs losing condition with a number of mortalities. About 5% of weaners were affected and the sows, while apparently healthy, were showing an increased return to service.

Post mortem examination on a clinically affected nine week old weaner pig with a PCV of 16% showed a markedly enlarged spleen, with evidence of two recent, but healed ruptures. There was a marked parasitaemia of extracellular organisms on erythrocytes, morphologically consistent with *E.suis.*

A further 18 clinically affected pigs were bled. These included litter mates of the previous weaner. One of these pigs died shortly after bleeding and was found to have a PCV of 13%, parasitaemia and similar post mortem findings including marked splenomegaly. Six of the 18 pigs had some degree of parasitaemia. Others showed erythrocyte fragments and increased reticulocytes suggestive of past infection.

Preserved blood samples were sent to the Australian Animal Health Laboratory for electron microscopy. These were examined by both TEM and SEM. The organisms on the surface of the erythrocytes were found to have ultra structural characteristics similar to those described for *E. suis* and *E. suis*-like organisms.

Since then we have also confirmed infection in a second commercial piggery near Darwin. We are currently developing an ELISA to determine the extent of this infection in domestic and feral pigs in the NT and hope also to develop a PCR which will confirm the identify of the organism and improve monitoring for this disease.

Probable neuronal ceroid-lipofuscinosis in a Border Collie

Helen Parkes and Anton Janmaat, Berrimah Veterinary Laboratories)BVL), NT Department of Primary Industry and Fisheries

A two year old male Border Collie showed behavioural changes and proprioceptive and visual deficits for about six months. The clinical signs increased in severity and the animal was euthanased. Post-mortem examination was unremarkable.

Sections of spinal cord, obex, medulla, cerebellum, midbrain, thalamus and cerebrum were processed for histological examination. The majority of neurons contain a variable amount of eosinophilic, granular material forming discrete inclusions in the cytoplasm. The material is PAS positive and is auto-fluorescent in unstained and H&E sections. The PAS stain quenches the auto-fluorescence. We have not examined the neuronal inclusions electron-microscopically as yet. Neuronal ceroid-lipofuscinosis has been described in the Border Collie breed.

Osteomas in "Leather-jackets"

Wendy Townsend, Queensland Department of Primary Industries, Yeerongpilly Veterinary Laboratory

One skeleton of a leather-jacket was received for post-mortem examination. A French chef who had become increasingly concerned about its abnormal appearance as he was eating the overlying tissue had submitted it. The skull and many fin rays were missing. Nodular bony swellings of variable size were observed over the spine and ribs of the remaining skeleton. These were most abundant over the neural and haemal spines. The majority of swellings were solid, however occasional hollow forms were noted.

"Osteomas" in fish are usually hyperplastic nodules that can occur at a high prevalence in some populations e.g pink snapper, leather-jackets and trevallies. They are considered by some better described as hamartomatous rather than neoplastic lesions. As far as can be ascertained, they cause no difficulties for the fish. Their cause is unknown, but they have been seen to occur at a relatively high prevalence in some populations of fish from unpolluted waters.

Sorghum ergot poisoning in feedlot steers

Jim Taylor, Queensland Department of Primary Industries, Toowoomba Veterinary Laboratory

Four of a group of eighteen, 14 month-old Hereford steers developed hyperthermia, panting and salivation while being fed a sorghum based ration. The sorghum had gross evidence of ergot and the grain contained 5mg/kg of di-hydroergosine.

Clinical samples were taken from the four affected animals with no significant haematological or biochemical changes.

Aortic-iliac thrombosis in lambs

Jim Taylor, Toowoomba Veterinary Laboratory

An owner submitted a 9-month-old Suffolk lamb with progressive hind limb paresis. The lamb as one of seven from a group of 40 that had developed hind limb paresis over the previous 6 months The affected lamb had hind limb paresis, coolness of the hind limbs and no detectable femoral pulse. At necropsy, there was focal mural thrombosis of the aorta in the thoracolumbar region and at its bifurcation into internal and external iliac arteries. Both kidneys had marked multifocal renal infarcts.

While aortic thrombosis is a condition reported in horses and cats, no reference could be found to the condition in sheep. No cause was apparent in the animal examined. Hopefully, any additional cases will be submitted for examination.

Glasser's disease in pigs held in lairage

Jim Taylor, Toowoomba Veterinary Laboratory

Two hundred and thirty four pigs were transported to a meatworks and held in lairage for slaughter the next day. The following morning five pigs were dead and 18 were showing nervous signs consisting of lateral recumbency, paddling and exophthalmos. One dead and two live pigs were submitted for necropsy. Lesions were restricted to the meninges with marked diffuse fibrinopurulent meningitis. *Haemophilus parasuis* was isolated from the brains of each animal.

Glasser's disease often manifests as a polyserositis affecting a number of serosal surfaces, but occasionally as in this case only a single serosal surface may be affected.

Indospicine toxicity in a dog

Peter Phillips, IDEXX Laboratories, 33 Flemington St, Glenside, SA 5065
Keith Locke, Sadadeen Veterinary Clinic, Alice Springs, NT 0871

Liver from a dog from a Northern Territory cattle station bordering the Simpson Desert was submitted for histopathology following the dog's demise despite the intensive treatment by the Alice Springs practitioner.

The history included a statement that this dog had never been fed horsemeat. A further check with the owners confirmed that the dog had been fed only dry food and beef.

Histopathology of the liver revealed hydropic degeneration of hepatocytes with obliteration of most sinusoidal spaces. The outstanding lesion was apparent collapse of periacinar hepatocytes with infiltration of macrophages containing light brown pigment appearing to obliterate the terminal efferent arterioles. Small channels of blood vessels and occasional neutrophils infiltrated the area. There was some deposition of fibrin in the periacinar region. This was consistent with lesions as described by Hegarty *et al* (1988) from dogs being fed meat from horses which had grazed *Idigofera linnaei* (Birdsville indigo), the toxic principle of which is the amino acid indospicine (6-amidino-2-hexanoic acid).

A further check on the dog's feeding habits could not preclude some carrion feeding of carcasses, but the owners did confirm that the dog had been eating galahs, which had been consuming the legume's seeds in the station horse paddock. Although no studies of the toxicity of Indospicine-containing bird meat have been done it is considered reasonable to suspect that the galahs may have been the source of the dog's Indospicine intake.

Ref: Hegarty MP, Kelly W R, McEwan D, Williams O J and Cameron R. 1988. "Hepatotoxicity to dogs of horse meat contaminated with Indospicine", *Aust Vet J* 65: 337-340.

Department of Primary Industries Water and Environment

Mount Pleasant Laboratory Report

Philip Ladds

Laboratory strength was reduced temporarily in April when David Taylor travelled to the UK to participate in Foot and Mouth Disease control work. Interesting cases were as follows:

Bovine. Woolly coated cardiomyopathy caused sudden death in a 2-day-old Polled Hereford calf. Mycotic abortion occurred in one herd and *Mortierella wolfii* was isolated from haemorrhagic lung lesions in a 5 year-old cow that died suddenly. Cyanobacterial hepatopathy (See ASVP 2001 Melbourne Proceedings) and severe hepatopathy with many deaths probably due to mycotoxins on the plant Rough Dog's Tail (*Cyanosurus echinatus*), was observed in several herds. In a severe outbreak of *Salmonella dublin* infection in a dairy herd, more than 50 calved died suddenly. Other submissions included *Haemophilus somnus* endocarditis and severe myocarditis in separate animals, probable Bovine Virus Diarrhoea abortion with positive serology but no obvious foetal lesions, and lingual calcinosis - resembling calcinosis circumscripta in the dog - in two animals at an abattoir.

Ovine and Caprine. In sheep, interesting diagnoses included yersiniosis as the cause of diarrhoea, ill-thrift and deaths in several flocks, eperythrozoonosis, multifocal granulomatous pneumonia caused by *Muellerius capillaries* on separate properties, severe necrotising hepatitis resulting from massive migration of immature flukes, and, as in cattle (see above), hepatopathy with massive necrosis considered due to mycotoxins on pasture with *Cyanosurus* sp.; on one property approximately 400 of 1000 sheep died and obvious photosensitisation was apparent in many survivors. In goats, outbreaks of verminous pneumonia (*M. capillaries*) and coccidiosis were diagnosed.

Avian. Diagnoses included lymphoid leukosis, histomoniasis (in one case concurrent aspergillosis) in turkeys, chlamydiosis with splenitis, hepatitis and serositis in a Purpled Crowned Lorikeet (*Glossopsitta porphyrocephala*) and yersiniosis causing 50% mortality in a group of canaries; here was concurrent granulomatous pneumonia caused by the respiratory mite *Sternostoma tracheocolum*.

Wildlife. Submissions included subcutaneous sarcoma and lymphosarcoma in Tasmanian Devils (*Sarcophilus harisii*), chronic myositis with terminal (*Corynebacterium ulcerans*) septicaemia in a platypus (that had probably been attacked by a dog), toxoplasmosis with blindness and severe chorio-retinitis in pademelons (*Thyogale billarderii*), multiple parasitism and trauma (suspected dog attack) in little penguins (*Eudyptula minor*), and pulmonary aspergillosis in several Pacific gulls (*Larus pacificus*).

Sudden deaths of four Australian fur seals (*Arctoephalus pusillus*) that were being relocated by road transport from salmon farms, were found to be due to asphyxiation, due in turn to massive aspiration of regurgitated food ingot the lungs.

Other species. Sudden death of an otherwise healthy alpaca at pasture, was due to a broken neck - the dislocation and haemorrhage being in the lower cervical region.

Rabbit calicivirus infection was diagnosed serologically on a number of occasions in rabbits submitted from most parts of the state. Wild, commercial and pet rabbits were all represented; severe hepatic coccidiosis was also diagnosed in several animals.

Multifocal encephalomalacia and *Enterococcus hirae* septicaemia in chickens

Malcolm Lancaster, VIAS, Attwood, Victoria

300 chickens in a batch of several thousand developed abnormal head posture in their first week post hatching. *Enterococcus hirae* was recovered from 6/6 livers and 1 of 2 brains cultured. Large areas of malacia were present in all brainstems examined and fibrin thrombi were present in associated capillaries.

There are many unanswered questions about this syndrome and the disease has not been reproduced experimentally. However, isolation of *Enterococcus hirae* was a consistent feature (NB. If API test strips are used this organism is identified as *Enterococcus durans*). Disease outbreaks are self-limiting.

Malacic lesions were found in the brainstem, optic tectum and cerebrum, distinguishing them from the malacia of vitamin E deficiency which occurs in the cerebellum. Furthermore, encephalomalacia due to vitamin E deficiency tends to occur in chickens 15-30 days of age, while this *Enterococcus*-associated malacia was found in birds 3-8 days old.

Microsporidiosis in Two Young Koalas

Judith S. Nimmo Wilkie, IDEXX/CVDL, Mount Waverley, VIC

Two six-month old koalas from a fauna park that had appeared normal on leaving the pouch a week previously were found dead. Three or four young koalas had died previously in a similar manner. There were no significant post mortem findings reported.

Microscopic lesions were restricted to the intestinal tract. Villi in the small intestine were irregularly stunted. Crypt epithelium was somewhat disorganised. The lamina propria was rather oedematous and congested and contained increased numbers of mononuclear inflammatory cells and a few granulocytic leucocytes. Enterocytes covering villi and also those sloughed into the lumen of the intestine, were markedly vacuolated and distended with retractile oval organisms. These were also Gram positive.

Electron microscopic studies showed the organisms to be consistent with Microsporidia. Studies are continuing to try to speciate the organism but this protozoa has not been identified in koalas before and it is not certain if this is a new species or if the organism is one from another host that has crossed species.

STATE REPORTS WESTERN AUSTRALIA - DAVID FORSHAW

Epizootic ulcerative syndrome (EUS)

Brad Chadwick, WA Dept of Fisheries, AHL (WA Dept of Agriculture), South Perth, WA

EUS, caused by the highly pathogenic fungus Aphanamyces invadans, has been diagnosed more frequently in WA this year than in the past. A number of fish species have been submitted with chronic ulcerative skin lesions, including ornamental species as well as farmed Golden Perch from the Albany area. There was prolific superficial growth with a secondary fungal species (Saprolegnia sp.) associated with most lesions, however silver stains clearly showed the much larger Aphanamyces hyphae that are pathognomonic for EUS, penetrating deep into the muscle layers. Laboratory culture for fungi failed to isolate the organism in both cases, however this occurs commonly as this fungus is slow and difficult to grow.

EUS is considered a disease of such significance that its occurrence is notifiable in many parts of the world, although it is endemic in WA. It is a slow and insidious infection which is usually fatal. The fungus enters the skin through any minor abrasions. As the fish becomes sick during the course of the infection, it is normal for secondary pathogens such as the white spot parasite and other gill parasites to take advantage and proliferate rapidly. There is no treatment for EUS. Clients were advised to attempt to gradually eliminate the infection by prompt removal of any fish showing typical skin lesions from the aquarium.

Novel Animal Papillomaviruses

Cassandra Hincks, School of Biomedical and Veterinary Sciences, Murdoch University, WA 6150 (C.Hincks@student-murdoch.edu.au)
Phillip Nicholls, School of Biomedical and Veterinary Sciences, Murdoch University, WA 6150 (Nicholls@numbat.murdoch.edu.au)

Animal papillomaviruses play an important role in both animal health and papillomavirus research, such as vaccine development and as models of papillomavirus immunology and biology. However most of the collective knowledge about these viruses is focused on human papillomaviruses. There is very limited documentation on animal papillomaviruses, even though there has been in excess of 60 types reported. Within this number, there are only a few of these viruses that are collectively found to infect the same species, whereas 80 different human papillomaviruses have been fully characterised. Currently, we are attempting to rectify the comparatively low detection of animal papillomaviruses.

A collection of suspicious lesions submitted from various veterinary laboratories and archive materials is being tested, via immunohistochemistry, electron microscopy and PCR, for possible papillomavirus infections. To date we have detected papillomavirus infections in the domestic pig (*Sus scrofa*), a horse (*Equus caballus*) and inverted and cutaneous canine (*Canis familiaris*) papillomas.

These and any other papillomaviruses detected will be sequenced to determine if they are new papillomavirus types. The confirmation and characterisation of these papillomaviruses are still been carried out along with more lesions being processed.

These and other novel animal papillomaviruses may provide insights into evolution, diversity and biology on this family of viruses. We would like to thank those of you who have contributed wart-like lesions to our study, and would like to use this opportunity to request paraffin blocks (on loan) from lesions you consider might have a papillomavirus aetiology.

Anonymous. (1996). Studies of Cancer in Animals. In "IARC Monographs on the Evaluation of Carcinogen Risks to Humans. Volume 64. Human Papillomaviruses", Vol. 64, pp 261-276. World Health Organisation International Agency for Research on Cancer, Lyon.

Nicholls, P.K., and Stanley, M.A. (1999). Canine papillomavirus-A centenary review. *J. Comp. Pathol*. 120, 219-233.

Stanley, M.A., Masterson, P.J., and Nicholls, P.K. (1997). In vitro and animal models for antiviral therapy in papillomavirus infections. *Antivir. Chem. Chemother*. 8, 381-400.

Sundberg, J.P., Junge, R.E., and Lancaster, W.D. (1984). Immunoperoxidase localization of papillomaviruses in hyperplastic and neoplastic epithelial lesions of animals. *AM.J.Vet.Res.* 45, 1441-1446.

Cutaneous Nocardiosis in a dog

Mandy O'Hara, School of Biomedical and Veterinary Sciences, Murdoch University, WA, 6150

SIGNALMENT AND HISTORY:
A 5y9m male neuter Rhodesian ridgeback cross had a chronic history of pruritic skin disease associated with atopy, which was complicated by *Staphylococcal* and *Malassezia* infections. The dog was placed on ketaconazole 200mg SID, cephalexin 500mg BID, and cyclosporine 100mg SID for 3 weeks before developing complications and having skin biopsies submitted for histopathology and microbiology.

GROSS PATHOLOGIC FINDINGS:
The dog developed multiple subcutaneous, painless nodules ranging in diameter between 1cm to 5cm located on the dorsal and lateral aspect of the trunk and head. On clipping these lesions pin-point, centrally located fistulae draining purulent to haemorrhagic exudate were identified.

HISTOPATHOLOGY:
Description: Skin punch biopsy
There was a severe, multifocally coalescing infiltrate of neutrophils and macrophages accompanied by lesser numbers of lymphocytes and plasma cells within the dermis and panniculus layers. Formation of a fistulous tract was evident within the central dermal

inflammatory infiltrate. There was marked dermal and hypodermal oedema with early fibroplasia surrounding the deep dermal and hypodermal inflammatory infiltrate. The epidermis was diffusely and regularly hyperplastic with moderate, diffuse spongiosis. Gram and modified ZN stains identified low numbers of gram positive, acid fast, extracellular, branching, braded filamentous bacteria in the areas of inflammation.

Diagnosis: Severe, chronic, multifocally coalescing, pyogranulomatous dermatitis and panniculitis with intralesional branching, beaded filamentous bacteria: *Nocardia asteroides*

LABORATORY RESULTS:
Bacteriology: *Nocardia asteroides* was cultured from skin biopsies

COMMENT:
Nocardia spp are saprophytic organisms found widely distributed in the soil and decaying organic matter (Lerner 1996). Soft tissue infections with *Nocardia spp* are uncommon in both humans and domestic animals. Cutaneous nocardiosis in humans is characterised by one of four clinical manifestations; (1) Mycetoma; (2) lymphocutaneous infection; (3) superficial skin infections such as abscesses and cellulitis, and (4) disseminated disease with skin involvement (Kalb RE 1985). In both animals and humans, cutaneous *Nocardiosis* may be associated with puncture wounds caused by animal/insect bites or injuries caused by plants (Fingland 1992) (Kalb RE 1985). Patients receiving immunosuppressive therapy for organ transplants are also at greater risk of developing *Nocardiosis* (Roberts SA 2000). Although there was no history of bite wounds or trauma from plant thorns, this dog had areas of broken skin attributed to self-trauma associated with atopic skin disease. This dog was also receiving cyclosporine therapy to manage his atopy, as previous administration of corticosteroids was associated with an episode of acute pancreatitis.

Cyclosporin is an immunosuppressant used to treat organ transplant patients to prevent graft rejection and in the treatment of autoimmune disease. Its effect is mediated by inhibition of the transcription of T-cell activation genes (Ho S 1996). Reduced production of IFN- and IL-2 T cells, results in reduced activation of macrophages and inhibition of the amplification of T-cell mediated immunity. These effects inhibit the development of acute transplant rejection or autoimmunity, however they predispose to the development of opportunistic infections.

N asteroides is a facultative intracellular pathogen whose virulence is associated with its:

1. Resistance to phagocytosis (filamentous log phase *Nocardia)*
2. Ability to inhibit phagosome-lysosome fusion
3. Ability to decrease lysosomal enzyme activity in macrophages
4. Ability to neutralise phagosomal acidification and resist oxidative killing mechanisms, presumably by producing surface associated superoxide dismutases and catalases
5. Complex cell wall glycolipids (Lerner 1996).

Ultimately, destruction of *Nocardia* infections requires the generation of a lymphocyte response that activates humoral and cell mediated immunity resulting in phagocytic and lymphocyte-mediated destruction of the organism (Lerner 1996). Although lymphocyte function assays or cyclosporine trough levels were not measured in this case, cessation of cyclosporine treatment

combined with antibacterial therapy has led to an apparent resolution of *Nocardiosis* in this dog. The clinical manifestation of nocardiosis in this case is most consistent with a superficial skin infection. It is hypothesised the combination of excoriations and face rubbing/feet chewing associated with this dog's atopic skin disease may have resulted in soil contamination of broken skin which has provided an entry site for the soil-borne *Nocardia*. Administration of cyclosporine to control the atopy may have facilitated the infection by reducing T-cell mediated amplification of the immune response.

REFERENCES:

J. Kirpensteijn and R.B. Fingland: Cutaneous actinomycosis and nocardiosis in dogs: 48 cases (1980-1990). Journal of the American Veterinary Association 201 (6): 917-920, 1992.

Ho S, Clipstone N, Timmermann L, Northrop J, Graef I, Fiorentino D, Nourse J and Crabtree G: The mechanism of action of Cyclosporin A and FK506. Clinical Immunology and Immunopathology 80 (3): S40-45, 1996.

Kalb RE, Kaplan MH, Grossman ME: Cutaneous nocardiosis. Case reports and review. J Am Acad Dermatol. 13 (1): 125-33, 1985.

Lerner, P.I: Nocardiosis. Clin Infect Dis 22 (6): 891-903, 1996

Roberts SA, Franklin JC, Mijch A, Spelman D: Nocardia infection in heart-lung transplant recipients at Alfred Hospital, Melbourne, Australia 1989-1998. Clin Infect Dis 31 (4): 968-72, 2000.

Squamous cell carcinoma of the udder skin in goats

Cleve Main, Animal Health Laboratories, WA Department of Agriculture, South Perth

Saanen goats in a flock close to Perth are affected by udder lesions that have reportedly been present for some years. Lesions begin as papules that progress to horny outgrowths over some weeks. The horny growths do not grow larger than 5mm but become "carcinomas" with age. Only two year and older are affected and the condition has appeared to act like infectious disease, spreading slowly through the flock. Originally lesions were present on one animal imported from interstate and slowly spread through the flock. Now affecting 20/100 animals. Lesion progress through hyperkeratosis with striking hyperplasia of the stratum spinosum, rete peg formation, and some hydropic change in some foci. Hyperkeratosis frequently extends to cutaneous horn formation. Epidermal hyperplasia becomes more marked with transition to squamous carcinoma in situ and accompanying mild chronic inflammatory change in the dermis. Lesions can coalesce to form massive plaque-like lesions grossly, involving only the skin of the udder but occasionally extending into the proximal teats. Immunohistochemistry for papilloma viruses courtesy of Dr Phil Nicholls at Murdoch University resulted in a negative result as have direct electron microscopic examination of crusty lesions, ruling out Orf and goat pox virus infections. UV induced squamous cell carcinoma is the presumed diagnosis.

Marshmallow toxicity in merino ewes and lambs

Cleve Main, Animal Health Laboratories, WA Department of Agriculture, South Perth

10/420 ewes dead and 6 of their lambs went down after driving and died following grazing a pasture of 90% marshmallow (Malva Parviflora). Affected animals may get up after resting but eventually go down again. At PM, brown malodorous abomasal fluid was noted. Histologically, thee was multifocal multiphasic myonecrosis in the heart. In skeletal muscle, milder more acute necrosis was seen. In the colon, widespread but patchy ectatic mucosal vessels and superficial mucosal erosions were seen but the significance of these is not clear.

ALT 521, 260 U/L (<30); CK 83000, 6130 U/L (<500); GGT 53, 68 U/L (23-67); GLDH 20.5, 11.4 U/L (<20); vit E 1.6, 1.68 mg/L (>1).

This comprises a rare well documented report of true marshmallow staggers.

Magpie leucocytozoonosis

Marc Kabay, Animal Health Laboratories, WA Department of Agriculture, South Perth

Two young magpies from the Perth zoo were euthanased, one because of a fractured foot, the other because of a severe pox infection of the beak area. An incidental finding in many organs was the presence of large numbers of large lobulated basophilic structures in leucocytes displacing and compressing the nucleus to a crescent at the edge of the cell. Electron microscopic examination shows these to be consistent with *Leucocytozoon* species. Despite very heavy infection in one bird, no lesions attributable to it were seen.

Canine hepatitis in a dog in quarantine

Marc Kabay, Animal Health Laboratories, WA Department of Agriculture, South Perth

A four month-old golden retriever in a quarantine station ex-UK was observed dull and listless with an elevated temperature, jaundice (elevated liver enzymes) and a PCV of 20. It subsequently developed bloody diarrhoea and died. At PM, 90% of the lungs were congested/haemorrhagic. The liver and kidneys were swollen and there was brown urine in the bladder and multiple serosal haemorrhages through the abdomen. Histologically, there was acute paracentral to midzonal hepatic necrosis with focal aggregates of lymphocytes and macrophages. Numerous basophilic intranuclear inclusion body present but only in necrotic areas casting some doubt about their authenticity. Multifocal glial nodules were seen in the cerebellum and there was haemorrhage into Bowman's spaces in the kidney. Immunoperoxidase testing for canine adenovirus at Cornell University was positive but viral culture attempts proved unsuccessful.

Dolphin cryptococcosis

John Creeper, Animal Health Laboratories, WA Department of Agriculture, South Perth

A dolphin was stranded and died while being transported to another location for treatment. At necropsy, the lungs contained large firm areas, the cut surfaces of which were very dense and paler than surrounding lung. Similar lesions were seen in the lymph nodes and in the meninges. Histologically, within the lesions, numerous yeast-like bodies with large capsules morphologically with *Cryptococcus* sp were seen. *Cryptococcus neoformans* var *gattii* was identified on culture at the State Health laboratories. The animal was apparently a non-estuarine species eliminating the potential link with Eucalyptus trees and raising the possibility of infection from marine birds. There are several papers in the literature on cryptococcosis in dolphins.

Potoroo mycobacteriosis

David Forshaw, Animal Health Laboratories, WA Department of Agriculture, South Perth

Progressive forelimb weakness/paralysis and ultimately generalised paresis was seen in a Gilbert's potoroo in a captive colony. Cryptococcosis was suspected as this diagnosis was made in an animal that had died previously showing similar signs. At necropsy, large granulomatous lesions were seen in liver and vertebral bodies around the cervico-thoracic junction. Histologically, these had necrotic centres containing masses of FBs. Microscopic granulomas were also seen through many organs but with few AFBs visible. *Mycobacterium intracellularis* was cultured from the liver and spinal cord lesions.

Porcine enteric mycobacteriosis

David Forshaw, Animal Health Laboratories, WA Department of Agriculture, South Perth

During an investigation into weaner ill thrift in a 2000 sow outdoor piggery on the south coast, 2/5 pigs had bronchopneumonia and from 3/5 salmonella were isolated from gut tissues. In one pig where no gross gut lesions were noted, histiocytic infiltrates in lamina propria and submucosa of ileum and colon were seen with many giant cells and large numbers of AFBs were seen in lesions. PCR testing of mycobacterium isolated from lesions indicated that the isolate does not fit into either the *M.tuberculosis* or *M.avium* complexes. Further typing is proceeding.

Bovine teat lesions

Cleve Main, Marc Kabay, Animal Health Laboratories, WA Department of Agriculture, South Perth

In successive years, a number of dairy cows from the south coast developed vesicular and ulcerative teat lesions originally thought to be caused by an exceptionally acidic teat wash. Lesions are said to appear initially as small vesicles and then rupture and develop ulceration. Histologic examination of punch biopsies shows acute epidermal oedema with superficial dermal eosinophilic vasculitis. Intracorneal pustules are filled with cell debris and fluid and imbedded in

some of the pustules are fragments of plant material which are attracting an intense eosinophil infiltrate. Vesicular exotic diseases were excluded and direct electron microscopy and herpesvirus serology did not indicate an aetiology. The lesions are suggestive of an allergic response, possibly to plant material.

DR TERRY NICHOLLS

All Society members will be saddened to hear of the recent sudden death of Terry Nicholls. Terry suffered a heart attack following his participation in a senior hockey game and died shortly after.

Terry graduated from Melbourne University in 1969. He entered private practice in Victoria and later in England before completing a PhD in viral disease of chickens at the Walter and Eliza Hall Institute in Melbourne in the early 70's.

Terry joined the Victorian Department of Agriculture as a Veterinary Pathologist and Research Scientist at the Benalla Regional Laboratory in 1977. While at Benalla, Terry's interests were wide-ranging and included research into Eperythrozoon ovis infection, lead poisoning in dogs, bovine mastitis. Terry was a leader in developing the ACIAR Project in Indonesia and Malaysia and he was a strong supporter of the National Fish Health Laboratory conceived by Dr John Copland.

He became the Acting Director of the Benalla Laboratory in 1983. His consensus approach to management and support of his staff was much appreciated. He attempted to involve all staff in understanding the management strategies of the laboratory and its budgetary challenges. Ultimately however, the lack of government support for veterinary services in Victoria that resulted in the exodus of so many good pathologists from that State, became intolerable and Terry moved to Canberra to join the BRS in 1988.

He expanded his interests in the regulatory environment of Canberra to include pesticide residue testing, antibiotic residue monitoring, and food safety. During this time, he retained a strong affiliation with pathology and laboratory service and in 1997 was co-author of an AOAC Symposium paper on Laboratory and Accreditation and Proficiency Testing in a Deregulation Market. Terry's vision was for government to support competitive laboratory participation in regulatory programs through maintenance of quality assurance programs.

At the time of his death, Terry had been given the task of reviewing the National Registry of Domestic Animal Pathology for Animal Health Australia. His terms of reference included undertaking an inventory of the Registry and to explore options for its future. Like every task he approached in his all too short life, Terry attempted to ensure all interested parties were kept informed of the process and had opportunity to contribute. His approach was always to aim for agreement rather than disharmony.

In Terry's death, Australian Veterinary Pathology has lost a champion. Always a strong supporter of the ASVP and committed to developing Australia's younger pathologists, Terry often found himself in the role of the defender of laboratory services amongst the Canberra bureaucracy. He recently remarked to me that he was "the only bugger in Canberra who knows what a laboratory looked like!" - while not perhaps entirely accurate, it is also not far from the truth. The society will miss him as an ally. Many of us will also miss him as a friend.

To his wife Joy and Guy, Paul, Toby, and Lucy we extend our deepest sympathy.

ROB RAHALEY.