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# Summary of Scientific Sessions

## Monday 21st March (p.m.)

| Room A | Paper |
|--------|-------|
| –      | Opening of Conference – Mr. G. David, President AVTRW |
|        | “The Future of Veterinary Research” |
|        | An introductory address by Prof. Lance Lanyon |
|        | Responses from invited Panel members: |
|        | Dr. Alan Doyle (Wellcome Trust), Dr. Peter Stevenson (Defra), Prof. Nigel Brown (BBSRC), Sir Brian Fender (HEFCE), Prof. Peter Holmes (SHEFC), Dr. Mary Kelly (Science Foundation Ireland), Judy MacArthur-Clark (RCVS) |
|        | Debate opened to the floor |
| 01–02  | Life-long Learning |

## Tuesday 22nd March

| Room A | Paper |
|--------|-------|
| 03–13  | Veterinary Education (theme session) – morning |
|        | Plenary Lecture – Prof. Remco Schrijver, Managing Consultant, Animal Sciences Group, Lelystad, The Netherlands |
|        | “Coping with an international outbreak of potentially zoonotic disease” |
| 14–18  | Animal Welfare (theme session) – afternoon |
| Room B | 19–30 Vaccinology (theme session: Comparative Veterinary Immunology Group) – all day |
| Room C | 31–37 Infectious diseases – morning |
|        | Foot-and-Mouth disease outbreak 2001: discussion 2001: discussion forum – morning |
| 38–41  | Veterinary Pharmacology (theme session) – afternoon |
Wednesday 23rd March

| Room   | Hours  | Session Description                                      |
|--------|--------|----------------------------------------------------------|
| Room A | 42–65  | New, evolving and emerging diseases (theme session) – all day |
| Room B | 66–82  | Veterinary Parasitology (theme session) – all day         |
| Room C | 83–102 | Veterinary Pathology (theme session) – all day            |
| Room D | 103–107| Veterinary Pharmacology (theme session) continued – morning |
Acknowledgements

The Association for Veterinary Teaching and Research Work thanks the following bodies for their most generous support of the scientific conference:

**Pfizer Animal Health**

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**Vetoquinol**

**Intervet UK**

**Schering-Plough Animal Health**

**Syngenta**
From the President of the Association for Veterinary Teaching and Research Work

Welcome to the 59th Annual Conference of the Association for Veterinary Teaching and Research Work, held again this year in Scarborough. For AVTRW members this has been the venue for many years, and in some cases for decades, to catch up on what is happening in animal science and veterinary teaching across the UK and Ireland. AVTRW, a specialist Division of the British Veterinary Association, and with affiliations to the Institute of Biology and the BioSciences Federation, provides a forum for multi-disciplinary research, allowing opportunities for scientists from a wide variety of fields of interest, and from many institutes, to meet, talk and learn from each other. This year’s themed sessions include New, Evolving and Emerging Diseases, Pharmacology and Animal Welfare. These, in addition to the regular appearance of Veterinary Pathology, CVIG and Education sessions ensure that the wide spectrum of interests of those involved in veterinary teaching and research are represented and reviewed.

The Association plays an important part in the communications between members and those responsible for veterinary research and teaching. There have been extensive consultations during the past year covering all aspects of the professional life of our veterinary and non-veterinary members. Not surprisingly issues relating to career development, renumeration, job security and recruitment of veterinary researchers are high on the agenda. AVTRW, with generous support from Pfizer, are promoting veterinary research careers via a series of roadshows during 2005 to try and address some of these issues. Other issues such as top-up funding, the new veterinary surgeons act, the defra animal health and welfare strategy and the potential shortage of large animal vets will also continue to be addressed by the Association. I am very grateful to colleagues from the funding bodies who have agreed to participate in our opening panel discussion. We look forward to a lively debate on the major issues for veterinary research.

The Plenary presentation this year will be by Dr. Remco Schrijver from Lelystad, The Netherlands. The title of his talk is: “Coping with an international outbreak of potentially zoonotic disease”. This lecture, which will describe the recent avian influenza outbreak in the Netherlands, will complement our themed session on new, emerging and evolving diseases and will be of considerable interest to those involved in the control of potentially zoonotic disease in the UK.

Finally, and most importantly, AVTRW Scarborough is fun! One of my pledges for my term of office was to reintroduce music to the evening activities, so all those musicians amongst you are invited to bring your instruments, or just your voice, to provide some rousing evening entertainment.

I am delighted to welcome you to our Conference, whether as a regular, or as a newcomer and feel sure that you will depart having made useful scientific exchanges and enjoyed three days in the convivial company of like minded colleagues.

I hope you have an enjoyable and productive Conference.

Graham David
Honorary President
AVTRW
March 2005
Abstracts

Monday 21st March 2005

Room A. Theme: Life-Long Learning

Room A: 16.30–17.00

01. Identifying the characteristics of veterinary surgeons who pursue a research career: An epidemiological study

MURRAY, J.K.*, FRENCH, N.P.*, FITZPATRICK, J.L.*, PINCHBECK, G.L.*

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bThe Moredun Research Institute, International Research Centre, Bush Loan, Penicuik, Midlothian EH26 0PZ

A retrospective, matched case-control study was performed to compare the characteristics of veterinary surgeons employed in veterinary research with those who had never held a research post. Cases were randomly selected graduates who were employed at Universities or Institutes that conduct research, and who played a major role in veterinary research projects during 2001–2003. Controls, matched by year of graduation, were veterinary surgeons that had not held any post that was primarily a research post since they graduated. Data were obtained for 173 matched sets. Conditional logistic regression analyses showed that variables significantly (P < 0.05) associated with a career involving research included; male graduates, graduates who had completed a summer studentship, graduates who had completed an internship, residency or houseman’s programme and graduates who held a veterinary diploma. Qualitative analysis showed that the main reasons for graduates entering a research career were the greater intellectual stimulation than was available in practice and having the opportunity to try a research career. Lack of funding and job insecurity were cited as the main factors which might cause a veterinary surgeon working in research to change career.

Room A: 17.00–17.30

02. How valid is clinical revalidation?

MICHELL, A.R.*

Department of Biochemistry and Pharmacology, Harvey Research Institute, St. Bartholomew’s Hospital Medical School, Charterhouse Sq., London E1 3M 6BQ

Periodic revalidation of performance is now expected of any self-regulating profession, by both public and politicians, just as it has been expected of pilots for decades. The radical step has been from the passive basis (‘nothing adverse is known’) to active assessment: ‘this doctor is fit to practise’. The nearer assessment approaches measurement of performance, the more valid will be that assessment; professional bodies will ultimately be judged on how well they assesses initial competence and how effectively they assure the public that competence is being maintained. ‘Trust me’ will no longer suffice, nor will CPD participation. Any future veterinary scheme would require acceptability (to public and profession), affordability (without public funds) and authenticity – ability to assess skills relevant to effective performance. But the overriding consideration may be political acceptability. A GMC scheme based on CPD, annual appraisals, and career development portfolios which included feedback from patients encountered vitriolic opposition from the ‘backwoodsmen’ and was watered down to little more than appraisal; a fatal error which will inevitably rebound on us. The Final Shipman Inquiry Report ridicules the diluted scheme: CPD records do not suffice and appraisal is almost irrelevant – nothing less than re-demonstration of competence is required.

* Note: * indicates that the presenter is a member of AVTRW; ** indicates that the presenter is not a member.
Tuesday 22nd March

Room A. Theme: Veterinary Education

Room A: 09.00–09.30

03. KEYNOTE: Introduction to the Higher Education Academy Centre for Medicine, Dentistry and Veterinary Medicine sponsored session

McCONNELL, G.**
Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH

The former Learning and Teaching Support Network is now part of the Higher Education Academy. The Centre for Medicine, Dentistry and Veterinary Medicine continues to build its activities and resources and some of these will be explored by Nigel Purcell, Senior Education Adviser. Work on portfolios is rapidly advancing. An overview of the current situation in UK and Ireland veterinary schools will be given, with a presentation from Newcastle on electronic portfolios offering ideas for future implementations.

Room A: 09.30–10.00

04. KEYNOTE: The resource archive for teacher trainers

PURCELL, N.**
Higher Education Academy Subject Network for Medicine, Dentistry and Veterinary Medicine, University of Newcastle, Newcastle upon Tyne NE2 4AB

The Resource Archive for Teacher Trainers is a bank of learning and teaching resources developed through LTSN-01 for use by staff developers and veterinary teachers to support:

- The delivery of short courses on teaching to veterinary staff involved in teaching in the clinical environment.
- The delivery of teaching certificate/diploma programmes.
- Veterinary teachers who wish to enhance their teaching.

The aim of this workshop is to introduce the LTSN-01 resource archive and to explore the ways in which it can be employed to foster effective teacher development. It will be of interest to all staff who would like to develop their own teaching skills and/or who are involved in educating veterinary educators.

By the end of the session participants will be able to:

- Outline the process by which the RAFTT database was developed.
- Understand how the database can be used to access resources to support:
  - teacher training programmes;
  - personal professional development in their teaching role.
- Contribute to the database.
- Decide whether to join the RAFTT community of practice.

Room A: 10.00–10.15

05. Using technology to improve animal welfare. Results of a randomized controlled trial comparing knowledge, preference and proficiency of students taught by traditional instructor demonstration or a multimedia CD

NAYLOR, J.M.**, ABUTARBUSH, S.M., PARCHOMA, G.
University of Saskatchewan, Saskatoon, SK, Canada S7N 5B4

Introduction. We developed an instructional CD on the technique of passing a nasogastric tube in the horse and evaluated it against traditional instruction in a randomized, controlled, blinded trial. Student performance, knowledge and preference were evaluated. Materials and methods. Fifty two of a total of 72 students in the WCVM class of 2005 enrolled. They were randomly assigned to either traditional or CD instruction. Each group spent one hour either with the traditional demonstration with horse and instructor or in the computer lab with the CD. All evaluations were performed by graders who had no knowledge of the instructional method. Results. Student preference: Students expressed a significant preference for the computer module than for traditional instruction but both groups felt well equally prepared to perform the procedure on a live horse. Student knowledge: Computer based learners scored significantly higher on a 10 question MCQ quiz than traditionally instructed students, mean scores were 9.6 versus 7.8, respectively. Student performance: There was no significant difference in the error rate between groups. Total times spent attempting to pass a nasogastric tube were not statistically different, 7.1 and 8.1 min for computer and traditionally instructed students, respectively. On their successful attempt computer based learners passed a nasogastric tube significantly quicker than traditionally instructed students; the times were 135 versus 251 s, respectively. Discussion/conclusions. Computer based learning improved instruction and delivers direct welfare benefits by reducing the number of horses required for demonstration purposes. The indirect welfare benefits may be more important – computer-
Room A: 10.15–10.30

06. Development and evaluation of ‘Catteries and Kennels’, a computer aided learning (CAL) package as an alternative to lectures for veterinary undergraduates

DENWOOD, M.J.**, DALE, V.H.M., YAM, P.S.
Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH

Introduction. To make the teaching of small animal housing less formal, a CAL package was developed to replace the first year undergraduate lectures at Glasgow. Materials and methods. An interface was designed using Macromedia Flash that was intended to be clear, easy to use and interactive. Digital images and video were gathered, with permission, from several local catteries and kennels. Fourteen veterinary students, nurses, clinical scholars and kennel assistants were subsequently asked to review the program and complete a questionnaire, comprising eight questions with 5-point Likert scales. Results. Most questions used 1 as the lowest rating and 5 for the highest rating. Median scores were: 5 for ease of use and presentation; 4 for interest, usefulness, animations and the integrated quiz; and 3 for the amount of information in the package (where 1 was too little, 5 being too much). All vet students gave a rating of 5 for the package as a suitable lecture replacement. Discussion/conclusions. Although the package has yet to be formally integrated into the undergraduate course, the authors are confident that it provides an enjoyable alternative to lectures on the subject, providing students with an interactive resource and enabling teaching staff to concentrate on less didactic teaching.

Room A: 10.30–10.45

07. A comparison of two cohorts of students’ attitudes towards the final year companion animal professional examination, before and after the introduction of OSCEs

DALE, V.H.M.*, MOULD, J., SULLIVAN, M.
Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH

Introduction. OSCEs were introduced in the undergraduate course in 2003–2004, replacing the previous professional examination format of clinical cases. An exit questionnaire was designed with a view to making a comparison between the two independent groups in their attitudes towards the examination format. Materials and methods. A questionnaire using a 10 point Likert scale was developed to measure students’ attitudes in 2003, distributed again in 2004. Comparisons between the cohorts were measured using the median as an average, and the Mann-Whitney U test to measure levels of significance. Results. The 2004 group did not differ significantly from the 2003 group in the amount of stress they claimed to have felt before the examination, but experienced a significantly greater amount of stress during the OSCEs. The 2004 group felt that the course had prepared them less well, although they had mock examinations, unlike their predecessors. The OSCE group also felt that their knowledge and competencies were less well tested; and the duration of individual stations were too short. Both groups had an average response to the overall duration of the examination, and there was no significant difference between the two groups’ overall rating of the examination. Discussion/conclusion. Further evaluations are needed to measure the longer term impact of OSCEs.

Room A: 10.45–11.00

08. Exploring diversity in veterinary programmes

RHIND, S.M.*
Veterinary Teaching Organisation, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG

Introduction. Veterinary education is continually evolving to meet the changing needs of the profession. This project involved visiting selected veterinary schools in North America to explore the diversity which exists in their curricula and observe and discuss their teaching and learning strategies. Materials and methods. Four veterinary schools in North America with varied curricula were visited during the autumn of 2004. Several days to one week were spent in each school and included meetings with students, faculty and administrators to discuss the details of each individual programme and the relative merits and challenges of each system. Results. Broadly, the overall curriculum models in the schools visited were of preclinical courses followed by a transition through paraclinical subjects to clinical work with varying levels of associated
integration. The use of problem based learning (PBL) ranged from short 2 week ‘blocks’, to whole courses, to almost complete PBL strategies. Courses on generic and ‘professional’ aspects of training were present or in the process of being introduced, usually running as a defined thread throughout each programme. Discussion/conclusions.

Comparing and contrasting the different approaches raises several issues for future debate which are of relevance to UK veterinary education. These include vertical integration (both extent and consequences of), PBL, tracking, the use of outcomes to guide curriculum design and the teaching and assessing of professionalism.

Room A: 11.30–12.00

09. KEYNOTE: ePortfolios: Design, implementation and evaluation

COTTERILL, S.*, McDONALD, A., DRUMMOND, P., HAMMOND, G.

School of Medical Education Development, University of Newcastle, Newcastle upon Tyne NE2 4AB

A customisable ‘generic’ electronic portfolio (ePortfolio) has been developed at Newcastle University as part of a collaborative FDTL4 project (http://www.eportfolios.ac.uk). The ePortfolio has been configured to be used in a range of different contexts. It was first applied in the undergraduate medical programme at the University of Newcastle from September 2003. The software is being continually developed as it has since been configured for Bioscience, Postgraduate Researchers and Contract Research Staff. The ePortfolio is also being used in Dentistry in a project involving 6 Dental Schools and 2 Postgraduate deaneries. The ePortfolio has potential applicability to veterinary education and this will be discussed. The paper describes the design of the ePortfolio and its application in a range of contexts where it is being used to support the evidencing of learning outcomes/skills, development and in the facilitation of personal development planning (PDP). The flexibility of the ePortfolio to address both ‘generic’ PDP and subject-specific requirements is appraised and findings from evaluation studies are summarised. Interoperability with other systems, including virtual learning environments, is also considered. This aspect is important in the veterinary context, where there is a need to support life-long learning and provide continuity between undergraduate and continuing professional development.

Room A: 12.00–12.15

10. Portfolio development and implementation: The Edinburgh experience

RHIND, S.M.A. a,*, SCOTT, P.R. b, McCONNELL, G.W. a

aVeterinary Teaching Organisation; bVeterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG

Introduction. In 2003, a personal and professional development portfolio was introduced into the BVM&S programme at Edinburgh. The portfolio has been designed to support the development of reflective practice and to provide a more defined structure to recording of learning experiences from clinical EMS. Materials and methods. The portfolio was introduced for first year in session 2003–2004. As the portfolio rolled through into 2nd year, it was further developed and a mentoring system was introduced whereby academic staff each mentor a small group of students. The portfolio comprises 3 separate sections each with a set of defined learning outcomes. Results. The project is still in the early stages with qualitative feedback suggesting a divergence of opinion from both students and staff on the merits of such a portfolio and reflective practice in general. Some students have prior experience of utilising portfolios from school and are therefore familiar with the general principle. The additional decision to include a staff mentoring system with the portfolio has also received variable feedback. Discussion/conclusions. The R(D)SVS portfolio will continue to roll out over the next few years and will be subject to a constant process of review and modification as required. We believe it will be a particularly valuable tool for enhancing and recording the learning experiences during clinical EMS. We also intend that in the near future the portfolio will be managed on-line.

Room A: 12.15–12.30

11. The first veterinary clinical skills centre in the UK

YAMAGISHI, B.J. a,**, WELSH, P.J.K. b, PEAD, M.J. b

aAcademic Support and Development, Royal Veterinary College, Royal College Street, London NW1 0TU; bVeterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA

In 2002, the RCVS drew up a list of essential day-one competences required of the new veterinary graduate. The Royal Veterinary College wanted graduates to be able to practise and be tested on each of these competences. Enthused by visits to medical schools’ clinical
skills centres, the RVC was awarded funding by Higher Education Funding Council for England to create its own centre. In 2004, the RVC opened the first veterinary clinical skills centre in the UK. Innovation was key to creating a centre that would be central to the clinical stages of the 5-year degree. Firstly, in the design of the Centre, where equine, farm animal and small animal patient facilities have to be incorporated. Secondly, in the area of veterinary models as these need to be developed from scratch. Thirdly, skills have to be broken down into their most basic component parts. Together with diagrams and photos, checklists have been produced, which provide step-by-step instruction to enable peer learning and self-assessment. So, students are able to visit the Centre in the morning and, for example, simulate radiographic positioning with a canine mannequin and that afternoon practise the same skill on a live dog in the RVC referral hospital.

Room A: 12.30–12.45

12. Using milk recording data to connect vet students with commercial dairy farms

HANKS, J.D.*, HOVI, M.

Veterinary Epidemiology and Economics Research Unit (VEERU), School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR

Over 100 veterinary practices now routinely download milk recording data for client herds from the internet into specialist herd management software. This gives potential access to individual animal data for approximately 50% of commercial dairy cows in Great Britain. Routine analyses are used to monitor current and long-term aspects of herd performance and provide timely and animal specific advice to farmers. In spite of the rapid uptake by veterinary practices, the training of undergraduate veterinarians in these skills is still almost insignificant. Newly qualified veterinarians are often unprepared for the commercial and multi-disciplinary realities of dairy production. Using existing milk recording data places no additional demands on the farmer beyond giving permission for data access. Thus a student could readily be assigned a commercial dairy herd and trained in appropriate analyses to follow over an extended period. In addition to a detailed knowledge of that herd this provides endless opportunities for benchmarking and comparison with herds monitored by fellow students.

Room A: 12.45–13.00

13. Virtual radiography of the horse

BOULOCHER, C.*, FRAMÉ, M., McCONNELL, G.

a CLIVE, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH; b Veterinary Clinical Studies, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG; c Veterinary Teaching Organisation, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Summerhall, Edinburgh EH9 1QH

The “Virtual Radiography of the Horse” is an interactive diagnostic imaging website that aims to illustrate radiographic techniques and create a digital bank of normal radiographs with anatomical features highlighted. The text is based on the “Equine Radiography Handbook 2004”, used to teach radiographic techniques of the horse at the Royal (Dick) School of Veterinary Studies, Edinburgh. Standard normal radiographs are complemented by three dimensional movies based on tomodensitometry data, using software more normally used for surgical planning in human medicine. The use of this tool for educational purposes is presented. Additionally, digital images and rotational movies are catalogued as Reusable Learning Objects in the repository of the University Edinburgh, making them available for use in tutorials or talks in several areas (diagnostic imaging, equine medicine or surgery). The association of three dimensional reconstructions (both radiographic and opaque bone within transparent skin) and radiographs is a new approach to teaching in veterinary medicine and is illustrative of the increasing importance of on-line learning resources. The use of medical reconstructive software is shown to be valuable in the new context of veterinary education.

Room A: 14.00–15.00

PFIZER PLENARY LECTURE: Dealing with an international outbreak of potentially zoonotic disease

SCHRIJVER, R.

Animal Science Group, Lelystad, The Netherlands
Room A. Theme: Animal Welfare

Room A: 15.30–16.00

14. KEYNOTE: Quality of life and animal welfare

SCOTT, E.M.*, WISEMAN, M.L.*, REID, J.*, NOLAN, A.M.*, FITZPATRICK, J.L. b

*University of Glasgow, Glasgow G12 8QW; bMoredun Research Institute, Penicuik

Chronic disease and pain have a huge impact on a person’s quality of life and we believe that this is also the case in animals, with significant welfare implications. Quality of life (QoL) is an abstract multiple-attribute complex construct, and in humans, reliable health related (HRQoL) measurement tools have been developed to assess the effects of chronic pain and disease using scientific methodology from the field of psychometrics. These tools have a pivotal role in the medical decision making process in relation to the treatment of chronic disease in man and we hypothesise that they will facilitate the development of evidence-based therapeutic options for painful chronic diseases in animals. Our work in dogs provides support for the hypothesis that chronic pain in dogs is associated with a wide range of behavioural disturbances, which impact significantly on QoL and which can be recorded using an instrument generated using psychometric principles. More recently, we have been exploring the possibility and opportunity for developing and applying psychometrically based QoL techniques for measurement of and ultimate overall improvement in quality of life for farm animals. We report on progress in the development and testing of such instruments.

Room A: 16.00–16.15

BVA – AWF Animal Welfare Lectureships – Introduction

KERR, K.

BVA Animal Welfare Foundation, 7 Mansfield street, London, NW

Room A: 16.15–16.30

15. Application of welfare assessment techniques to clinical practice

MAIN, D.C.J.**

Introduction. Informal welfare assessment is an integral part of veterinary clinical practice. However, the application of scientific methods to welfare research is now well advanced. Furthermore, the application of these principles to clinical situations is becoming more important. Assessment methodology. Formal welfare assessments aim to generate credible results that are valid (measures are relevant to welfare), feasible (can be applied to a clinical situation) and reliable (they can be consistently assessed). The extent to which these systems have been developed, validated and applied is variable. Farm animal systems are being used in farm assurance certification systems (www.vetschool.bris.ac.uk/animalwelfare) and are being subjected to scientific scrutiny within an EU funded project (www.welfarequality.net). Discussion/conclusions. The application to farm animal systems has generated key findings such as parameters should include more than just health concerns and that carers need to be actively involved in the development of solutions and in the monitoring of their success. An important future application would be the evaluation of veterinary interventions within either clinical audit systems or evidence-based clinical research. The net result of applying such formal welfare assessments should be a measurable improvement in the welfare impact of husbandry systems and veterinary interventions.

Room A: 16.30–16.45

16. Avian chemoreception: Implications for poultry welfare?

McKEEGAN, D.E.F.**

Glasgow University Veterinary School, Bearsden Road, Glasgow G61 1QH

In recent years an accumulation of convincing anatomical, neurophysiological and behavioural evidence has demonstrated the existence of functional chemical senses in a wide range of bird species, including the domestic fowl. Thus the detection of environmental
chemical stimulants has relevance for the welfare of poultry, but previously has been largely neglected. A research program investigating neurophysiological and behavioural responses to environmental pollutants (e.g., ammonia, an irritant) and gas mixtures used in controlled atmosphere stunning (e.g., carbon dioxide, potentially nociceptive) has provided insights into the welfare challenges posed by these chemical environments. Electrophysiological experiments to determine olfactory and trigeminal thresholds in hens have illustrated that their chemical senses can respond to stimuli experienced during normal production. For example, while exposure to ammonia at levels routinely encountered in commercial situations is unlikely to result in pain, such exposure constitutes a potent, chronic olfactory stimulus that eventually alters olfactory thresholds. Related behavioural studies have demonstrated aversion responses that do not depend on the stimulus being nociceptive. Mounting evidence that chemosensory cues are of importance to birds means that we must consider the impact of the chemical environment when making welfare recommendations in modern poultry production systems.

Room A: 16.45–17.00

17. Attitudes of veterinary students to current ethical and welfare issues confronting the veterinary profession

DUNCAN, J.S.*

Faculty of Veterinary Science, University of Liverpool

As part of the BVSc. undergraduate research module a survey based study is being undertaken which has been designed to assess veterinary students' attitudes to current ethical and welfare issues confronting the veterinary profession. The students' attitude to the selected issues will be correlated with a number of factors including an empathy score, year of study, sex, chosen career path. A report detailing the aims and background to the study, study design and preliminary data analysis will be presented.

Room A: 17.00–17.15

18. Associations between ‘in parlour’ behaviour and welfare in dairy cattle

McMULLAN, A.J.**, LUCAS, B.*, FITZPATRICK, J.L.*, NOLAN, A.M.*, McGAUGHAY, A.*, NICOL, C.J.*, SCOTT, E.M.*

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bDepartment of Veterinary Clinical Studies;  
cDepartment of Preclinical Studies, University of Glasgow, Glasgow;  
dSchool of Veterinary Science, University of Bristol, Bristol

Inflammatory disease is probably a major source of pain and, consequently, reduced welfare in ruminant species. In an ongoing study of the assessment of pain in dairy cows, we have studied disease (mastitis and lameness) incidence and severity, using markers including somatic cell counts (SCC). Furthermore, we hypothesised that behavioural indicators will be associated with inflammatory disease likely to induce pain in dairy cows. Behavioural studies have also been undertaken to provide information on pain related behaviours. Behaviours assessed included ‘in parlour’ behaviours such as the number of leg lifts during the milking process and while in the parlour, the number of leg shuffles during the milking process and while in the parlour, the number of tail swishes and the cows reaction to cluster removal. A total of 311 cows have been studied to date. Preliminary results indicate statistically significant associations between shuffling and condition score, (negative association), between total leg lifts and locomotion score (positive association) and between total leg lifts and average SCC (positive association). There also exist significant positive associations between tail swishing and shuffling of feet (both during the milking process and during time spent in the parlour) and between tail swishing and lifting of feet (both lifts during the milking process and total number of lifts). The seasonal relationship between behaviours and welfare is also being considered. This work is ongoing.

Room B. Theme: Comparative Veterinary Immunology Group: Vaccinology

Room B: 09.00–09.30

19. Development of cattle vaccines against bovine tuberculosis

VORDERMEIER, M.***, BUDDLE, B.*, HEWINSON, R.G.*

*a TB Research Group, Department of Bacterial Diseases, Veterinary Laboratories Agency, Weybridge, New Haw, Addlestone, UK;  
bAgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand

In 1997, an independent scientific committee chaired by Prof. John Krebs, concluded that vaccination of cattle offered the best long-term solution for controlling the disease in the National Herd and that priority should be given to the development of a cattle vaccine against bovine TB
Bovine (B)RSV is a major cause of bronchiolitis and pneumonia in young calves. An important problem encountered in the development of some BRSV vaccines has been the occurrence of enhanced disease following virus challenge. Since natural BRSV infection does not predispose to augmented respiratory disease following a subsequent infection, live virus vaccines probably provide the safest approach to vaccination against BRSV. Previous studies have demonstrated that vaccination of calves with recombinant vaccinia viruses (rVV) or bovine herpes virus 1 (rBHV-1) expressing the F or G glycoproteins of BRSV induced a significant reduction in BRSV infection and protect against lung pathology. However, DNA vaccination offers many advantages over recombinant virus vectors, including the lack of an immune response to the virus vector, the potential for neonatal vaccination and the potential for induction of long-lived immunity. Although milligram doses of DNA need to be used, vaccination of calves with DNA encoding the F protein significantly reduced nasopharyngeal excretion of BRSV and protected against the development of pulmonary pathology. However, compared to live virus infection, the immune response induced by DNA vaccination is slow to develop. Recent developments in reverse genetics have provided the opportunity...
to manipulate the genome of BRSV in order to develop stable, live attenuated BRSV vaccines. Studies on BRSVs which contain gene rearrangements, deletions or mutations have provided information on host/pathogen interactions in the bovine respiratory tract and have identified a number of promising live, attenuated BRSV vaccine candidates. For example, studies using mutant BRSVs in which either or both of the non-structural (NS1 and NS2) genes have been deleted have demonstrated a role for the NS genes in regulating interferon (IFN)-α/β and have suggested a role for IFN-α/β in the development of acquired immunity.

Room B: 10.30–11.00

22. Vaccination against feline immunodeficiency virus

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Many experimental strategies have been adopted in experiments to protect cats from FIV infection by vaccination. A number of these have been successful, in particular inactivated virus vaccines and more recently DNA vaccines. The interest in developing a vaccine arose both because FIV is a common cause of morbidity and mortality in pet cats and because the feline virus provides the only natural model for its counterpart in man, human immunodeficiency virus (HIV). HIV infection remains an urgent priority for world health care and an effective vaccine is urgently required to halt the current pandemic of acquired immunodeficiency syndrome (AIDS). Shortly after the discovery of FIV and its characterisation as a lentivirus, attempts were made to produce a vaccine and success was soon achieved with relatively simple inactivated virus or inactivated virus-infected cell vaccines. Further development of this approach led to the introduction in 2002 of the first commercial vaccine against FIV (Fel-O-Vax FIV), in the USA. With an estimated prevalence of the infection of up to 25% in some populations of pet cats, an effective FIV vaccine could have a significant impact on animal welfare. Of greater significance is the possibility that a similar strategy might produce an effective vaccine against HIV. This presentation will review some of the successes achieved with vaccines against FIV and highlight some of the remaining problems to be addressed by future research. The potential for the use of similar strategies in the development of HIV vaccines will also be discussed.

Room B: 11.30–12.00

23. Vaccinal control of Marek’s disease

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Marek’s disease (MD) is a lymphoproliferative disease of chickens, caused by oncogenic (serotype 1) strains of Marek’s disease herpesvirus (MDV). Early in infection, MDV actively replicates in lymphocytes, causing destruction of lymphoid tissues. At about 7 days a switch to latent infection occurs in T lymphocytes, which harbour the MDV genome with limited viral antigen expression. These cells carry the virus through the bloodstream to the visceral organs, peripheral nerves and feather follicle epithelium. MDV replication in feather tissues results in shedding of cell-free virus with skin and feather debris, and this is the source of infection for other chickens. In the viscera and nerves of susceptible genotypes of chicken, latently infected lymphocytes become transformed and proliferate forming gross lymphoid tumours. The resulting morbidity and mortality is responsible for a decrease in productivity and major economic losses. Prevention of MD is crucial, since there are no anti-viral drugs to reduce mortality in an infected flock. MD is, to date, the only virus-induced tumour disease for which an effective vaccine is available. Viruses of all three MDV serotypes have been used as cell-associated live vaccines, administered to day-old chicks via the sub-cutaneous or intra-muscular route using a semi-automated device. Many hatcheries now use an in ovo delivery system to administer the vaccine to embryonated eggs three days before hatch. While serotype 2 and HVT (herpesvirus of turkeys, serotype 3) are naturally non-oncogenic, serotype 1 vaccine strains are attenuated by serial passage in tissue culture so that, while remaining infectious, they do not induce lymphoid atrophy or tumour formation. Vaccination against MD was introduced in 1970, and HVT vaccine provided excellent protection. However, during the 1970s, there was a decrease in the efficiency of monovalent HVT vaccine due to interference from homologous maternal antibodies and to the emergence of MDV field strains of increased virulence. In the mid-1980s a ‘bivalent’ vaccine (HVT + the serotype 2 strain SB-1) was introduced, and this provided better protection than either of the individual components used alone, a phenomenon known as protective synergism. With further increases in virulence of field viruses, the ‘Rispens’ vaccine (serotype 1 strain CVI-988) was introduced for widespread use in the 1990s. It is the most protective vaccine against virulent field strains, probably because it is closely antigenically-related to the field strains. However, field viruses continue to evolve towards pathotypes of greater virulence, possibly driven by the selection pressures imposed by vaccination, and the challenge will be to find a more efficient vaccine. With this in mind, the possibility of using genetically engineered vaccines has been investigated. Fowl pox virus has been successfully used as a vector for delivery and expression of the gB gene of MDV in chickens, eliciting neutralising antibodies, decreasing MDV viraemia, and giving protection against virulent MDV strains. Attempts to improve the efficacy of the HVT vaccine by insertion of MDV genes or...
chicken genes involved in the immune response, have shown some promise. Recent bacterial artificial chromosome (BAC) technology has permitted the cloning of the whole MDV genome which could potentially be genetically attenuated by deleting virulence-associated genes. Vaccine virus stocks are prepared in tissue culture using primary cells derived from large numbers of pathogen-free eggs. Use of permanent cell lines for preparation of vaccine stocks is being investigated to reduce costs and labour. The infected cultured cells must be slowly frozen in the presence of a cryopreservative agent. Being cell-associated, the vaccines must be stored at −196 °C in liquid nitrogen. Only the HVT vaccine is also available as a cell-free freeze-dried product which can be stored at 4 °C. Each batch must be tested for safety and potency in chickens. The potential generation of MDV DNA vaccines using BAC technology, would make production significantly easier and cheaper. Vaccine viruses do not prevent super-infection by challenge viruses, but establish a persistent infection which reduces early viraemia and protects against tumour formation, and hence mortality, after subsequent exposure to pathogenic strains. However, multiplication and excretion of the challenge virus from feather tissues still occurs and the virulent strain shed by vaccinated birds is still oncogenic to non-vaccinated birds. However, although infected and infectious, a vaccinated animal will not develop tumours, and infection has no economic consequences. An ideal vaccine would be one that prevented replication, or at least shedding, of the virulent virus. Inoculation of vaccine into day-old chicks is followed a few days later by viral virus spreading in the bloodstream. This phase of viraemia determines induction of immunity. Maximal protection needs an interval of 1–2 weeks between vaccination and exposure to virulent strains. T cells are considered to play the major role in vaccinal protection, and there is evidence for a two-step mechanism of protection. Firstly, the vaccine virus has antigens similar to virulent strains and these antigens (including the MDV glycoproteins) stimulate an immune response to virulent strains, resulting in decreased viraemia, and inhibition of viral replication. Initial resistance to virus replication and spread leads to decreased malignant transformation and reduced immunosuppression. Secondly, MDV tumour antigens stimulate the immunological rejection of tumour cells by cytotoxic T cells. Vaccine breaks, defined as the sub-optimal vaccinal protection of a flock, can have several causes. Early exposure of young chicks, or exposure of chicks to hypervirulent MDV strains, can overcome vaccinal protection. Suppression of cell-mediated immunocompetence, due to environmental stress or concurrent infections with immunosuppressive agents such as chicken anaemia virus, can decrease vaccine efficacy. Loss of vaccine activity, by inappropriate storage, handling and administration, is another factor that contributes to lowered protection. In young chicks, the effects of vaccine are severely compromised by homologous maternal antibodies which delay vaccine virus replication, establishment of latency and hence immunity. The major challenge is to develop a vaccine which prevents replication and shedding of the challenge virus, and which does not drive an evolutionary selection for increasing virulence of field strains of MDV. This will require further research into the mechanism of vaccinal protection at the tissue, cellular and molecular levels, which is largely unknown at present. In the meantime, comparison of vaccine delivery routes, re-vaccination regimes, and optimal choice of vaccine to complement the genotype of different breeds of chick should maximise our ability to control Marek’s disease.

Room B: 12.00–12.30

24. Novel approaches to vaccination against Infectious Bursal Disease Virus in the chicken – in ovo DNA vaccination and cytokines as vaccine adjuvants

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In ovo delivery of vaccines has proved very successful and is widely adopted by the poultry industry in some countries. However, vaccination in the perinatal period bears the risk of the agent being neutralized by maternal antibodies. It has been reported that DNA vaccines administered to neonatal mammals are able to stimulate immunity and protect in the presence of maternal antibody. We have therefore explored the potential use of in ovo DNA vaccination for protection against the important poultry pathogen, infectious bursal disease virus (IBDV). The coding sequence for VP2 protein from IBDV was introduced into the plasmid pCI-neo and expression confirmed in vitro. After in ovo injection into the amniotic fluid plasmid DNA localised to the proventriculus and thymus. DNase activity was demonstrated in the amniotic fluid and plasmid DNA could be protected in vitro with use of cationic liposomes. Complete protection against IBD was stimulated by priming in ovo with a DNA vaccine (pCI:VP2) followed by boosting the chick using the fowlpox recombinant (pFIBD1) that also contains the VP2 gene. This protection was not invoked by the individual experimental, only the prime-boost combination. Antibodies to IBDV could not be detected using this prime-boost vaccination strategy, even after the chicks had been challenged with virulent IBDV. The data show that, although not protective on its own, a DNA vaccine can successfully prime an immune response when delivered in ovo. The role of the interleukin-18 (IL-18) in the immune response to viral infections is becoming clear in mammals, in particular its role in driving inflammatory (and therefore anti-viral) responses. IL-18 also shows promise as a vaccine adjuvant in mammals. The aim of this study was to investigate the potential of chicken (ch) IL-18 to act as a vaccine adjuvant with a recombinant vaccine (pFIBD1) against IBDV. The fowlpox genome contains several candidate immunomodulatory genes, including an IL-18 binding protein (IL-18bp). There are two ORFs in the fowlpox genome which encode potential IL-18bp – ORF073 and ORF214. We knocked out (Δ) the potential IL-18bp genes in pFIBD1 and inserted (Δ:) the cDNA encoding chIL-18 into pFIBD1 in the non-essential ORF030, generating several constructs – pFIBD1:chIL-18, pFIBD1ΔORF073, pFIBD1ΔORF073:chIL-18, pFIBD1ΔORF214 and pFIBD1ΔORF214:chIL-18. The subsequent protection from challenge, as measured by viral load and bursal damage, with virulent IBDV given by these altered pFIBD1 strains were compared to that given by the wild-type pFIBD1. The results show that chIL-18 can act as a vaccine adjuvant.
25. The comparative efficacy of commercially available BVDV vaccines in cattle challenged with European BVDV Type 1

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Introduction. Bovine viral diarrhoea virus (BVDV) is an important cattle pathogen with a global distribution. Materials and methods. A novel inactivated BVDV vaccine (PregSure BVD™, Pfizer Animal Health S.A.), and three other commercially available BVDV vaccines were administered to three to seven month-old calves according to the manufacturer’s instructions. A similar number of calves were given saline as a control. All animals were negative for BVDV serum antibody and virus before enrolment. All animals were challenged intranasally 21 days after completion of the recommended two dose immunisation course with $10^5$–$10^6$ TCID50 of a heterologous noncytopathic strain of a European BVDV type 1. Results. PregSure BVD™ stimulated a significantly [P < 0.05] higher geometric mean neutralising antibody titre against a BVDV-1a strain (heterologous to the inactivated vaccines but homologous to Vacoviron™) than Bovilis™ and Bovidec™ at seven and 21 days after completion of the basic vaccination, and higher than Mucobovin™/Vacoviron™ at seven days after vaccination. All saline control animals remained seronegative until challenge. A higher percentage (44.4%) of animals was protected from post-challenge leucopaenia following vaccination with PregSure BVD™ than with any of the other three vaccines (0–30%). Discussion/conclusions. PregSure BVD™ was shown to be superior to competitor BVDV vaccines following challenge with a heterologous noncytopathic European BVDV type 1 strain under controlled laboratory conditions.

26. The formation of antigen-presenting cell (APC) clusters in association with CD4+ T cells in the lamina propria of the neonatal pig intestine

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APC/T cell interaction is likely to be important in determining the outcome of immune responses in the intestine, since the appropriate expression of either tolerance or an active response is critical in maintaining mucosal integrity. 4-colour immunofluorescence was used to analyse interactions between APCs (including dendritic cells and endothelium) and CD4+ T cells in the intestine of the pig. To test for co-localisation of particular cell subsets or molecules, observed values were compared against predicted values based on the null hypothesis that each element of positive staining was randomly and independently distributed. Monocyte-derived APCs associated with MHCII+ endothelium at significantly greater levels than predicted by the null hypothesis (p < 0.0001), to form APC clusters. In the absence of MHCII these interactions did not occur. Analysing the association of CD4± T cells with these MHCII+ APC clusters showed that T-cells were 20- and 8-fold more likely to be associated with dendritic cells or with dendritic and endothelial cells, respectively, than predicted by the null hypothesis (p < 0.002). Again, these interactions did not occur in the absence of MHCII. We hypothesise that mature APCs interact with MHCII+ endothelium to form a complex antigen-presenting environment, which determines the outcome of presentation to mucosal T cells.

27. Regulation of survival of intestinal T-cells by collagen matrices

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Studies in several species have shown that CD4+ T-cells from normal intestinal lamina propria are preferentially susceptible to apoptosis after activation, while those from inflammatory bowel disease intestine are not. Susceptibility to apoptosis has been regarded as important in preventing damaging mucosal immune responses to food antigens. However, mucosal T-cells occupy micro-environments
containing matrix components which are not included in conventional tissue culture. We have examined the ability of matrix components to rescue lamina propria T-cells from activation-dependent cell death. Isolated splenic and lamina propria leucocytes were cultured with and without polyclonal activation with conA, either in tissue culture medium or in type 1 collagen. After 3 days numbers of CD4+ T-cells and expression of CD25 were determined.

Higher cell density was necessary for optimum activation in gels. While activation resulted in decreased survival of lamina propria cells in the absence of collagen ($p = 0.035$), numbers of CD4+ cells increased after activation in collagen gels ($p = 0.033$). We conclude that apoptosis of activated intestinal T-cells is likely to be regulated by matrix components and is more complex than previously considered.

Room B: 16.00–16.15

28. Comparative efficacy of enzyme-linked immunosorbent assay and hemagglutination inhibition tests for measuring humoral immune response against egg drop syndrome virus in layer chicks

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An indirect enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against egg drop syndrome (EDS) virus was performed. Virus identification was done through haemagglutination inhibition (HI) test using known antisera. Anti-chicken immunoglobulins were raised in goats and purified by ammonium sulphate ($\text{NH}_4\text{SO}_4$) precipitation technique. These goat-anti-chicken immunoglobulins were conjugated with 10mg of horseradish peroxidase. Four hundred serum samples were collected from layer flocks vaccinated against EDS and specific antibodies were determined by using a horseradish conjugate for ELISA. HI test was also performed for determination of specific antibodies. The indirect ELISA was sensitive, specific and reliable method than HI test for the detection of antibodies in this research.

Room B: 16.15–16.30

29. Vaccination against brucellosis: Development of non-living vaccines

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Introduction. Sheep and goat brucellosis caused by Brucella melitensis is a sustained problem in areas of the Mediterranean and Middle East. The current recommended vaccine is a live attenuated strain, Rev. 1, which although efficacious is less than ideal. The vaccine strain is beset by problems of residual virulence, pathogenicity in accidental hosts (including man), and interference with diagnostic and surveillance measures. The development of an efficacious non-living, defined vaccine is therefore a valuable goal in research toward improving control of this economically significant zoonosis. Materials and methods. Novel candidate antigens were selected from the B. melitensis 16M genome data. DNA vaccines were produced for each candidate. The protective efficacy each of these vaccines against B. melitensis challenge was established using a BALB/c infection model. Correlates of protective immunity were investigated for candidates showing protective efficacy. Results. Two out of five selected candidates were found to be protective when delivered as 4 doses of naked DNA at 3 week intervals. The protection generated by these vaccines was equivalent to that generated by the recommended live vaccine, Rev. 1. Specific antibodies were detected using ELISA, and IFN-γ ELISPOT techniques indicated generation of antigen specific CD4+ and CD8+ T cells in vaccinated mice. Discussion. Two protective DNA vaccines have been developed, confirming the promise of this approach for combat of intracellular pathogens. The immune response mediating the protective effect has been assessed. These vaccines now need to be assessed in natural hosts such as sheep and goats.

Room B: 16.30–16.45

30. Ability of rabies vaccine strains to elicit cross-neutralising antibodies

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In Europe, in addition to classical rabies virus (RABV), two European Bat Lyssaviruses (EBLV-1 and EBLV-2) have been identified ($n > 700$). EBLVs have also been identified in 3 human, 1 stone marten and 5 sheep. An additional concern is cat-bat inter-
actions; a cat in Denmark was found to have anti-EBLV-1 antibodies and at least 16% of bat lyssavirus surveillance submissions at VLA involve cat contacts. Cats therefore represent a potential ‘spill-over’ host but do not appear to be important in epizootic terms. This study evaluated the ability of antibodies to rabies vaccines to neutralise EBLVs. We examined vaccinated dog and cat sera in two categories; (i) FAVN-RABV titres $\geq 0.5–5.0$ IU/ml (5–23% PETS sera) and (ii) $>5.0$ IU/ml. All sera in group (ii) $>5.0$ IU/ml ($n=53$), were able to neutralize both EBLVs. In group (i); dog and cat sera ($n=79$, 54), only a proportion were capable of neutralizing either EBLV-1 (87%) or EBLV-2 (53%) or both EBLVs (47%). The emergence of new bat lyssaviruses in Eurasia, their continuing occurrence in Europe and the recent UK cases ($n=5$) emphasizes the need for continual lyssavirus surveillance and assessment of the capability of rabies vaccines to elicit protective immunity in recipients.

**Room C. Theme: Infectious Diseases**

**Room C: 09.15–09.30**

31. Rapid detection of *Mycoplasma dispar* and *Mycoplasma bovirhinis* using allele specific polymerase chain reaction protocols

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Viruses and bacteria, including mycoplasmas, are major contributors to respiratory disease in cattle with nearly 2 million animals affected in the UK annually resulting in huge economic losses. Whilst it is estimated that *Mycoplasma bovis* is associated with one quarter of these cases *M. dispar* is also a proven pathogen but is rarely isolated because of its fastidious growth requirements in vitro. *M. bovirhinis*, on the other hand is frequently isolated, but is not believed to be a primary pathogen. However until their true occurrence is known it is difficult to determine their significance. We describe an allele specific PCR based approach for the rapid detection of two bovine mycoplasma species. Specific and universal oligonucleotides were used in combination to detect the presence of single nucleotide polymorphisms within the 16S ribosomal DNA sequence. Presence of mycoplasma 16S rDNA is indicated by the production of a single control fragment of 785 bp. Positive samples generate alternative specific products, of 583 and 358 bp for *M. dispar* and *M. bovirhinis* respectively, over the same region. This technique provides a reliable and sensitive method which, although widely used in human genetic screening, has not been documented for diagnosis of bacterial infection.

**Room C: 09.30–09.45**

32. Contribution of biofilm formation to disease caused by *Mycoplasma bovis* and *Mycoplasma mycoides* subsp. *mycoides* SC

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Although *Mycoplasma bovis* and *Mycoplasma mycoides* subsp. *mycoides* SC are important bovine pathogens very little is known about their mechanisms of disease. We propose that the formation of biofilms may explain the chronic and often persistent nature of mycoplasma disease. A biofilm can be defined as a layer of cells adherent to a surface normally surrounded by a polysaccharide matrix. Biofilms may form on the surface of the lung, within a joint, in the oral cavity, in the intestinal tract or even in the environment. Biofilms have been found in every bacterial species studied but to date, have not been described in mycoplasma. The most important and widely studied property of biofilms is their vastly increased resistance to antimicrobials and to host defences. Compared with unattached cells, biofilms are commonly 10–1000 times more resistant. As well as enabling chronic infection of hosts, biofilms may cause bouts of acute infection when cells are periodically released from the biofilm. We have studied biofilm formation in *M. bovis* and the causative agent of CBPP, *M. m. m. SC*. Although *M. bovis* formed a prolific biofilm *M. m. m. SC* was unable to form a biofilm. Biofilms were significantly more resistant to stress, particularly drying than broth grown *M. bovis*. 
Contagious bovine pleuropneumonia (CBPP), an OIE list A disease, affects many countries in sub-Saharan Africa. Frequent animal movements and poor or non-existent laboratory facilities in these areas make diagnosis of CBPP very difficult. We developed and evaluated a pen side test, the latex agglutination test (LAT), for the rapid diagnosis of CBPP in the laboratory and in the field. A capsular polysaccharide (CPS) was extracted from the causative agent of CBPP, Mycoplasma mycodies subspecies mycodies small colony variant. This antigen was then bound to latex beads approximately 0.8 μm in diameter. Testing of over 200 sera from CBPP free cattle showed a specificity of 95% while testing of sera from CBPP affected herds in Namibia showed sensitivity levels comparable to the OIE approved complement fixation test. The LAT also gave very clear results on whole blood taken directly from affected cattle at the pen-side. The CPS LAT provides a rapid and valuable screening test for the detection of CBPP in affected but sometimes symptomless cattle and requires little training or scientific knowledge to perform.

Introduction. The site of Chlamyphila abortus persistence between infection and abortion remains unknown. Possible persistence after abortion, with consequences in the transmission of Ovine Enzootic Abortion has been hypothesised. In this survey we examined if chlamydial genome can be detected in ovine uteri.

Materials and methods. Uterine tissue samples from 304 ovine genital tracts, collected at an abattoir using a sterile technique, were examined using a PCR for chlamydial genome. The pregnancy status/stage was determined by examining the embryo/fetus size and morphology. Samples from gravid uteri belonged to the first 100 days of gestation. The clinical history of the animals unknown. Results. The total prevalence of chlamydial genome detection was 30.9%, with a significantly higher prevalence in the pregnant animals (46.9%, \( P > 0.0001 \)). Higher detection rates were recorded during early gestation compared to mid-gestation. Discussion/conclusions. Our results indicate that there is evidence of some degree of persistence of infection in the uterus although they do not specify the origin of such infection. The role of uterine persistence in disease transmission remains unclear but it may explain previous evidence of chlamydial vaginal shedding during oestrus. Chlamydial uterine persistence may also affect the development of local immune mechanisms and improve our understanding of local protection mechanisms against abortion.

Introduction. Caseous lymphadenitis (CLA), caused by the bacterium Corynebacterium pseudotuberculosis, is increasing in prevalence amongst British sheep and goats. Under certain circumstances C. pseudotuberculosis organisms can be excreted in the milk of CLA infected animals. Caseous lymphadenitis is a recognized, although rare, zoonosis and milk has been suggested as a possible vector of infection to humans. The organism displays certain structural characteristics likely to increase resistance to thermal destruction. This makes it necessary to establish whether milk contaminated by C. pseudotuberculosis is made safe through the use of standard dairy pasteurization techniques. Materials and methods. Goat's milk samples spiked with cultures of C. pseudotuberculosis to different concentrations and with pus from CLA lesions, were passed through a pilot pasteurization plant under standard conditions. Serial samples were taken from the pasteurized milk and bacteriological enrichment techniques were employed to detect the presence of any...
surviving organisms. Results. Initial results indicate that no viable \textit{C. pseudotuberculosis} organisms were present within the spiked milk samples following standard pasteurization. Discussion/conclusions. On the basis of these initial findings, commercial pasteurization ensures the safety of milk produced by dairy goats and sheep infected with CLA.

\textbf{Room C: 10.30–10.45}

36. Teat-end lesions and their relationship to intramammary infection in two dairy farms (organic and conventional)

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A healthy udder is the important feature of the dairy animal, because of the impact on the dairy farmer's economy. Udder health can be evaluated in several ways, the occurrence of mastitis, individual cow somatic cell count (SCC), and bacterial scoring. Each method poses different problems. In recent study 240 lactating and non-lactating cows teat health was assessed with regard to intramammary infection. Teats were grouped in 5 general categories: normal teat-end, smooth chronic ring lesions, rough chronic ring lesions, acute teat-end lesions, and non-classified. Only 22% of teats were classified as normal, 55.5% had smooth chronic ring lesions, 13% had rough, chronic ring lesions, 1.5% had acute teat-end lesions, and 8% could not be classified. Milk samples were taken from each teat of all cows and their bacterial profile assessed. The profiles were significantly different ($p > 0.001$) in cows presenting acute and chronic teat-end lesions compare to healthy cows.

\textbf{Room C: 10.45–11.00}

37. Genetic diversity of isolates of \textit{Treponema} spp. associated with bovine digital dermatitis, and implications for epidemiology of the condition

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Introduction. Bovine digital dermatitis is an emerging disease which was first identified in the UK in 1987. It is estimated that over 80% of dairy farms are currently infected. As a leading cause of infectious lameness, it is of increasing animal welfare and economic concern. The involvement of \textit{Treponema} spp. in the condition is highly probable; however, the difficulty of culturing these organisms in vitro has meant that the aetiology, pathogenesis and epidemiology have not yet been well elucidated. Materials and methods. Lesion tissue samples were obtained by punch biopsy on 10 farms in Cheshire, Gloucestershire and Northern Ireland. Subsequent culture resulted in 23 pure \textit{Treponema} spp. isolates. To assess genetic diversity, 16S rRNA sequence analysis, enzyme profiling and flagellin gene sequencing were performed. A direct ELISA was utilised to investigate serological reactivity patterns. Results. Clustering of isolates into phylogenetically distinct groups was consistently observed with all microbiological techniques performed. A similar pattern was reflected by the serology. Discussion. Our results are the first to demonstrate the substantial antigenic heterogeneity of digital dermatitis associated \textit{Treponema} spp. The implications for the epidemiological investigation of digital dermatitis, particularly related to case definition and diagnostic procedures, will be further discussed.

\textbf{Room C: 11.30–12.30}

Foot-and-Mouth Disease control in 2001: Clearing the smoke

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Presentation followed by discussion forum
Room C. Theme: Veterinary Pharmacology

Room C: 15.15–15.45

38. Measurement, judgement and evidence-based medicine: The value of comparative clinical science

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In striving to avoid ‘postcode privilege’ in treatment, the NHS has put increasing constraints on clinical judgement, indeed the combined effect of ‘NICE’ reports and fear of litigation for non-standard therapy almost amount to a straightjacket. This approach may be politically expedient but it is scientifically and ethically questionable. Evidence gleaned from peer reviewed papers essentially establishes the response of average patients, 19 times out of 20. If you are not average or you are number 20, your clinical welfare or survival may depend on your clinician’s willingness to try something different. Moreover genetic differences in response to drugs are a flourishing area of research. Such debates seem a luxury in veterinary medicine, plagued by such a dearth of high quality clinical evidence. Yet we have the important advantages of an instinctively comparative approach and the ability to use our target species for experimental as well as clinical research. Above all, comparative clinical science offers veterinary medicine the chance to capitalise on innovative concepts and techniques from human medicine but also to educate human medicine to the benefits of perceiving man as one among many mammalian species, and the intellectual dangers which have accrued from failing to do so.

Room C: 15.45–16.15

39. Setting dosage schedules of antimicrobial drugs for use in veterinary medicine: Is there a role for PK-PD?

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Knowledge of plasma concentration-time effect relationships (pharmacokinetics) for antimicrobial drugs linked to some measure of antimicrobial efficacy and potency (usually MIC) has led to the use of surrogate markers in experimental models of infection and in clinical trials as predictors of the outcome of therapy. AUC/MIC and $C_{\text{max}}/\text{MIC}$ ratios provide the best correlation for drugs with concentration dependent killing mechanisms of action and $T > \text{MIC}$ is used for drugs with predominantly time dependent killing properties. There are several considerations which indicate that these surrogate can be applied to rational dosage schedule design, but they must be applied recognising potential pitfalls and problems as follows: (1) relating pharmacokinetic-pharmacodynamic (PK-PD) surrogates to clinical outcome is less satisfactory than correlation to bacteriological outcome; (2) the required numerical value of a selected surrogate marker is “drug and bug” specific (with variation not only between bacterial species but also between strains) and there is also dependence on inoculum size; (3) the numerical value of a particular surrogate which is optimal for efficacy is unlikely to be optimal for preventing the spread and emergence of resistance; (4) some drugs, e.g., newer macrolides/triamilides have unusual PK properties and/or additional non-antimicrobial actions which contribute to outcome; (5) organisms existing in a protected environment, e.g., in biofilms will not obey the normal rules of PK-PD; (6) studies conducted in disease models in the target species may or may not be representative of animals with disease under field conditions and the variability in drug PK and PD can only, in the final analysis, be allowed for by taking measurements under field conditions, i.e., using population PK-PD modelling. Despite these constraints, PK-PD integration and modelling approaches will be increasingly used to set dosage schedules, not least because of their superiority, in most circumstances, to dose titration studies.

Room C: 16.15–16.45

40. Pharmacokinetic/pharmacodynamic (PK/PD) relationships as applied to macrolides and related drugs: An overview

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The use of macrolide antimicrobials, in both human and veterinary medicine, has become well established over many years, most notably for the treatment of respiratory infections. More recently, a number of related classes, namely the ketolides, azalides and triamilides, have
been developed and introduced for clinical usage. These newer compounds tend to have improved pharmacokinetic characteristics that optimise their antimicrobial effect. Macrolide antimicrobials are generally bacteriostatic and act through direct inhibition of essential protein biosynthesis by interaction with the bacterial ribosome. Traditionally, the antibacterial effect of macrolides, such as erythromycin, spiromycin, tylosin and clarithromycin, has been related to the time pathogens are exposed to an appropriate amount of active moiety, measured in terms of the time (T) that the drug concentration is above the minimum inhibitory (MIC) of each specific pathogen and the killing rate is not increased by increasing drug concentrations. A time-dependent (T > MIC) activity is also shown by other bacteriostatic drugs such as tetracyclines and lincosamides and bactericidal drugs such as the beta-lactams. This is in marked contrast to bactericidal compounds such as the fluoroquinolones and aminoglycosides that exhibit concentration-dependent killing. For these the ratio of the MIC to either the peak plasma concentration (C<sub>max</sub>:MIC) or the area under the plasma drug concentration-time curve over 24 h (AUC<sub>24</sub>:MIC) provides a better PK/PD predictive parameter. Recent evidence would suggest a bactericidal activity under some circumstances for some macrolides and for newer compounds such as azithromycin and dirithromycin. AUC<sub>24</sub>/MIC is more highly correlated with clinical efficacy than T > MIC. The newer macrolides tend to have a prolonged half-life in target tissue, which contributes to a longer drug exposure time for pathogens, but lower plasma-drug concentrations than those found in body tissues. Indeed, plasma levels may sometimes be below the MICs of the pathogens against which good clinical efficacy is well established. The applicability of the different PK/PD models to these circumstances will be discussed and improvements to the measuring of tissue fluid concentrations suggested for future veterinary application.

### Room C: 16.45–17.15

#### 41. Selamectin is equipotent to ivermectin as a P-glycoprotein substrate in human, canine and rodent tissue

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**Introduction.** 33% of Collies have a high brain disposition of ivermectin due a genetic mutation in the mdr gene coding for the P-glycoprotein (P-gp) efflux pump on blood-brain barrier endothelia. We studied the transport of ivermectin, selamectin and moxidectin in P-gp-expressing human intestinal epithelial cell monolayers (Caco-2), rat colonic mucosae and canine peripheral blood lymphocytes (PBL) in order to assess P-gp sensitivity. Materials and methods. Caco-2 monolayers were grown on Transwells®. Fresh canine PBL were isolated by Ficoll-Hypaque gradient, while rat colonic mucosae were mounted in Ussing chambers. Results. Fluxes of the P-gp substrate, rhodamine-123 (Rh), across Caco-2 showed that ivermectin and selamectin had IC<sub>50</sub> values of 0.1 μM, but for moxidectin it was 10 μM. [H]-ivermectin, [H]-selamectin and [H]-moxidectin displayed secretory/absorptive ratios of 7.5, 4.7 and 2.6. Secretory transport of [H]-ivermectin and [H]-selamectin, but not [H]-moxidectin was blocked by the P-gp inhibitor, verapamil. Similar data was seen in rat colon. Ivermectin, selamectin, and verapamil, but not moxidectin, inhibited efflux of Rh from PBL. Conclusions. It is likely that P-gp interaction at the blood-brain barrier is similar for ivermectin and selamectin. Differences in distribution of avermectins between plasma and CNS are due to the different doses, formulations and delivery routes used.
Commercial poultry are kept intensively in huge numbers, they have a short lifespan, rapidly reach sexual maturity and are frequently moved around the world. In some regions, they live in close contact with humans and other animal species or are allowed contact with wild birds. They are susceptible to many viral diseases, some of which are immunosuppressive and others zoonotic or capable of transmission to other non-avian hosts. These are some of the reasons that new diseases of poultry emerge relatively frequently. In addition, new variants of existing agents appear which challenge current methods of control and sometimes pose a hazard to man. In the last 30 years, ‘new’ virus diseases have included egg drop syndrome ‘76 and avian rhinotracheitis and it is likely that they originated from other avian species. On the other hand, new variants of well-known diseases include avian leucosis J, infectious bronchitis and highly pathogenic avian influenza and infectious bursal disease. The new variants can arise due to mutation or recombination and perhaps emergence from chickens in developing countries. Recently, West Nile virus has appeared in USA and Europe but the chick appears to be a dead-end species. All these challenges emphasise the importance of surveillance and readiness to apply control measures. Of particular value is molecular epidemiology in investigating disease sources.

Introduction. Goose parvovirus was confirmed as the cause of substantial mortality in young goslings. The isolation of goose parvovirus has not been reported in Britain since 1981. Materials and methods. Two affected farms were rearing birds for Christmas. Clinical signs included weakness, head shaking and anorexia leading to diarrhoea and nasal discharge. The eyelids became swollen and then stuck together. Death usually occurred within 48 h. 50% mortality was seen by 4 weeks of age. Both farms had been supplied by the same breeder. The investigation consisted of post-mortem examinations, histopathology, virology, farm visits and serology. Results. Gross findings included diphtheritic lesions in the oral cavity and on the tongue, fluid large intestinal contents, perihepatitis and pallor of the heart. Serology (agar gel precipitin) for goose parvovirus antibodies was positive in birds from the two farms and the breeder unit. Virology confirmed the presence of goose parvovirus and molecular analysis identified goose parvovirus of wild type identical or very closely related to a recent isolate from elsewhere in northern Europe. Discussion/conclusions. Enquires suggested that the infection may have been widely dispersed via goslings. Goose parvovirus is a highly contagious disease and latent infections can occur. It is possible that the disease may be seen in the 2005-breeding season. Acknowledgements: The molecular analysis was performed by Ceva-Phylaxia Veterinary Biologicals, Budapest, Hungary.
counts for nearly 50% of all undiagnosed cattle disease (Anon, 2002). The low diagnosis rate for enteric disease would support the investigation of other potential causal agents. In humans, noroviruses, a subset of the Caliciviridae are the most common cause of infectious non-bacterial gastroenteritis. Bovine diarrhoea samples (n = 398) supplied by 8 VLA regional laboratories were tested using RT-PCR to detect bovine noroviruses. An access database was created to link epidemiological information about the source animal from the VLA database, Farmfile, with the PCR results. Univariate and bivariate analyses were undertaken in STATA8.0. In total 11% (44/398) samples were positive for bovine noroviruses. No significant associations were demonstrated between outcome (PCR result) and exposure variables. However, case fatality and morbidity and mortality rates were higher in affected groups. The prevalence of bovine noroviruses and coronaviruses were comparable in this study. This suggests that further work needs to be undertaken to investigate the importance of noroviruses as enteropathogens of cattle.

45. Bovine enteric caliciviruses: Relationships, diversity and recombination

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Bovine enteric caliciviruses were first recognised during the 1970s but not until recently were some identified as noroviruses. The genus Norovirus of the Caliciviridae encompasses viruses that cause high profile outbreaks of gastroenteritis in man, suggesting a possible zoonosis. We studied the prevalence and diversity of bovine noroviruses in the UK plus their relationship to the well-studied human noroviruses. We found that: all bovine norovirus sequences formed a third genogroup (III) that was distinct from the two known human norovirus genogroups (I and II); 11.6% of 398 bovine diarrhoea samples contained bovine noroviruses; the bovine noroviruses (genogroup III) formed two genetic clusters represented by Bo/ Newbury2/76/UK and Bo/Jena/80/DE; the majority of UK sequences were Newbury2-like but 5 polymerase sequences were Jena-like. However, sequence analysis of a UK virus, Thirsk10, identified a chimaera between the Newbury2- and Jena-like viruses. This strongly supports the notion that recombination is part of the natural evolution of the genus Norovirus of the Caliciviridae. We conclude that bovine and human noroviruses are distinct; there was no evidence that the UK bovine noroviruses are a public health risk; two distinct clusters of bovine noroviruses exist but genomic recombination can occur, having implications for their diagnosis and immunological control.

46. A longitudinal survey of an outbreak of sleeping disease in farmed rainbow trout (Oncorhynchus mykiss)

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Introduction. Sleeping disease (SD), caused by salmonid alphavirus (SAV) was first reported in the UK in 2002. Since then outbreaks of SD have occurred each year, with disease apparently endemic on some sites. In the absence of published descriptions of the course of field infections of SD, a longitudinal study of a natural outbreak was performed to investigate the virological, serological and histopathological changes and the relationship between these. Materials and methods. Following the introduction of rainbow trout to a farm for on-growing, sequential sampling was carried out over a 20 week period. Results. Viraemia was detected on four consecutive weeks from week 6 with a peak of 60% positive at week 7. Mean viral titres were >10^6 TCID50/ml. Seroconversion was first detected at week 9. Mean neutralizing antibody titres peaked at approximately 1:100. Typical sequential histopathological changes affecting the pancreas, heart and skeletal muscle were observed. Cumulative mortalities in the sampled population was 6.3%, but levels of up to 47% were recorded in other affected populations on the same site. Water temperatures between weeks 6 and 10 were 12.5–13.5 °C. Discussion/conclusions. The study produced useful information on the temporal relationship between virological, serological and histopathological changes over the course of an outbreak in relation to the diagnosis, pathogenesis and epidemiology of SD.
Room A: 10.30–10.45

47. Virological, serological and histopathological evaluation of fish strain susceptibility to experimental infection with salmonid alphavirus

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Introduction. Pancreas disease (PD) caused by Salmonid alphavirus (SAV) has re-emerged as a significant cause of mortality in farmed Atlantic salmon (Salmo salar L.) in Ireland and elsewhere. A recent epidemiological investigation of PD indicated an association between salmon strains and mortality levels. To compare strain susceptibility to SAV infection an experimental trial with smolts of three different strains was performed. Materials and methods. Three distinct strains of Atlantic salmon, (A, B and C) were allocated to 3 separate seawater tanks. Fish were challenged intraperitoneally and sampled over a 42 day period. Histological examination of pancreas, heart, skeletal muscle and brain was performed and lesions scored. Virus isolation and serology by end-point virus neutralization testing was performed on serum samples. Results. Significant differences in mean lesion scores in pancreas, heart and skeletal muscle were observed. Discussion/conclusions. Significant strain differences in relation to response of SAV challenge were detected. Further investigations are necessary to elucidate the mechanisms underlying these differences.

Room A: 10.45–11.00

48. Assessing UK farmer attitudes to biosecurity on sheep and cattle farms

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Farmer attitudes to improved biosecurity on cattle and sheep farms in the UK were studied using a farmer focus group survey, with eight focus groups. The data was analysed using qualitative data software and the theory of reasoned action, as the analytical framework. While there were some regional differences in farmer views of biosecurity, farmers had a clear and uniform understanding of the concept. The main drivers for improvement among the farmers were: expected financial gain, expectations of improved animal health and welfare, professional pride, desire to practice good husbandry and to improve farmer reputation (by getting rid of ‘bad farmers’). The main barriers for improved biosecurity were: a perceived poor efficacy of measures in the absence of action by others, high potential/perceived cost, perceived increase in bureaucracy and paperwork and fear of losing independence. There was a lack of trusted referents. While advice and support from local veterinarians was trusted most, they were seen as potentially biased in their advice on biosecurity. In conclusion, biosecurity and its implementation at farm level are complex and value-laden issues. There is a need to address the farmer perception that only farmers are expected to do something about biosecurity and that farmer efforts will be meaningless due to lack of action or wrong action by others.

Room A: 11.15–11.30

49. Disposal of ship and aircraft waste: Is there a risk to British livestock from foot and mouth disease?

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Meat contained in ship and aircraft waste brought into Great Britain (GB) has been identified as a possible vehicle of foot and mouth disease (FMD) virus. GB livestock may come into contact with contaminated waste that has not been disposed of appropriately, resulting in possible livestock exposure to any virus present in the waste material. A quantitative risk assessment (QRA) model has been developed which estimates the annual probability (and thus frequency) of waste from a ship or aircraft resulting in GB livestock becoming exposed, and infected with FMD, and the factors that contribute to that risk. The model results indicate that, the frequency of infection in GB as a result of illegal waste is estimated at a mean value of 0.0007 per year with 90% certainty that the frequency of
infection is between 0.0001 and 0.002. This translates to, on average 1 infection in every 1429 years, with 90% certainty that the frequency is between 1 in every 500, and 1 in 10,000 years as a direct result of ship and aircraft waste. The model also provides key insights into the factors within the risk assessment that provide the greatest contributions to the risk of infection.

Room A: 11.30–11.45

50. Improving veterinary surveillance in Vietnam – a knowledge management approach

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This study investigated veterinary surveillance in Vietnam and examined possible solutions to improve the system. The study approached the issue as one of ‘knowledge management’ encompassing many human issues, not just information technology issues. Approaches were borrowed from business management and social science to focus on the behaviour and motivation of the stakeholders in the system, including ten focus groups, several key informant interviews, and a participatory intervention. Stakeholders’ differing needs for animal disease information were demonstrated. The stakeholders had sensible views on possible solutions to failings in the system. Key problems identified were over-reliance on the passive receipt of reports from non-government commune animal health workers and lack of local ownership and utilisation of information. An intervention to introduce more active field surveillance by District Veterinary staff and introduction of basic methods to extract ‘local value’ from data collected at local level was tested. The trial increased the number of disease investigations, including one of HPAI, and local analyses were deemed useful by District staff. These interventions were cost effective and could be effective in improving the veterinary surveillance system in Vietnam. They have already been included in the current WB/FAO/JSDF Project on Avian Influenza Emergency Recovery.

Room A: 11.45–12.00

51. Contagious caprine pleuropneumonia: A new disease threat to Europe

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The OIE list B disease contagious caprine pleuropneumonia (CCPP), caused by Mycoplasma capripneumoniae, can have a very serious impact on goat herds with morbidity and mortality rates of over 90% in newly affected regions. The disease has been identified in the Middle East, the Indian subcontinent, North and East Africa but is almost certainly under reported because of the fastidious nature of the causative mycoplasma. We report new outbreaks in the Thrace region of Turkey, several kilometres from the Greek Border which probably began in August 2002 and appears to be spreading in the region. Morbidity and mortality rates are reported to be very high in both adults and kids. The disease affected goats of all ages which showed a reluctance to walk, a fever of over 41°C and accelerated respiration with frequent coughing. The lungs of dead and euthanised animals have shown characteristic “port wine” lesions with abundant pleural fluid, fibrin and unilateral hepatisation; in one herd alone nearly 150 of 400 adults and over 100 of 400 kids died in 2004. Naturally infected goats, bought from one the herds, were able spread the disease to 90% of healthy contacts in less than two weeks.

Room A: 12.00–12.15

52. Detection of bovine tuberculosis in spill-over hosts

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Tuberculosis caused by Mycobacterium bovis is characterised by the progressive development of granulomatous lesions in lymph nodes, lung tissue and other organs. Cattle and other bovine species, including buffaloes and bison, are susceptible to the disease, but nearly all warm-blooded animals can be affected. Species are not all equally susceptible; some are spill-over or end hosts and others act as reservoirs (maintenance hosts). In Great Britain, bovine TB primarily affects cattle; however, the disease has also been found in badgers and deer. Infection in other farm and domestic animals, such as sheep, goats, llamas, pigs, dogs and cats, is not uncommon worldwide.
Humans are also susceptible. Diagnosis in cattle depends largely on the results of regular tuberculin testing and/or characteristic lesions being recognised at the slaughterhouse. This surveillance regime is not as rigorous for the other species and is dependant on veterinarians recognising the signs in the live animal or at post mortem. Confirmation of disease is the same and is made by identifying M. bovis on culture. However, there are other Mycobacterium species that occur in the environment, which can cause granulomas in immuno-compromised hosts, and these also need to be identified.

Room A: 12.15–12.30

53. An investigation of the immune profile of pigs during infection with Brucella suis or Yersinia enterocolitica 0:9

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Introduction. Brucella suis is a bacterium that causes brucellosis in swine. Current diagnosis relies on serological tests such as Rose Bengal Test (RBT) and competitive ELISA (cELISA). These tests are neither 100% specific or sensitive. False positive serological reactions (FPSR) are often attributed to cross reactive antigenic epitopes in the lipopolysaccharide of organisms such as Yersinia enterocolitica serotype 0:9. Materials and methods. 20 Gottingen Mini-pigs were experimentally infected with either B. suis or Y. enterocolitica 0:9; or a sham inoculation control. Whole blood from these animals was stimulated with Brucella specific antigen. Levels of IFNγ produced was measured using Biosource cytokine ELISA. The results were compared with RBT and cELISA. Bacterial culture techniques confirmed active infection.

Results. Expressed as % positive.

Discussion. Serological reactivity from the Yersinia infected animals confirm the current problems with FPSR. Use of Brucellergen antigen stimulation and IFNγ ELISA was shown to improve diagnostic specificity. However, the sensitivity of diagnosis of true Brucella infection was slightly reduced. This suggests a combination of standard serological tests with a confirmatory test using IFNγ would improve the specific diagnosis of B. suis infection.

Room A: 12.30–12.45

54. Detection and identification of Chlamydophila abortus.

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Enzootic abortion in ewes (EAE) is routinely diagnosed by detecting elementary bodies of Chlamydophila abortus in smears prepared from infected placenta. C. abortus can also be isolated from infected material in McCoy cells or embryonated eggs; these techniques are considered the `gold standard. Currently, the polymerase chain reaction (PCR) is increasingly being used for the detection and differentiation of Chlamydia sp. Primers targeting the genes encoding the inter spacer regions of the 16S and 23S ribosomal RNA, the conserved regions of the genes encoding the outer membrane proteins (omp1 and omp2), and the genes encoding the polymorphic membrane proteins (pmp) have been used to improve sensitivity and specificity of detection. In the present study 26 placental samples obtained from aborting ewes and goats were used to compare the sensitivity and specificity of PCR based on primers specific for omp1, omp2, pmp and 16S-23S rRNA interspacer gene with isolation in McCoy cells and embryonated eggs. Impression smears of all the samples were stained with Ziehl–Neelsen (MZN) before culture and found to be positive. C. abortus was detected by PCR in 25 of 26 samples (96.1%) using primers specific to omp1, in 12 of 26 samples (46%) using primers specific to omp2, in 13 of 26 samples (50%), using primers specific to pmp and in 24 of 26 samples (92.3%) using primers specific to the 16S-23S interspacer gene. All samples were positive for C. abortus by culture in McCoy cells and embryonated eggs. However, in 5 of 26 samples a second passage was required for the demonstration of inclusions in McCoy cells. These results indicate that isolation in cell culture or embryonated eggs is marginally more sensitive than PCR for the detection of C. abortus in field samples but it is more time-consuming and labour-intensive. Of the primers tested those specific to the omp1 gene were more sensitive. All of the primers used were specific to C. abortus, with no amplification of DNA extracted from cell cultures infected with Chlamydophila pecorum.
Room A: 12.45–13.00

55. A trial investigating the effect of organic acids in weaner pigs’ diets on Salmonella prevalence in finisher pigs

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Introduction. Future EU regulations will prevent the use of antibiotics as in feed growth promoters; the aim of this study was to examine the effect of a commercial in-feed organic acid product on the prevalence of Salmonella infection on a chronically infected pig farm. Materials and Methods. A group level intervention study (2 x 2 factorial design) was carried out with the addition of in-feed organic acids to groups either in the field, sheds, both or neither (control group). Salmonella levels were tested using 2 sets of pooled faecal samples per group at different stages and serum samples taken from individuals before leaving for finishing units. These serum samples were matched to the results of meat juice samples taken from the same individuals at slaughter. Results: Salmonella levels were highly variable between the groups, and the results of pooled faecal samples showed no significant reduction in salmonella prevalence in any of the treatment groups when compared to the control (for all groups p > 0.05, fisher’s exact). This was also reflected in serum and meat juice samples taken from individual pigs. Conclusions: The farm had underlying clinical problems in addition to salmonella and this study failed to demonstrate beneficial effects of in-feed organic acid supplements on salmonella prevalence, either at the group or individual pig level.

Room A. Theme: New, Evolving and Emerging Diseases (continued)

Room A: 14.00–14.30

56. KEYNOTE: New and emerging diseases of small companion animals

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Emergence or re-emergence of infectious disease in the UK companion animal population may be due to novel pathogens, or more frequently by increased contact between source of infection, competent vectors and susceptible animals through travel, environmental changes or husbandry practices. Between 2000 and 2004, more than 120,000 small animals travelled into the UK under the Pet Travel Scheme, many dogs being infected with leishmaniosis, babesiosis, or ehrlichiosis. Blood transfusions, direct contact (by biting?) and alternative arthropod vectors have consequently been incriminated in spread of infection to non-travelled dogs. The prevalence of sub-clinical infections in the travelled canine population is unknown. Over the past 4 years, the occurrence of mycobacterial disease (M. bovis, M. microti, M. avium intracellulare) in small animals in SW England has increased. In addition, potentially fatal canine angiostrongylosis previously restricted to SW England and south Wales, is being increasingly diagnosed in domestic dogs and foxes SE England and the Midlands. The major epidemiological aspects including risks to owners, companion animals, wildlife and livestock are currently unknown. The unknowns in these examples reflect the serious deficit in funding for infectious disease research including zoonoses in small companion animals, compounded by difficulties in sampling healthy animals and restrictions placed on research by charities.

Room A: 14.30–14.45

57. Thermophilic Campylobacter species in private household pets

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Campylobacteriosis is an important cause of infectious gastro-enteritis in humans. Dogs and cats may act as a reservoir for infection and a wide variety of prevalences has been detected in both asymptomatic pets and pets with signs of gastro-intestinal disease. Rectal swabs were collected from 46 household pets (40 dogs, 6 cats) at the University Veterinary Hospital, Dublin. Eight dogs and one cat showed signs of diarrhoea. Samples were cultured for Campylobacter spp by direct and filtration methods. An overall prevalence of 23/46 (50.0%) was found, with Campylobacter cultured from 8/9 (88.8%) symptomatic pets and 15/37 (40.5%) pets with no gastro-intestinal signs. Eleven of the 23 pets with Campylobacter isolated (47.8%) presented for routine vaccination. This illustrates the importance of asymptomatic carriers. Multiplex PCR will be performed for Campylobacter species differentiation and identification on each isolate.
Room A: 14.45–15.00

58. Risk factors for Salmonella prevalence on UK pig farms – a cross-sectional study

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An abattoir survey in 2003 showed that 23.4% of UK finisher pigs were infected with Salmonella, which is similar to that reported in 1999/2000. Following the example of other EU countries, the British pig industry recently set up the Zoonoses Action Plan (ZAP) scheme which aims to reduce Salmonella prevalence, and thereby any costs to public health which entail from this. As one of several VLA projects which support these goals, we selected over 400 farms at random from the databases of national farm assurance schemes, thought to cover over 91% of UK production. On over 100 units which were able to take part, pooled pen faecal prevalence and neck muscle seroprevalence of Salmonella in samples were determined and detailed management questionnaires completed. We will present results of analysis of these, estimating the relative importance of various risk factors for farm level prevalence. These results will be discussed in the context of current guidance given to farmers to reduce Salmonella, and the economic implications. We will outline an ongoing intervention study involving some of these farms, which aims to test these results.

Room A: 15.00–15.15

59. Use of a real time RT-PCR to investigate epidemiology of sheep pestiviruses

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Introduction. The pestiviruses of sheep (border disease virus; BDV) and cattle (bovine virus diarrhoea virus; BVDV) may infect sheep and cause economically important disease. While infection in non-pregnant sheep is usually sub-clinical, infection of pregnant ewes can cause abortion or the birth of immunotolerant persistently infected (PI) lambs, which are an important source of infection. Conventional diagnosis relies upon ELISA testing to demonstrate anti-pestivirus antibody in a flock or pestivirus antigen in the blood of PI animals. Use of PCR allows pestivirus genotyping, which is relevant given the recent reports of BVDV-2 in UK cattle and historical reports of BVDV-2 in sheep. Materials and Methods: Both a classical two step nested RT-PCR and a one-step real time RT-PCR were developed to differentiate BDV and BVDV types 1 and 2. 62 ovine samples provided for BDV antigen detection were examined by ELISA and with both RT-PCRs. Results. Correlation between the ELISA and RT-PCR assays was generally good. The two step classical nested RT-PCR and the one-step real time RT-PCR concurred in their genotyping results, though the classical assay appeared to be more sensitive. In 35 samples BDV was detected and in 7 samples BVDV was detected. In some cases more than one sample from a flock was tested; in terms of outbreaks 79% were due to BDV and 21% were due to BVDV-1. BVDV-2 was not detected in any samples tested. Discussion/conclusions. The majority of pestiviruses in sheep in the UK are BDV, but a significant proportion are BVDV-1. Both nested and real time RT-PCR are suitable for detection of PI sheep and for rapid genotyping of pestiviruses present.

Room A: 15.30–15.45

60. The development of multiplex Taq Man PCR assay to detect some of the most important abortifacient bacterial species to the bovine host

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A multiplex real-time Taq Man PCR assay was developed to detect four of the most important abortifacient bacterial species to the bovine host, namely, Brucella abortus, Salmonella enterica, Bacillus licheniformis and Arcanobacterium pyogenes. Four singleplex Taq Man® assays were initially developed to detect these bacterial species and then incorporated into a multiplex reaction. Each of the four singleplex assays and the multiplex assay were assessed for sensitivity using a $\log_{10}$ dilution series of quantified genomic DNA from the four abortifacient bacterial species. Results showed a decrease in the detection limit by a factor of 10 in the multiplex assay when compared to the singleplex IS711, stn and kerA assays. Replicates ($n=8$) at each starting genomic DNA concentration used in the singleplex and multiplex reactions revealed that the results are highly reproducible for each assay.
Currently there is no treatment or pre-clinical diagnosis for (OPA) and it is invariably fatal. All available data on the prevalence and distribution of OPA are based on retrospective histopathological reports. A flock with a history of OPA was recruited for a 3-year longitudinal study of JSRV infection in about 200 ewes and their offspring. A JSRV PCR test to detect JSRV provirus in the blood was optimised and used to identify JSRV infection. The PCR test was repeated every three months. The results for the first two years of the study will be reported: total positives in a single bleed varied from 31% to 59% for the breeding ewes and 25–58% for the lambs. The rate of infection of the ewes and of the lambs increased significantly after the first test. A higher proportion of older animals were positive. JSRV incidence in the ewe population was higher in the period prior to lambing. A positive result for the ewe was associated with a higher risk of the lamb being positive. Lambs from both negative and positive mothers became JSRV positive as early as 20–30 days after birth. A higher proportion of Texel-cross ewes and their lambs were positive compared to the other 2 breeds in this flock.

62. Detection of chlamydial genome in ovine genital tracts in an abattoir survey

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Introduction. The site of Chlamyphila abortus persistence between infection and abortion remains unknown. Possible persistence after abortion, with consequences in the transmission of Ovine Enzootic Abortion has been hypothesised. In this survey we examined if chlamydial genome can be detected in ovine uteri. Materials and Methods: Uterine tissue samples from 304 ovine genital tracts, collected at an abattoir using a sterile technique, were examined using a PCR for chlamydial genome detection. The pregnancy status/stage was determined by examining the embryo/fetus size and morphology. Samples from gravid uteri belonged to the first 100 days of gestation. The clinical history of the animals unknown. Results. The total prevalence of chlamydial genome detection was 30.9%, with a significantly higher prevalence in the pregnant animals (46.9%, \( P > 0.0001 \)). Higher detection rates were recorded during early gestation compared to mid-gestation. Discussion/conclusions. Our results indicate that there is evidence of some degree of persistence of infection in the uterus although they do not specify the origin of such infection. The role of uterine persistence in disease transmission remains unclear but it may explain previous evidence of chlamydial vaginal shedding during oestrus. Chlamydial uterine persistence may also affect the development of local immune mechanisms and improve our understanding of local protection mechanisms against abortion.

63. European bat lyssaviruses in the United Kingdom

MARSTON, D.A.**, McELHINNEY, L.M., BROOKES, S.M., JOHNSON, N., SELDEN, D., JOLLIFFE, T.A., PARSONS, G., WAKELEY, P.R., FOOKS, A.R.

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Since 1987 nearly 5000 dead or euthanased bats have been submitted to the VLA under the Defra funded 'passive' surveillance programme in the United Kingdom. Of these submitted cases, only 4 have been identified as being positive for European Bat Lyssaviruses (EBLV). All 4 cases were Daubenton’s bats and all were positive for EBLV-2. A recently developed Taqman assay was successfully used to rapidly genotype the isolates responsible in the two cases reported in Surrey and Lancashire in 2004. In the first case, the infected bat was found in the centre of Staines. In the second case, the bat had been found in Lancashire a year previously, then stored in a freezer before being submitted to the VLA in October 2004. The sequence data from both isolates show high homology to the other EBLV-2s isolated in the UK. The distribution of viral RNA was determined within selected organs of each bat and the Taqman assay was used to estimate the relative viral loads. EBLV-2 is endemic in the UK population of Daubenton’s bats at an estimated level of 3–8%. Spill over infections of EBLVs from bats to mammals have been documented, but there has been no evidence for host adaptation.
Room A: 16.30–16.45

64. Bat Lyssavirus active surveillance

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European Bat Lyssavirus type 2 (EBLV-2) is the only lyssavirus that has been detected in the UK (n = 5, 4 bats and 1 human), other than imported cases, since 1902. There have been 13 cases in mainland Europe associated with Myotis bat species (M. dasycneme and M. daubentonii) and one other human case. EBLV-1 is more prevalent (>700 cases) and generally associated with Serotine bats (Eptesicus serotinus) plus one confirmed and one suspected human case. EBLV-1 has not been isolated in the UK. In Scotland and England (n = 29 sites) serum samples and oral swabs were collected from Daubenton’s bats (n = 347) and in England (n = 6 sites) samples were collected from serotine bats (n = 51). Antibody prevalence was determined using specific (EBLV-1 or –2) fluorescent antibody virus neutralisation tests, and tissue culture and PCR were used for virus isolation from swabs. At EBLV-2 a priori sites (n = 2), 6–12% of bats were seropositive. When all samples were included this decreases to 3–8%. Pilot EBLV-1 seroprevalence data suggest that previous exposure levels may be similar (2%). All oral swab tests were negative suggesting that virus was not being shed in the saliva. Future surveillance for EBLV-1 & -2 will be undertaken throughout the UK.

Room A: 16.45–17.00

65. Occurrence of Mycoplasma bovis in pneumonic cattle in Britain between 2000 and 2004

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The Mycoplasma Group of the VLA (Weybridge) is the national reference centre for animal mycoplasmas performing identification and serological tests on clinical samples submitted by the VLA regional centres. Of the 1327 bovine isolates received, mainly from pneumonic lungs between 2000 and 2004, just over half (670) were identified as Mycoplasma bovis. M. bovis is a significant pathogen of young cattle causing pneumonia as well as arthritis, mastitis, otitis media and occasionally, abortion. The majority of M. bovis isolates, which appear increasingly resistant to antibiotic treatment, were identified by conventional cultural and serological tests such as the growth inhibition test, although molecular methods including PCR and a PCR/DGGE method are being used increasingly. The ELISA test detected antibodies to M. bovis in 22% of approximately 10,000 serum samples taken from pneumonic cattle tested during the same period. While the highest number of isolates are seen in early winter, M. bovis is increasingly seen throughout the year.

Room B. Theme: Veterinary Parasitology – from parasite biology to practical application

Room B: 09.00–09.30

66. KEYNOTE: How do parasites hid their spots?: A proteomics solution

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Parasitic worms such as gastro-intestinal nematodes and liver fluke are responsible for major diseases of worldwide livestock. For example, recent data estimates that 40–80% of cattle are now infected with liver fluke (Fasciola hepatica) in England and Wales, respectively. The failures to control parasitic worm infections are complex, correlating with recent climate change, poor drug efficacy, developing drug resistance, outdated diagnostics and predictive models, and the continued lack of commercial vaccines. A better understanding of basic parasite biology is pre-requisite to uncover new and alternative means of control. Proteomics (protein array and mass spectrometry) has opened up opportunities to systematically investigate parasitic-host interactions. The seminar will review the progress made at Aberystwyth University in incorporating global, targeted and functional proteomics strategies to increase understanding of the establishment of parasitic worms in mammalian hosts. Examples of differential expression, protein–protein interactions, in vivo analysis, individual parasite fingerprints and mechanistic-based proteomics will be assessed.
67. The effects of transforming growth factor-\(\beta_1\) on the proteome of mouse bone marrow derived mast cells

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Introduction. Gastrointestinal nematode infections in rodents and ruminants elicit Th2 responses that are characterised by mast cell recruitment and expression of mucosal-specific mast cell chymases. In mice, the presence of active transforming growth factor (TGF)-\(\beta_1\) in the gut epithelium is necessary to generate the mucosal mast cell phenotype. In vitro homologues of mucosal mast cells can be generated by culturing bone marrow stem cells in the presence of stem cell factor, interleukin-9 and TGF-\(\beta_1\), Materials and methods. Bone marrow derived mast cells (BMMC) were grown in culture in the presence or absence of TGF-\(\beta_1\) and cellular extracts analysed by two-dimensional gel electrophoresis.

Results. Mast cell proteases-1 and -2 were highly upregulated in TGF-\(\beta_1\) treated cells, making up approximately 40% of the total soluble protein. The chaperone calnexin was also upregulated. SerpinB1a and galectin-1 were found to be less abundant following TGF-\(\beta_1\) treatment. Quantitative real-time PCR and FACS analyses confirmed the downregulation of galectin-1. Conclusions. Proteomic analysis of mast cells in vitro has given insights into how mast cells may behave in situ under the influence of TGF-\(\beta_1\). Downregulation of galectin-1 will influence the interaction between mast cells and the basement membrane and may facilitate their eventual migration into the epithelium.

68. Characterisation of the expression of the sialyltransferase, SIAT-4C (ST3Gal-IV) in response to selected cytokines in vitro

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Mucus is composed of mucins, secretory antibodies, anti-proteases, antimicrobial peptides, lactoferrin and trefoil peptides. Mucins, the main constituent of mucus, are glycoproteins containing up to several hundred carbohydrate chains attached to the peptide (apomucin) by O-glycosidic linkages. Typical human mucins contain fucose, galactose, N-acetylgalactosamine, N-acetylgalactosamine, mannose, sulfate and sialic acid. Infection of mice and rats with Nippostrongylus brasiliensis has been shown to cause transient alterations in the glycosylation patterns of the intestinal mucins. Respiratory tract infections in man have been shown to cause increased sialylation of mucins. Expression profiling using microarrays identified the up regulation of the sialyltransferase, SIAT-4C (ST3Gal-IV) in the jejunum of BALB/c mice during infection with Trichinella spiralis. The aim of the present study was to characterise the expression of SIAT-4C (ST3Gal-IV) in response to selected cytokines in vitro. The human colonic adenocarcinoma cell line (LS174T) and the human respiratory muco-epidermoid carcinoma cell line (NCI-H292) were used. Cells were incubated for 48 h with one of the following cytokines TNF-z, IL-\(\beta\), IFN-z, IL-4, IL-9 or IL-13. RNA was extracted, reverse transcribed and amplified by PCR using published primers for ST3Gal-IV. The results and the significance of findings will be discussed.

69. Characterisation of cyathostomin beta tubulin gene sequences and expression patterns

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Introduction. Anthelmintic resistance is currently a major veterinary research priority. Resistance to benzimidazole (BZ) anthelmintics threatens the control of parasite species worldwide, including cyathostomin, the primary equine parasitic pathogens. BZs target beta tubulin, and both point mutations and selection for specific isoatypes cause resistance in ruminant nematodes. Cyathostomin BZ resistance mechanisms however remain unknown, and only isotype 1 has been described. Materials and methods. Full-length beta tubulin isotype 2 cDNAs were cloned and sequenced from 2 cyathostomin species, and compared to isotype 1 from the same cDNA population. Partial isoype 2 genomic DNA sequence was PCR-amplified, and intron-exon boundaries were determined. Expression of both isoatypes was analysed in four cyathostomin life cycle stages, by semi-quantitative RT-PCR. Results. The isotype 2 cDNAs each encoded a 450 amino acid protein with an isotype 2-specific carboxyl-terminal, and clustered phylogenetically with trichostrongylid isotype 2 beta tubulins. Slight differences in genomic organisation were observed between the two cyathostomin isoatypes. Constitutive temporal expression of isoype 1 was revealed, whilst isotype 2 was only detected in adult RNA. Discussion. This study presents the first description of a cyathostomin beta tubulin isoype 2 gene, which will allow investigation of the role of both cyathostomin isoatypes in BZ resistance.
70. Upregulation of intelectin by goblet cells in response to TH2 stimuli

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Introduction. Inteletin 2 (Itln2), a novel 37–40 kD lectin, expressed by goblet and Paneth cells, is highly upregulated in the jejunum of nematode infected mice. An ovine homologue of Itln 2 is expressed by airway goblet cells in sheep harbouring the lungworm *D. filaria*. It is likely that Itlns play an important role in the innate immune response to nematode infection. Materials and methods. A human goblet cell-like line (LS174T) was used to determine whether TH2 or TH1-specific cytokines influenced expression of Itlns. Cells were cultured in medium containing interleukin-1β (IL-1β), IL-4, IL-9, IL-13, tumour necrosis factor α, or interferon γ. The cells were harvested when they became confluent and RT-PCR reactions were carried out using primers for human Itln 1 and 2. Results. There was increased expression of both human Itln 1 and 2 only in those cells grown in IL-4 or IL-13. Interestingly a change in morphology was also noted in these cells under light microscopy. Conclusion. This data, using a goblet cell line, suggests that Itln expression may be controlled by TH2 cytokines. This adds further weight to the suggestion that Itln is important in the innate immune response to nematode infections.

Room B: 10.30–10.45

71. Leucine aminopeptidase of the blood fluke *Schistosoma mansoni* and its homologue in *Fasciola hepatica*

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The ability of helmint parasites to survive in their host is dependent on the acquisition of nutrients via a number of endo and exoproteases produced by the parasite gut to facilitate digestion. Although the endoproteases of both *Schistosoma mansoni* and *Fasciola hepatica* have been widely reported, less is known about the final breakdown of host peptides to amino acids, which can then be absorbed by the parasite. A functional recombinant *S. mansoni* leucine aminopeptidase (LAP) was expressed in insect cells. This recombinant LAP shared biochemical properties, including pH optimum for activity and substrate specificity, with the major aminopeptidase activity in soluble extracts of adult worms. The pH range in which the enzyme functions and the lack of a signal peptide indicate that the enzyme functions intracellularly. Immunolocalisation studies showed that the *S. mansoni* LAP is synthesised in the gastrodermal cells surrounding the gut lumen. Accordingly, it is proposed that peptides generated in the lumen of the schistosome gut are absorbed into the gastrodermal cells and are cleaved by LAP to free amino acids before being distributed to the internal tissues of the parasite. Due to the high level of similarity between these LAP homologues, it is likely that this enzyme plays the same crucial role in *F. hepatica*. Thus, LAP is a vaccine candidate worthy of investigation against both helmint parasites.

Room B: 10.45–11.00

72. Role of adjuvants in the protection produced by recombinant vaccines for the control of liver fluke infection in sheep

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∗ Institute of Infectious Disease, University of Technology, Sydney, Australia

Introduction. Fasciolosis (Liver fluke infection) causes clinical and subclinical disease in ruminants worldwide. The development of an effective vaccine against *Fasciola hepatica* is of increased importance due to the rise in drug resistance, as well as an increased incidence of disease in certain areas. We have used recombinant *Fasciola hepatica* cathepsin L1 (rFhCL1) and thioredox peroxidase (TPx) as vaccine antigens to experimentally immunise sheep against *Fasciola hepatica*. Finding an appropriate adjuvant to enhance the immune response to vaccine antigens is crucial. Materials and methods. Two experiments were carried out in sheep, a field trial and an experimental challenge infection. In both cases sheep were immunised on three occasions using rFhCL1 and/or TPx. Various adjuvants were used including Freund’s adjuvant, Aluminium hydroxide and Quil A. In the field trial, sheep were infected naturally by grazing on fluke-infected pasture. Experimentally infected sheep were dosed with 200 *F. hepatica* metacercariae. Immunological parameters, faecal egg counts (FEC), fluke burdens and liver enzymes were monitored. Results. The magnitude of the immune response, both humoral and cellular, produced following vaccination varied greatly between adjuvants used. The response to vaccination using Aluminium hydroxide was poor relative to Quil A and Freund’s. There was correlation between the magnitude of response and reduction in FEC and total fluke burden. Discussion/conclusions. The adjuvant used in vaccination of sheep with a recombinant vaccine for liver fluke infection plays a crucial role in its success.
73. The efficacy of nitroxynil and triclabendazole administered synchronously against juvenile triclabendazole-resistant *Fasciola hepatica* in sheep

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Triclabendazole has been widely used in the treatment of liver fluke infections in cattle and sheep for the last 20 years. It is the only flukicide that claims efficacy against all stages of liver fluke, from early immature to adults. However, triclabendazole resistance has been reported in many countries and appears to be an increasing problem. Previous studies have shown synergy between triclabendazole and other flukicides against triclabendazole-resistant (TCBZ-R) isolates. A study was conducted to investigate whether synergy exists between nitroxynil and triclabendazole, when administered at their recommended doses, in the treatment of immature (4 week-old) TCBZ-R *F. hepatica*. In total, 68 indoor-fed lambs were used in the study. Lambs were allocated to six groups and dosed with either 250 triclabendazole-susceptible (TCBZ-S) metacercariae (Groups 1 & 3) or 250 TCBZ-R metacercariae (Groups 2, 4, 5 & 6). Treatments were administered 28 days after infection. Groups 3 and 4 were dosed with triclabendazole; Group 5 with nitroxynil, Group 6 with triclabendazole and nitroxynil and Groups 1 and 2 were left untreated. Two lambs from each group were euthanased and examined 48 h post-treatment, while the remaining lambs were euthanased and examined eight weeks post-treatment (12 weeks post-infection). Within groups dosed with TCBZ-R metacercariae, similar numbers of fluke were recovered eight weeks post-treatment (53, 63, 43 and 51 mean number of flukes recovered in Groups 2, 4, 5 & 6, respectively). The study failed to show efficacy of combined nitroxynil and triclabendazole treatment against immature TCBZ-R *F. hepatica* infection.

74. Prevalence of *Fasciola hepatica* infection in dairy herds in England and Wales using a bulk milk tank ELISA

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Measuring antibodies in bulk tank milk samples by ELISA provides a simple, low cost method of assessing the disease status in herds of dairy cattle for both control and surveillance purposes. A serum antibody detection ELISA developed to diagnose *Fasciola hepatica* infection in cattle was adapted and validated for use with bulk tank milk samples. The prevalence of infection in 61 dairy herds was established using serum or faecal egg counts from a proportion of individual cattle within each herd. The correlation between the ELISA and herd seroprevalence was 0.83. Using a cut off value of 27 Percent Positivity, the bulk tank ELISA identified herds in which more than 25% of the cows were infected, with a diagnostic sensitivity of 96% (95% Confidence Intervals, 89%, 100%) and a diagnostic specificity of 80% (95% Confidence Intervals, 66%, 94%). Using 623 bulk tank samples from herds across England and 445 samples from Wales, the prevalence of *F. hepatica* infection in dairy herds was established as 48% (95% Confidence Intervals, 46%, 54%) in England and 86% (95% Confidence Intervals, 84%, 90%) in Wales.

75. Infection of cattle with *Neospora caninum* in early and late gestation – parasite distribution and lesions in the foetus and placenta

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Introduction. The apicomplexan parasite *Neospora caninum* is an important cause of bovine abortion throughout the world. It has been hypothesised that the parasite could be causing abortion by multiplying uncontrollably in an immunologically immature foetus. This hypothesis
Materials and methods. Three groups of six pregnant heifers were orally infected with a nominal dose of 40,000 sporulated oocysts at days 70, 120 and 210 of pregnancy. Immune responses were measured throughout gestation. In addition foetal viability was monitored at least fortnightly. Viability of the oocysts was confirmed by bioassay in gerbils using titrated doses of \( N. \) caninum oocysts. Results. Fifteen cows developed Neospora-specific antibody responses and all animals developed lymphoproliferative responses. One cow infected at 120 days of pregnancy aborted; brain from the foetus was positive for \( N. \) caninum DNA by PCR. Four animals infected at 210 days gave birth to live, congenitally infected calves at term. One animal infected at 210 days aborted but the cause of the abortion was not established. The remaining animals gave birth to uninfected calves at term. Conclusion. This study demonstrates experimentally that oocyst infection can cause abortion, or can congenitally infect calves.

Room B: 12.15–12.30

76. Neospora caninum: Oocyst challenge of pregnant heifers at different stages of pregnancy

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Introduction. Previous work has shown that infection of pregnant animals with Neospora caninum tachyzoites at 70 days gestation resulted in foetal death but at 210 days resulted in the birth of live congenitally infected calves. In another study, infection of pregnant cows with 600 \( N. \) caninum oocysts at 70 days of pregnancy, resulted in the birth of live, unfedborn calves at term. The objective of this study was to investigate the response of pregnant cattle to oral infection with \( N. \) caninum oocysts at different stages of pregnancy. Materials and methods. Three groups of six pregnant heifers were orally infected with a nominal dose of 40,000 sporulated \( N. \) caninum oocysts at days 70, 120 and 210 of pregnancy. Immune responses were measured throughout gestation. In addition foetal viability was monitored at least fortnightly. Viability of the oocysts was confirmed by bioassay in gerbils using titrated doses of \( N. \) caninum oocysts. Results. Fifteen cows developed Neospora-specific antibody responses and all animals developed lymphoproliferative responses. One cow infected at 120 days of pregnancy aborted; brain from the foetus was positive for \( N. \) caninum DNA by PCR. Four animals infected at 210 days gave birth to live, congenitally infected calves at term. One animal infected at 210 days aborted but the cause of the abortion was not established. The remaining animals gave birth to uninfected calves at term. Conclusion. This study demonstrates experimentally that oocyst infection can cause abortion, or can congenitally infect calves.

Room B: 12.30–12.45

77. Vaccination against Neospora-associated abortion in cattle

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Neospora caninum is a frequently diagnosed cause of abortion in dairy cattle throughout the world, yet there is, at present, no satisfactory method of controlling the disease. Transmission of the parasite occurs exogenously via ingestion of oocysts and endogenously via transplacental spread of the tachyzoite stage of the parasite to the foetus. Vaccination would be the ideal control strategy to prevent exogenous infection. Here, we have established proof of principle, that immunisation with live tachyzoites inoculated intravenously to heifers protects against subsequent foetopathogenic challenge with a heterologous strain of the parasite. Cattle were immunised 10 weeks prior to gestation with intravenous inoculation of 10³ tachyzoites of the Nowra isolate of \( N. \) caninum. Following artificial insemination, cows were challenged at 70 days of gestation with an intravenous inoculation of 10³ tachyzoites of the NCiv isolate of \( N. \) caninum. Blood samples were taken throughout the experiment and foetal viability was monitored by trans-rectal ultrasonography. Foetuses of immunised cows survived to the end of pregnancy and were clinically normal at birth. Moreover, no evidence of \( N. \) caninum infection was detected in these calves. In contrast, foetal death was recorded in five out of six naive cows challenged with NcLiv at 70 days of gestation. These results demonstrate conclusively that immunisation with an intravenous inoculation of \( N. \) caninum tachyzoites protects against foetopathy induced by exogenous challenge during pregnancy.
Room B. Theme: Veterinary Parasitology – from parasite biology to practical application (continued)

**Room B: 14.00–14.15**

**78. GRA2 is a marker of acute Neospora caninum infection in cattle infected via different routes**

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Introduction. Neospora caninum infection can occur via two routes: endogenous transmission occurs when an existing infection in the dam undergoes recrudescence resulting in the infection of the foetus in utero, whereas exogenous transmission occurs through ingestion of oocysts. The mode of transmission is an important factor in deciding upon preventative control measures, as well as in enabling successful production of vaccines and chemotherapeutic agents. How and when the animal becomes infected is critical in determining whether abortion will occur. It is therefore important that we are able to identify the route of infection in order to gain a more thorough understanding of the importance of transmission in Neospora-associated abortion. Presently the only means we have of achieving this is to use antibody avidity ELISAs that give a retrospective indication as to whether or not an infection is recent. Materials and methods. We have used ELISA and western blot to test panels of sera derived from cattle that have been infected with the parasite via different routes. We have also looked at sera from infected individuals. Results. Preliminary studies using ELISAs showed that acutely infected animals had an antibody response to this antigen whilst in chronically infected animals the response was low or, in individuals that had been orally dosed with oocysts, not detectable. Also, Western blots probed with the same panels of sera, showed that there was a differential recognition of GRA2 and other antigens. Conclusions. We have shown that the presence of anti-GRA2 antibodies is dependant on the mode of transmission or the chronicity of infection. These data have implications for improving our understanding of the role different routes of transmission have in causing abortion and for implementing effective control measures.

**Room B: 14.15–14.30**

**79. Is the immune response to Neospora caninum incompatible with pregnancy in cattle?**

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Introduction. Neospora caninum is a protozoan parasite that is the most frequently diagnosed cause of abortion in dairy cattle in the UK. Why infected cattle abort is not known but it has been suggested that it is due either to induction of a cell mediated response at the foetalplacental interface that is incompatible with foetal survival or due to uncontrolled parasitaemia that develops in an immunologically immature foetus. The aim of this project is to determine the role of cytokines released at the materno-foetal interface in the pathogenesis of N. caninum associated abortions. Materials and methods. Cytokine specific real time quantitative PCR assays were used to measure expression of cytokines in the maternal caruncles and foetal cotyledons from cattle infected early or late in gestation. Results and discussion. High levels of interferon gamma were detected in the caruncle tissues from two cows infected early in gestation and whose foetuses were killed by the infection. In six other cows, whose foetuses were still alive when the mothers were euthanised, there was no significant difference in any cytokine tested other than IL4. There was a significant increase in expression in interferon gamma, IL4, IL10, IL12, TNFalpha and IL18 in the caruncles of cows infected late in gestation but whose foetuses were alive when the animals were euthanised. These results suggest that both pro-inflammatory and regulatory cytokines are produced at the materno-foetal interface, following infection with N. caninum. It is not clear if the pro-inflammatory cytokines contribute to the death of the foetus or are the result of parasite induced tissue necrosis.

**Room B: 14.30–14.45**

**80. ACME works – the key to sustainable control of parasites in sheep in Scotland**

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Introduction. Parasitic gastroenteritis and anthelminthic resistance are increasing in sheep flocks in Scotland. A radical approach to the problem is therefore required to protect animal welfare and sustain the viability of the sheep industry in Scotland. Materials and methods.
Helminth data and clinical history was obtained from diagnostic submissions from sheep flocks throughout Scotland to SAC Veterinary Centres. Collation of this data with results of on-farm investigations, field and experimental studies, was used to provide advice to veterinary surgeons and sheep farmers via farmer/veterinary meetings, technical information, popular articles and scientific publications. Results. Surveillance figures indicate that the number of outbreaks of PGE and Nematodirus disease in sheep has increased steadily over the last ten years and seasonal figures for 2004 indicate that PGE is now occurring during the greater part of the year including the winter months, threatening the welfare of sheep in many areas. Although the situation is not as serious as yet in the UK, a survey in Scotland in 2002 showed that over 80% of lowland farms had BZ resistance, a threefold increase since the last survey nine years previously. The interim results from a small survey on farms may have worms that are resistant to this anthelmintic and multiple resistance has been seen in both goats and sheep. Discussion/conclusions. Based on the above and Defra guidelines for sustainable worm control strategies for sheep the following guidelines are proposed:
• Adopt a quarantine strategy.
• Check the efficacy of the anthelmintics that are being used.
• Monitor to decide when to treat and what to treat against.
• Ensure best practice regarding the use of anthelmintics.
• Work out a control strategy with veterinary input.
• Reduce dependence on anthelmintics.
• Kill effectively when using anthelmintics.
• Select strategies that help preserve susceptible worms.

Room B: 14.45–15.00

81. Molecular epidemiology of cryptosporidiosis in cattle – a cross-sectional study

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Introduction. Cryptosporidium is an important cause of diarrhoea in humans and animals. Some species have been shown to be potentially zoonotic. The aim of this study was to determine prevalence and risk factors for Cryptosporidium infection in unweaned calves from farms in an area of Cheshire and to investigate the distribution, variation and zoonotic potential of genotypes. Method. 41 farms were visited in April/May 2004 and voided faecal samples were collected from unweaned calves. Farm, pen, individual animal and stool level variables were recorded. Samples were screened for Cryptosporidium oocysts using three different methods and positive isolates were assigned to species using PCR-RFLP of 18S rRNA gene. Intra-species variation will be investigated by sequencing of the 18S rRNA, COWP, GP60 and HSP70 genes. Results. A total of 215 samples were collected. When all screening methods were considered, the animal-level prevalence was 38%, with 73% of farms having at least one positive animal. On these farms, prevalence ranged from 17% to 100%, with a mean of 45%. Risk factors for infection have been identified, including age of the calf. PCR-RFLP of the 18S rRNA gene has so far identified all isolates to be C. parvum. Conclusions. The zoonotic agent, C. parvum is widespread in this population. Risk factor analysis may lead to intervention strategies.

Room B: 15.00–15.15

82. First characterisation of Cryptosporidium andersoni from cattle in the United Kingdom

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Cryptosporidium Reference Unit, NPHS Microbiology Swansea (Velindre NHS Trust), Singleton Hospital, Sketty, Swansea, SA2 8QA

Introduction. Profuse numbers of cryptosporidia were detected in a routine diagnostic faeces sample, submitted to the VLA, from a freshly calved dairy cow with diarrhoea. This unusual finding prompted the referral of the sample to the UK Cryptosporidium Reference Unit (National Public Health Service, Swansea) where the species was identified as Cryptosporidium andersoni. This is the first reported detection and molecular characterisation of C. andersoni from cattle faeces in the UK. Materials and methods. Genus-specific immunofluorescence microscopy and differential interference contrast microscopy were performed on the faecal samples. Species identification was by nested PCR-RFLP and DNA sequence analysis of a portion of the small subunit rRNA gene. Results. Large Cryptosporidium oocysts were identified in the faeces of the dairy cow, which were confirmed as C. andersoni by molecular characterization. Discussion. C. andersoni has been found in cattle faeces in other European countries, but this is the first report from the UK. No overt illness is generally seen with C. andersoni infections although abomasitis, reduced milk yield and poor weight gain have been reported. It is not known if the organism was the primary cause of diarrhoea in this case as the cow was freshly calved and had metritis. No other enteric pathogens were found.
83. KEYNOTE: Veterinary Pathology Liaison Group of the Royal College of Veterinary Surgeons

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Changes in the organisation of the veterinary profession have gathered pace in recent years. There is a worldwide shortage of veterinary pathologists; there are proposed changes to the Veterinary Surgeons Act; the Professional Development Phase (PDP) is planned, Continuing Professional Development (CPD) and the profession must also operate in a developing European environment. Historically, there has never been a Certificate or Diploma course in veterinary pathology under the auspices of the Royal College of Veterinary Surgeons (RCVS). At the inception of specialist qualifications in the RCVS, veterinary pathology was already offered by the Royal College of Pathologists (RCPath), so there was no perceived need for a similar qualification. In the late 1990s, the European College of Veterinary Pathologists (and later the European College of Veterinary Clinical Pathologists) were formed. In 2003, an approach was made to form a group that could act as a link between the broad range of veterinary pathologists and the RCVS. The group hopes to help with recruitment, support and development of veterinary pathology in the modern era. The group has 6 members and reports to the Education Committee and SFEC in the RCVS.

84. Comparison of two different sampling techniques to demonstrate viral pathogens in post mortem bovine pneumonia cases

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Introduction. The diagnostic process in post mortem bovine pneumonia cases involves demonstrating the presence of viral pathogens such as IBR, BVD, RSV and PI3. The fluorescent antibody test (FAT) is routinely used for this purpose and can be done on slides from lung impression smears or swabs. The aim of this study is to determine which technique gives the best detection rate. Materials and methods. Both sampling methods are carried out on all suitable bovine pneumonia cases submitted to Thirsk Veterinary Laboratory Agency (VLA) for diagnostic purposes from October 2004 till February 2005. Swabs are taken from the trachea and bronchi and tissue samples are collected from the lung and trachea. The tissues and swabs are then processed for FAT as per prescribed VLA standard operating procedures. McNemar’s test will be used to determine any statistically significant difference. Results. Results so far suggest no significant difference in the detection rate between the two methods. FAT’s from swabs are easier to interpret. Discussion/conclusion. Indications are that the tissue and the swab methods give equal recovery rates. The swab method is recommended because of easier interpretation. FAT on a single swab taken from the tracheal bronchus appears to be adequate to demonstrate viral involvement.

85. Expression of jaagsiekte sheep retrovirus antigens in the lung of ovine pulmonary adenocarcinoma affected sheep: Pathogenic implications

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Ovine pulmonary adenocarcinoma (OPA) is caused by a retrovirus known as jaagsiekte sheep retrovirus (JSRV). In OPA-affected animals there is no evidence of circulating JSRV-specific antibodies and the reduced response to ConA indicates an alteration in systemic
immunity. The expression of JSRV antigens in the lungs of OPA clinically affected sheep was examined. The further step was to investigate whether sheep naturally infected with JSRV and without clinical signs can develop antibodies against JSRV capsid (CA) and envelope (SU) proteins, after immunisation with newly synthesised fusion proteins. Immunohistochemistry examinations on lung sections of OPA affected animals showed JSRV-CA protein in the cytoplasm of a proportion of ATII neoplastic cells as already described in previous studies. Immunolabelling with JSRV-SU immune-serum showed consistent reactivity in the neoplastic epithelial cells, most intense at the apical surface of the tumour cells. Anti-CA antibodies were produced in all the JSRV viraemic and non-viraemic animals. No antibodies to JSRV-SU were detected in the sera of non viraemic animals. The failure to induce specific immune-response to JSRV-SU can be a consequence of the expression of endogenous retroviral sequences closely related to JSRV. The finding of consistent expression of JSRV-SU protein in tumour cells is consistent with the hypothesis of a possible role of JSRV envelope in proliferation.

**Room C: 10.00–10.15**

86. Development and validation of in situ hybridisation for the detection of porcine circovirus 2 (PCV2) and the application of this technique to the first outbreak of Post-weaning multisystemic wasting syndrome in New Zealand

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Post-weaning multisystemic wasting syndrome (PMWS) is associated with porcine circovirus 2 infection, causes high mortality and morbidity in 4–12 week old pigs and has emerged as a global threat to the swine industry. The diagnostic criteria for PMWS include the demonstration of characteristic clinical signs, histopathological lesions in tissues (including lymph nodes, tonsil, lung and kidney) and the demonstration in these lesions of PCV2 antigen by immunohistochemistry and/or PCV2 nucleic acid by in situ hybridisation. New Zealand MAF submitted tissues to the VLA for confirmatory diagnosis of PMWS, from an outbreak that would represent the first official report of PMWS in New Zealand. Immunohistochemistry facilitating interpretation of infection in individual cell types.

**Room C: 10.15–10.30**

87. Tracheal organ cultures for the study of Feline herpesvirus induced functional and morphological changes in respiratory epithelium

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*Introduction.* Feline herpesvirus-1 (FeHV) is primarily an epitheliotropic virus and induces extensive necrosis of respiratory epithelium. The aim of this study was to determine the effect of FeHV on respiratory epithelial cell viability and function. *Materials and methods.* Tracheas were collected, with informed owner consent, from cats euthanased for reasons other than respiratory disease. After 24 h in tissue culture, tracheal sections were incubated with a high dose of FeHV. Cilia movement, viral replication (evaluated by reisolation from serial dilutions of culture media) and viral load (determined by qPCR), as well as morphological changes and viral antigen expression (evaluated by light microscopy including immunohistochemistry) were examined over a variable time scale up to 120 h post infection. *Results.* Tracheal rings remained viable in culture and were infected in vitro with FeHV. Infection led to exponential viral growth in the early phase. Tracheal epithelial cells were not diffusely infected; instead, foci of infected epithelial cells, exhibiting intranuclear inclusion bodies prior to necrosis and detachment, were observed. Infection of individual cells was confirmed by the presence of viral antigen. At later time points, tracheal sections were largely devoid of epithelial cells. *Discussion.* Results of our study show that tracheal rings are suitable for in vitro studies of the effect of FeHV on the respiratory epithelium. This in vitro system can now be used to assess the effect of FeHV on the function of infected cells, prior to necrosis.
Previous studies indicate that γδ T lymphocytes, although comprising a small percentage of the pulmonary leucocyte population, have a critical role in the initial, innate response to bacterial infection including regulation of the movement and function of inflammatory effector cells. This role is investigated using a well-established murine infection model where a range of immunopathological parameters between control mice and mice with targeted disruption of their T-cell receptor δ-chain gene [γδ knockout (ko) mice] are compared. Following aerosol challenge with *Bordetella pertussis* knockouts indicated accelerated pulmonary injury in ko mice compared to controls despite lower contemporaneous bacterial loads. Higher densities of perivascular neutrophils in tissue sections and greater numbers of leucocytes and greater albumin and myeloperoxidase levels in broncho-alveolar lavage (BAL) samples in ko mice indicate differences in lung pathology in the two mouse phenotypes are mediated by differing degrees of leucocyte trafficking into infected alveoli. This in turn suggests a regulatory influence for γδ T cells in the choreography of the early inflammatory response to Gram-negative bacteria. Their function would appear to include the prevention of exaggerated, harmful inflammatory host responses through their influence over the migration of inflammatory effector cells such as neutrophils.

**Room C: 10.30–10.45**

**88. γδ T cells regulate the early host response to *Bordetella pertussis* infection in the murine respiratory tract**

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**Room C: 10.45–11.00**

**89. Poxvirus infection in a black Eastern Gray squirrel (*Sciurus carolinensis*)**

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Introduction. A female adult squirrel was found dead and submitted for post mortem examination because of its severe skin lesions. Materials and methods. Performed were gross and microscopic examination of carcass and tissues, skin scrapings and bacterial and fungal culture and histological data from control animals from a single source over a 7 year period was performed. Data were analysed using Fisher’s exact or χ² tests. There was no statistical difference in the incidence of gastric mineralisation between males (77.4%) and females (69.3%) or between animals dosed via oral (73.4%) or non-oral routes with vehicle control (73.2%). However, there was a significant (*P* < 0.0001) decrease in the incidence of gastric mineralisation in studies of 52 weeks (20.8%) compared to 4 weeks duration (80.5%). The data suggest that age is at least one of the factors determining the incidence of gastric mineralisation. Data from this analysis will be used to inform a prospective study of other potential factors including age at start of study, severity of mineralisation, diet, clinical chemistry and concurrent pathology in the stomach.

**Room C: 11.30–11.45**

**90. Factors affecting the incidence of focal mineralisation of the gastric mucosa of Beagles**

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Focal mineralisation of the lamina propria of the gastric fundus is a common incidental finding in Beagle dogs in routine safety studies. In the general domestic dog population gastric mineralisation is less common but is occasionally seen with uraemia. As the underlying cause of mineralisation in clinically normal experimental animals is unclear this study aimed to investigate factors determining the incidence of mineral deposition. A retrospective analysis of histopathological data from control animals from a single source over a 7 year period was performed. Data were analysed using Fisher’s exact or χ² tests. There was no statistical difference in the incidence of gastric mineralisation between males (77.4%) and females (69.3%) or between animals dosed via oral (73.4%) or non-oral routes with vehicle control (73.2%). However, there was a significant (*P* < 0.0001) decrease in the incidence of gastric mineralisation in studies of 52 weeks (20.8%) compared to 4 weeks duration (80.5%). The data suggest that age is at least one of the factors determining the incidence of gastric mineralisation. Data from this analysis will be used to inform a prospective study of other potential factors including age at start of study, severity of mineralisation, diet, clinical chemistry and concurrent pathology in the stomach.
Room C: 11.45–12.00

91. Isolation of *Mycoplasma bovis* from brain lesions of an Irish calf

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*Mycoplasma bovis* is a primary cause of pneumonia, arthritis and mastitis and has also been linked to other clinical disease of cattle including keratoconjunctivitis and otitis media. *Mycoplasma bovis* is not usually associated with infections of the brain. A 10 month old friesian male calf was submitted with a history of clinical signs of ataxia, depression, apparent blindness and severe weight loss which did not respond to antibiotic treatment. Prior to euthanasia on humane grounds, the calf was dull, lethargic, in very poor body condition and grinding its teeth. Post-mortem examination revealed a three-inch diameter space-occupying lesion in the left atrium, which protruded through the left atrio-ventricular valve into the left ventricle. In addition the cerebral hemispheres of the brain contained multifocal lesions of necrotic material. The presence of an endocarditis lesion together with lesions with similar histopathological appearance in the brain was suggestive of an infectious etiological agent, which did not primarily generate a purulent inflammation. However, blood agar plates inoculated with aseptically collected samples from the lesions remained apparently sterile after 48 h of aerobic and microaerophilic incubation at 37 °C. ZN stained, Gram stained and PAS stained fixed tissue sections revealed no acid-fast, gram-positive bacteria or fungi. Mycoplasma colonies were isolated after 72 h from both lesions and identified as *Mycoplasma bovis*.

Room C: 12.00–12.15

92. Atypical scrapie – confirming the unconfirmable?

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Since 2002, active surveillance of the sheep slaughter population has been undertaken in Great Britain to fulfil the requirements of EU legislation. In 2002/2003, 29 201 sheep were tested using the Bio-Rad Platelia ELISA on brainstem and confirmed by immunohistochemistry (IHC). In addition to the 24 positive cases identified by the ELISA test, there were an additional 28 positive cases that could not be confirmed using statutory diagnostic IHC with R145 (UK statutory diagnostic antibody (raised to a C terminal epitope). However, by application of a panel of antibodies, limited PrP^d^ staining was identified in the nucleus of the spinal tract of the trigeminal nerve (V). In the 2004/2005 survey, IHC has been performed using 3 different antibodies on all ELISA positive cases and over 700 ELISA negative cases. The same pattern has emerged, with approximately half of the ELISA positive cases containing staining in the V area of the obex only with antibody 2G11 (raised to a core epitope). Interestingly, a high proportion of the atypical cases carried alleles associated with resistance to scrapie and of the type being selected for in the National Scrapie Plan. There have been a number of publications from other EU countries reporting a similar phenomena, but only with a very small number of animals. Investigation into PrP^d^ staining in other brain areas has demonstrated PrP^d^ staining and has led to changes in tissue collection in order to collect cerebellum where possible to support disease confirmation. These data raise several important questions. Does this staining represent something new or a previously unidentified form of scrapie? Is it infectious? What might the clinical signs be? What are the implications for the National Scrapie Plan? Statutory changes have already been made for sample collection and should aid in disease confirmation.

Room C: 12.15–12.30

93. Subpial myelopathy following parenteral copper administration in sheep

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The cause of neurological disease following parenteral copper administration was investigated in 10 sheep flocks. Between 1% and 4% of ewes developed ataxia, paresis and recumbency 2–3 days following intramuscular injection of copper-containing com-
pounds into the neck, and died or were killed humanely 3 days – 12 weeks later. Eighteen of these sheep were examined post mortem; histological examination of the neuraxis revealed circumferential subpial white matter necrosis and Gitter cell accumulation in cervical spinal cord with variable involvement of subpial neuropil in thoracic spinal cord and medulla oblongata. This subpial lesion does not appear to have been recorded previously in sheep. However, marked degeneration of spinal subpial white matter has been reported to follow infusion of various copper compounds into the CSF (e.g., via lateral ventricle) in cats and rabbits, whereas administering iron containing compounds by the same route caused no histological change. The striking similarity between these lesions in cats and rabbits and those observed in the sheep strongly suggest these cases of ovine myelopathy arose as a consequence of high local copper concentrations, either by diffusion of copper compounds for example via intervertebral foramina or possibly following inadvertent epidural or subdural introduction of copper.

Room C: 12.30–12.45

94. Ovine toxoplasmosis – the possibility of vertical transmission

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Introduction. Significant economic losses due to toxoplasmosis occur when seronegative ewes suffer a primary infection in pregnancy following ingestion of sporulated Toxoplasma gondii oocysts contaminating pasture, food stores or water. However, recent reports from the UK suggest a high prevalence of congenital infection with T. gondii associated with lamb mortality in the same flocks in successive years.

Materials and methods. Blood samples were collected pre- and post-lambing from a flock of 29 T. gondii seropositive and 15 T. gondii seronegative Scottish Blackface ewes known to be free of Chlamyphila abortus and from their offspring prior to suckling. These samples were tested by ELISA and IFAT, respectively, for antibodies to T. gondii. Results. Positive antibody titres to T. gondii were detected in precolostral blood samples from three lambs (one single lamb from a seronegative ewe and twin lambs from a persistently seropositive ewe). There was no association between seropositivity in dams or lambs and mortality.

Discussion/conclusions. These findings suggest that vertical transmission of T. gondii by persistently infected dams may have occurred in a small proportion of the flock (<4.5% of pregnancies). This very low level of transmission is in accord with published work by other workers and is at odds with the findings of Duncanson et al. (2001) and Williams et al. (2005).

Room C: 12.45–13.00

95. High throughput reconstruction of ventricular architecture from diffusion tensor magnetic resonance imaging of canine ventricles for the use in virtual cardiac tissues

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The geometry and fibre architecture of canine hearts are reconstructed and visualised from diffusion tensor resonance imaging data sets, as a high throughput route to providing anisotropic ventricular geometry that can be incorporated into electrophysiological ventricular virtual tissues. Computational simulation of normal and abnormal electrophysiology, electromechanics and electromagnetics of the mammalian and human ventricles requires a high resolution (grid steps <0.2 mm) description of the cardiac geometry as discretely localised heterogeneities, such as transmural blood vessels radically alter electrophysiology by pinning re-entrant waves. Myofiber orientation is the main determinant of the distribution of stress throughout the cardiac wall during ejection. Most public domain computational models are based on methodical histological reconstructions and are “illustrative only”. This is particularly true for myofiber orientation where determination of orientation is difficult by standard histological techniques. Diffusion Tensor MRI provides an automatable technique from which the fibre architecture can be algorithmically extracted with a resolution five times greater than histological techniques. Here the technique has been successfully applied to quantitatively extract the fibre orientation from four canine hearts. The results further demonstrate that DT-MRI datasets can be directly input into Partial Differential Equation Solvers of ventricular virtual tissues.
96. Local production of acute phase proteins in milk in an experimental model of Staphylococcus aureus mastitis

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Introduction. The pathophysiological changes of the acute phase proteins (APP) haptoglobin (Hp) and serum amyloid A (SAA) and mammary associated SAA3 (M-SAA3) in milk and serum were related to alterations in the expression of their mRNA in liver and mammary tissue. Materials and methods. The concentrations of Hp and SAA or M-SAA3 were determined by ELISA in milk and serum from cows in an experimental model of Staphylococcus aureus induced mastitis. The expression of the mRNA coding for these proteins was assessed by quantitative PCR in mammary and hepatic tissue from infected animals. Results. The concentration of Hp and M-SAA3 in milk increased within 18 h of S aureus infection, with peak concentrations occurring after 3 days at concentrations (mean ± SEM) of 20.9±11.9 and 27.9±10.1 µg/ml for M-SAA3 and Hp, respectively. At 48 h after infection the relative increase of M-SAA3 mRNA (1500×β-actin) in the mammary tissue was greater than the increase in Hp mRNA expression (20×β-actin), whereas in hepatic tissue this was reversed. Conclusion. Inoculation of mammary glands with S aureus was associated with local production of APP within a few hours. In mammary tissue upregulation of mRNA for M-SAA3 was greater than that of mRNA for Hp.

97. RNA interference – the long and short of it all

MARTINEAU, H.M.*, PYRAH, I.T.

RNA interference (RNAi) is an intracellular mechanism which causes sequence specific post transcriptional gene silencing. The reaction is triggered by the introduction of double stranded (ds) RNA into the cytoplasm of the cell, and results in the specific targeted destruction of mRNA and protein production. It is a highly specific mechanism, which when successfully manipulated results in the knockdown of single or multiple genes so providing a quick and convenient method of gene function analysis. Within the pharmaceutical industry, investigation into its potential for use in target validation and as a therapeutic tool is ongoing. This talk will outline the details of a project designed to investigate the use of RNAi in the drug development process. Successful in vitro delivery of fluorescently labelled scrambled si (short interfering) RNA molecules will be shown and methods of determining gene knockdown following targeted siRNA administration illustrated. The potential for in vivo delivery and assessment of systemic pathology will then be discussed and the overall value of RNAi as an adjunct to toxicological pathology given.

98. Observations on three recent outbreaks of bovine herpesvirus-1 (BoHV-1) infection in cattle in the UK

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Respiratory signs are the commonest manifestations of BoHV-1 in cattle in the UK. Recently, three unusual outbreaks of BoHV-1 were investigated. In one, 130 dairy cows aborted 4–8 weeks following severe milk drop, conjunctivitis, nasal discharge and coughing. High BoHV-1 antibody titres were detected in maternal sera, BoHV-1 antigen in multifocal necrotising lesions in foetal tissues and BoHV-1 was isolated from placenta. In another, two yearling heifers had chronic anteroventral purulent pneumonia and multifocal ulcerative oesophagitis. BoHV-1 was isolated. Abundant BoHV-1 antigen was present in foci of necrotising oesophagitis and pneumonia, the latter...
superimposed on chronic purulent bronchopneumonia. The third report involves severe necrotising laryngitis and anteroventral bronchopneumonia in two 18 months old fattening bulls. BoHV-1 was isolated from the larynx and BoHV-1 antigen demonstrated immunohistochemically in association with necrotising lesions in larynx and lung. These cases are notable for the marked involvement of the larynx without similar lesions in the trachea. Latent BoHV-1 reactivates during episodes of stress, however, the clinical and pathological findings in these outbreaks suggest that BoHV-1 was the primary cause of the necrotising lesions. It is likely that pre-existing and secondary bacterial infections contributed to the severity of clinical disease in two herds.

**Room C: 14.45–15.00**

99. Low dose Mycobacterium bovis infection in cattle results in pathology resembling to that of high dose infection

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To understand the relevance of cattle to cattle transmission for bovine tuberculosis, it is important to study cattle experimentally infected with low doses of *M.bovis* that result in pathology resembling that of natural infection. Recent pathogenesis studies of *M. bovis* have focused on early stages of granuloma development in cattle. Previously our laboratory has reported on *M. bovis* granuloma classification by stage of lesion advancement within bovine lymph nodes. In this study we have applied this classification system to lymph nodes harvested from cattle intratracheally infected with low doses of *M. bovis*. Our results showed that 50% of cattle infected with only 1 cfu of field strain *M. bovis* developed advanced granulomas in thoracic lymph nodes. The degree of lesion advancement and granuloma distribution was similar between the lowest dose group (1 cfu) and the highest of the 4 groups (1000 cfu). The number of acid fast bacilli identified within the granulomas was similar among all groups. These studies indicate that large, necrotic granulomas can develop secondary to infection with the smallest of infective doses.

**Room C: 15.00–15.15**

100. Water dropwort (*Oenanthe crocata*) poisoning incident in horses in 2004

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Water Dropwort (*Oenanthe crocata*) is one of the UK’s most toxic plants, the distinctive roots (‘Dead Men’s Fingers’) being the most toxic part. This paper presents one non-fatal and one fatal case of poisoning in two ponies sharing sparse grazing in Berkshire. Clinical findings, pathological changes in the second case which died, and chromatographic evaluations of plant materials are presented. The first pony became recumbent and exhibited seizuring. After treatment it recovered. Six days later a companion pony also commenced seizuring during which it fell onto a parked vehicle and died shortly after. Postmortem examination revealed terminal bruising and abrasions as well as some older incidental change. No definite CNS changes were identified but a very recent tear in the base of the aorta was considered likely to have contributed to death. The stomach contained unusual rubbery white plant root material. A search of the small area where the two ponies had been grazing revealed the presence of Water Dropwort, samples of which were submitted for identification and evaluation. Chromatography of the tuberous roots and the gastric contents revealed a similar substance in both, the convulsant poison oenanthotoxin. These cases indicate that a site inspection is warranted when unaccountable seizuring occurs in horses at pasture.

**Room C: 15.15–15.30**

101. Chronic foot pain in the horse – is it caused by bone or tendon pathology or what?

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The aetiology of palmar foot pain and navicular disease remain poorly understood. In this study feet were selected from horses euthanased with a minimum 2-month history of unilateral or bilateral forelimb lameness (group L, n = 32) and from age matched control horses
group N, \( n = 19 \). The feet were examined by MRI before dissection. Histological examination was undertaken on samples from the distal phalanx (DP), including insertions of the deep digital flexor tendon (DDFT) and distal sesamoidean impar ligament (DSIL), also the navicular bone (NB) and insertion of the collateral sesamoidean ligaments (CSLS), the DDFT from the level of the proximal interphalangeal joint to 5 mm proximal to the insertion, synovial membrane from the palmar pouch of the distal interphalangeal joint (DIP) and navicular bursa; also the collateral ligaments (CLS) of the DIP joint and the DSIL. The severity of histological lesions from each site were graded and results compared between groups. There were no significant age-related changes in either group L or N, but there were significant differences between groups for lesions of the flexor aspect of the NB, proximal and distal borders and medulla of the NB, the DSIL and its insertions and navicular bursa. It was concluded that abnormalities of multiple structures of the foot were associated with chronic foot pain. It is postulated that adaptive and reactive changes will occur in the navicular apparatus in all horses, but pain results when significant lesions develop in the above sites. We believe that the inter-relationships between the NB and its supporting ligaments as well as the opposing DDFT deserve further detailed study. It is possible that vascular and matrix changes within the distal DDFT may proceed or occur concurrently with changes in the flexor fibrocartilage of the NB.

Room C: 15.30–15.45

102. A case–control study to identify pathological changes associated with fatal condylar fractures in racing Thoroughbreds

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Introduction. There is evidence that condylar fractures of the distal MCIII in racehorses are the result of fatigue related micro-damage at the fracture site. A case-control study was undertaken to identify if pathological defects in the articular cartilage of distal MCIII are associated with condylar fracture of MCIII. Materials and methods. Cases were defined as horses euthanased as a result of a condylar fracture of MCIII sustained during a race at one of the 59 UK racecourses. Controls were those horses that had been euthanased or died during racing for reasons other than limb or pelvic injury. The severity of cartilage abnormalities on the distal articular surface was assessed in both case and control MCIII’s during post-mortem examination at the University of Liverpool. Results. 21 cases and 28 controls were submitted. Presence and severity of fissures, ulceration and discoloration were similar between case and control MCIII’s. A greater percentage of control MCIII’s had punctate lesions present on the palmar halves of the condyles than the case MCIII’s. Discussion: The results suggest that subchondral bone pathology may be more important in the development of condylar fractures. The bone mineral density of distal MCIII using DEXA and micro-CT scanning will also be compared.

Room D. Theme: Veterinary Pharmacology (continued)

Room D: 09.15–09.45

103. Pharmacology of equine digital blood vessels: Implications for the pathophysiology of lamininitis

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Equine laminitis is a painful condition thought to resul from ischae-mia-reperfusion injury of the dermal laminae, resulting in the break-down of the connective tissue bond between dermal and epidermal lamellae. Rings of digital artery and vein have been used under iso-metric tension recording to determine receptors present on these ves-sels regulating their tone. This model has enabled identification of receptor types for mediators by methods using selective agonists and antagonists. The role of the endothelium can also be examined in this model. These vessels represent conductance and capacitance vessels rather than resistance vessels. We have also developed a perfused hoof model to determine the responses of small resistance vessels within this circulation. This is a more physiological model where mediator access to the vessels is limited to their intimal surface and the vessels are subjected to the shear forces from fluid flowing through them. These model systems have shown that this circulation is exquisitely sensitive to vasoconstrictor effects of 5-hydroxytryptamine (5-HT), a biogenic amine released by platelets. The pharmacology of the receptors mediating 5-HT responses differs on the arterial and venous sides of the circulation and 5-HT may selectively constrict the venous side of the circulation. Dietary amines release 5-HT from platelets, suggesting they could be the link between gastrointestinal events and digital ischaemia.
104. The role of platelet activating factor in LPS-induced equine digital hypoperfusion

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Introduction. LPS infusion induced digital hypoperfusion temporally related to increases in plasma thromboxane (TxB2) and 5-HT concentrations. In vitro, LPS-induced equine platelet 5-HT release is dependent on leucocyte-derived platelet activating factor (PAF). The study aim was to determine the in vivo role of PAF in LPS-induced equine digital hypoperfusion. Materials and methods. Blood flow in the lateral digital artery and vein of six adult Thoroughbred horses was measured using Doppler ultrasonography for 300 min following infusion of LPS (E. coli 055:B5; 30 ng/kg over 30 min) 20 min after receiving either the PAF antagonist WEB 2086 (3 mg/kg i.v.) or saline (control). Serial blood samples were taken for measurements of TxB2 (radioimmunoassay) and free 5-HT concentration (HPLC). Values were compared between treatment groups using one way repeated measures analysis of variance. Results. Control LPS infusion resulted in 84% and 87% reductions in digital arterial and venous blood flow, respectively. Plasma TxB2 and 5-HT concentrations peaked at 60 and 75 min, respectively, coinciding with the onset of the digital hypoperfusion. WEB 2086 pre-treatment had no significant effect. Discussion and conclusions. LPS-induced TxB2 and 5-HT release in vivo is relatively resistant to PAF antagonism.

Room D: 10.00–10.15

105. The role of calcitonin gene-related peptide in equine acute laminitis

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Equine acute laminitis is hypothesised to be due to ischaemia, so determining the involvement of vasoactive mediators such as calcitonin gene-related peptide (CGRP) in the regulation of equine digital blood flow is of importance. The aims were to characterise the responses of equine digital arteries (EDA) and veins (EDV) to hCGRP and to determine its source. There was no difference between the relaxant responses of endothelium-intact and -denuded vessels to hCGRP. Both EDA(e+) and EDA(e-) had significant relaxant responses to hCGRP, while EDV(e-) contracted in response to hCGRP. Further studies are needed to fully characterize the importance of CGRP in regulating equine digital blood flow.

Room D: 10.15–10.30

106. Low dose lipopolysaccharide induces p38 MAP kinase activation in equine platelets and leukocytes

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Products derived from activated platelets or leukocytes are likely to participate in lipopolysaccharide (LPS)-induced vasoconstriction in the horse. This study has examined the involvement of p38 MAP kinase in LPS-induced activation of equine cells. Platelets and leukocytes isolated from the blood of healthy Thoroughbred horses (n = 6) were exposed to LPS (10 pg/ml – 10 µg/ml) and phosphorylation of p38 MAPK examined by Western blotting at 5 min post stimulation. Platelets were also pre-incubated for 1 h with the p38 inhibitors, SB203580 or PD169316 (0.001–10 µM) and TxB2 release measured by radioimmunoassay 22 h after addition of LPS (1000 pg/ml; n = 4). LPS caused a dose dependent increase in phospho-p38 expression in equine platelets (maximum of 181±78% above basal at 50 pg/ml; EC50 = 14 ± 2 pg/ml) and leukocytes (maximum of 166 ± 44% above basal at 10 µg/ml; EC50 = 18 ± 4 ng/ml). LPS (1000 pg/ml)-induced TxB2 release (3.18 ± 0.14% above basal) was inhibited by SB203580 (maximum of 75 ± 0.14% at 10 µM) and PD169316 (maximum of 95 ± 0.61% at 1 µM). These results demonstrate that activation of p38 MAP kinase in equine platelets is approximately 1000-fold more sensitive to LPS than that in equine leukocytes and TxB2 release may be mediated via activation of this kinase.
107. Extraction and characterisation of tyrosine decarboxylase from equine caecal lactobacillus spp

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Introduction. Amine compounds such as tyramine and tryptamine are produced by equine hindgut bacteria in response to carbohydrate overload and cause vasoconstriction in the equine digit. Therefore, these compounds have been implicated in the pathophysiology of acute laminitis. The purpose of this study was to characterise the tyramine decarboxylase enzyme. Methods: Lactobacillus salivarius from the caecum was grown in MRS broth. Dialysed extracts from lysed bacteria were added to tyrosine in acetate buffer containing 0.2 mM pyridoxal-5-phosphate. After incubation at 37 °C for 30 min in the presence and absence of the selective decarboxylase inhibitor, benserazide, tyramine production was quantified by HPLC. The rate of reaction vs substrate concentration was used to calculate the $K_m$ and $V_{max}$. Results. The optimal pH for the enzyme reaction was 5.5, and the optimum temperature was 37 °C. The apparent $K_m$ for the reaction was $0.24 \pm 0.06$ mM and the $V_{max}$ was $10.9 \pm 0.9$ nmoles/min/mg protein. Benserazide (1 $\mu$M) caused a 38.2% decrease in reaction velocity. Conclusions. Lactobacillus salivarius contains an aromatic L-amino acid decarboxylase which produces tyramine. The optimum conditions for this enzymic reaction are consistent with other inducible amino acid decarboxylases. Understanding the kinetics of this reaction may provide further understanding of the processes in the equine hindgut leading to the onset of acute laminitis.