Bristol, RVC and Glasgow students scoop top honours at 2013 awards

Amy Smith, a third year veterinary science student at the University of Bristol, has been announced as the overall winner of the 2013 MSD Animal Health Connect Awards, with Camilla Brocklehurst from the Royal Veterinary College, and Alissa Edoff from the University of Glasgow being awarded second and third places respectively.

Amy was awarded the 2013 Connect trophy and a cheque for £3,000 for her project which investigated the primary culture of porcine intestinal epithelial cells, while Camilla received a cheque for £2,000 for her project which assessed the effect of turn direction on body lean angle in the horse in trot and canter. Alissa was awarded a prize of £1,000 for her study of grey squirrels in Scotland as tick hosts and reservoir hosts of *Borrelia burgdorferi*, the agent of Lyme disease.

“This year marked the 22nd anniversary of the Connect Awards and once again the quality of research and depth of knowledge demonstrated by each of the students was extremely impressive,” said Dr Mike Francis, Executive Director of Research and Development at MSD.

“The projects covered a range of veterinary topics, and it was encouraging to see the level of enthusiasm that the students had for their chosen fields of study,” Dr Francis added.

“The high standard of presentations and associated written reports made it difficult to select the final three winners, but Amy, Camilla and Alissa stood out by demonstrating a clear understanding of the science behind their projects.

Amy’s project showed that it is possible to effectively isolate the crypts from both the colon and jejunum of porcine intestines, and in doing so made good progress towards developing an effective primary culture for the isolation of intestinal epithelium. She also observed that the Connect Bursary gave her the opportunity to experience working within a research laboratory environment for the first time.

Camilla used a GPS-aided inertial measurement unit to quantify the body lean angle of horses when turning in trot and canter. Her work showed that horses lean marginally less than predicted – potentially due to the presence of laterality or underlying subclinical lameness – and hopes that her work will be of use in the future assessment of adaption mechanisms to lameness.

Alissa’s project proved that grey squirrels can be infected by, and are potentially competent to transmit a number of different *Borrelia* species. This includes *B. garinii*, a strain traditionally thought to be transmitted only by birds, raising new questions about host specificity and its underlying mechanism in this important tick-borne pathogen.

The students subsequently presented the findings of their projects to a panel of judges at MSD’s UK headquarters in Milton Keynes where the best three projects were awarded prizes of £3,000, £2,000 and £1,000.

The Connect Bursaries are designed to give veterinary students the opportunity to undertake their own research projects and to provide a better understanding of the work carried out within MSD’s research and development facilities in the UK. One student from each of the UK’s vet schools received a bursary of £1,000 towards the cost of their project with an additional bursary – also worth £1,000 – awarded to an equine specific project.

Dr Mike Francis with (left to right) Alissa Edoff, Amy Smith and Camilla Brocklehurst
Amy Smith, a third year veterinary science student at the University of Bristol, was announced as the overall winner at the 2013 MSD Animal Health Connect Awards for her research project entitled “Primary culture of porcine intestinal epithelial cells.” Amy received the 2013 Connect Trophy and a cheque for £3,000.

The intestinal mucosa is organised into villi and crypts in the small intestine and crypts in the large intestine. The intestinal epithelium is a cell monolayer representing the barrier between the intestinal lumen and underlying stroma. Intestinal epithelium is the site of absorption and secretion within the intestine.

Epithelial cells also release cytokines and express receptors which facilitate inflammatory and immune responses.

These cells are therefore of interest for the study on immune mediated disease, host-pathogen interactions, nutrition and the development of mucosal vaccines and therapies.

The majority of work with cultured epithelial cells has used immortalised cells, which behave differently to those in vivo: high levels of differentiation, rapid turnover and dependence on their natural cellular interactions making intestinal epithelial cells difficult subjects for primary culture.

Amy’s project therefore aimed to develop a method – initially developed by the Roslin Institute – for the isolation and primary culture of porcine colonic epithelium.

Amy’s work proved that is it is possible to effectively isolate the crypts from both the colon and jejunum. In doing so, she successfully isolated multi-cellular structures which were identified as crypts and made good progress towards developing an effective primary culture.

Equine Bursaries:
Emma Peal (University of Nottingham): The factors affecting the immune response to equine influenza vaccination in donkeys.
April Lawson (University of Bristol): Immunopathological effects of Anoplocephala perfoliata’s excretory/secretory antigenic component.

Lana Bordcosh (University of Edinburgh): Characterising and differentiating adipose and bone marrow derived mesenchymal stem cells.
Michaela Wegg (University of Liverpool): Characterisation of effect of local anaesthetics on TRP channel activity in canine chondrocytes by calcium fluoroscopy.
Rebecca Davenport (University of Nottingham): Host response to footrot in sheep.

Connect Bursaries:
Jocelyn Bisson (University of Cambridge): Examination of erythropoietin levels in dogs affected by transmissible venereal tumour.

Connect Award Winner 2013:
Amy Smith (University of Bristol): Primary culture of porcine intestinal epithelial cells.

Second Place:
Camilla Brocklehurst (Royal Veterinary College): Effect of turn direction on body lean angle in the horse in trot and canter.

Third Place:
Alissa Edoff (University of Glasgow): Grey squirrels as tick and reservoir hosts of Borrelia burgdorferi, the agent of Lyme disease in Scotland.

The 2013 Connect Bursary projects:

Connect Bursary Awards 2013
Overall Winner

Amy Smith, a third year veterinary science student at the University of Bristol, was announced as the overall winner at the 2013 MSD Animal Health Connect Awards for her research project entitled “Primary culture of porcine epithelial cells.” Amy received the 2013 Connect Trophy and a cheque for £3,000.

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Merged RGB immunofluorescence image of a branching crypt (x40) showing positive staining for Cytokeratin-18 (green), E-cadherin (red) and nuclei (blue)
Connect Bursary Awards 2013
Second place

Camilla Brocklehurst, a recent graduate from the Royal Veterinary College, came second in the 2013 MSD Animal Health Connect Awards for her project which studied the effect of turn direction on body lean angle in the horse in trot and canter. She was awarded a prize of £2,000.

Turning is commonly used as a diagnostic aid in lameness exams for horses. The forces experienced on the circle differ from those in a straight line, necessitating an inward lean of the body and asymmetric head/pelvic excursion. To better understand gait adaptations to lunging – in normal horses – the effect of turn direction on body lean in trot and canter warrant further investigation.

Camilla therefore set out to test the hypothesis that, on average the observed body lean angle would, independent of gait, be consistent with the ratio between gravitational and centripetal acceleration, but with variations for individual horses showing differences between reins.

Camilla observed 20 horses which were instrumented with a GPS-aided inertial measurement unit to quantify body lean angle, speed and circle radius in trot and canter in both directions. The difference between predicted (from speed and circle radius) and observed body lean was compared between horses, directions and gaits using a generalised linear model.

The mean speed and circle radius were 3.3m/s and 4.9m in trot, and 4.5m/s and 5.7m in canter. The mean observed body angle was -1.1 degree (range -7.6 to 5.4 degrees) in trot and -0.8 degrees (range -7.6 to 8.3 degrees) in canter and was significantly different between horses and directions, but not between gaits. Interactions between horse and direction and horse and gait were also found to be less significant. Camilla found that horses leaned marginally less into the circle than predicted, and surmised that her study provides additional evidence for horse specific adaptations showing significant differences between horses and turn directions but not between gaits. She concluded that this may relate to the presence of laterality or to underlying subclinical lameness.

Connect Bursary Awards 2013
Third place

Alissa Edoff came third and received £1,000 for her 4th year project which assessed grey squirrels in Scotland as tick hosts and reservoir hosts of Borrelia burgdorferi, the agent of Lyme disease.

The grey squirrel, Sciurus carolinensis, is an invasive species in the UK and a competent reservoir host for some species of Borrelia burgdorferi sensu lato (B. burgdorferi s.l.). However, it is not known how prevalent Borrelia infection is in grey squirrel populations in Scotland, or which bacteria are involved.

Alissa’s study aimed to quantify and characterise the prevalence and diversity of B. burgdorferi s.l. infection in grey squirrels by xenodiagnosis and ear biopsy.

Two hundred and twenty four squirrels were trapped from eight regions in Scotland, with 90% found to be carrying ticks. All ticks were identified as Ixodes ricinus: 78.3% of the ticks were larvae, 21.5% were nymphs and 0.2% were adults.

One hundred and thirteen squirrels were tested by ear biopsy and xenodiagnosis using larval pools. Twenty four (21%) were positive by ear biopsy and 3% were positive by xenodiagnosis.

B. garinii, a species normally associated with birds, was the most common infection, being found in 62% of positive ear biopsies. Infections with B. afzelii (29%) and B. burgdorferi sensu stricto (4%) were also detected. B. garinii and B. afzelii were detected in larval pools indicating transmission of these species to feeding ticks.

Two squirrels tested positive by xenodiagnosis and negative on ear biopsy.

These results show that grey squirrels can be infected by, and are potentially competent to transmit, a number of different Borrelia species. This includes B. garinii, a strain traditionally thought to only be transmitted by bird hosts, raising new question about host specificity and its underlying mechanism in this important tick-borne pathogen.
Connect Bursary Awards 2013

Bursaries

- April Lawson from Bristol University used her Equine Connect Bursary to study the immunopathological effects of Anoplocephala perfoliata’s excretory/secretory antigenic component.

Anoplocephala perfoliata is the most common cestode parasite found in horses. It resides predominantly in clusters at the ileo-caecal junction and has a reported prevalence ranging from 20-80%.

There is an association between A. perfoliata infection and spasmodic colic which increases with higher worm burdens. Heavy burdens are also linked to conditions such as ileal impaction and ileo-caecal intussusception which can require surgical intervention.

Gross and histological pathological changes occur at and around the site of attachment. These include oedema, hypertrophy of the mucosa and muscle layers and degenerative changes in the myenteric ganglia.

A. perfoliata produces a range of excretory and secretory (E/S) products, including several enzymes and glycoproteins which stimulate an immune response and antibody production. These products may have important functions relating to the longevity of parasite survival and pharmacological actions mediating the associated pathological findings. Additionally, it is unclear whether the neural damage seen in caecal mucosa stems from direct E/S product toxicity or bystander neurotoxicity owing to inflammatory mediator release.

To build on her previous work, April assessed the effects of A. perfoliata E/S products on the cytokine transcription by equine lymphocytes activated in vitro by Concanavalin-A, on the proliferation of Jurkat/K562 cells and on damage to monolayer integrity of Caco-2 cells.

A series of laboratory tests showed that the effect of E/S on cell proliferation was remarkably potent. Neither heat inactivation nor exhaustive dialysis of the E/S removed appreciable amounts of the activity. The E/S component also caused a significant loss of transepithelial electrical resistance over time, indicating a rapid loss of Caco-2 monolayer integrity – this supports the idea that E/S causes pathological effects to the caecal mucosa during A. perfoliata infection.

April’s work also showed that E/S components suppressed T-lymphocyte activation and cytokine production with a concurrent shift away from a Th1 and Th17 T-cell differentiation towards a Th2 anti-parasite response. She surmised that this could be due to the metabolic environment and complex feedback mechanisms between the Th subsets. April also deduced that the acute reaction of A. perfoliata E/S on lymphocyte cytokine transcription may not fully reflect chronic E/S influences that perhaps cause further immunomodulation.

- Jocelyn Bisson, a final year veterinary student at the University of Cambridge, made an examination of erythropoietin levels in dogs affected by canine transmissible venereal tumour.

Canine transmissible venereal tumour (CTVT) is unusual both in the way it is transmitted and its interaction with its host. Jocelyn’s project investigated the effect of CTVT on serum erythropoietin (EPO) levels by studying six dogs from a wild population in Ooty, Tamil Nadu, India, along with 13 unaffected control dogs from the same population.

CTVT is a clonally transmissible genital tumour found in dogs and bitches. Once endemic in the UK, it is now no longer present but has been recorded in dog populations on six continents and is particularly prevalent in groups of stray dogs.

CTVT is transmitted by physical transfer of neoplastic cells during intercourse or by biting, scratching or licking. It is one of only two known naturally occurring clonally transmissible tumours; the other being Tasmanian Devil Facial Tumour which poses a threat to the survival of the Tasmanian Devil species.

Through a process of blood testing, packed cell volume (PCV) measurement, dehydration assessment, radioimmunoassay, tissue sampling and statistical analysis, Jocelyn found that in naturally occurring CTVT cases in a stray population, serum EPO is significantly raised when compared to levels in unaffected control dogs in the same population.

This reveals evidence of a novel, adaptive, host: tumour interaction – a finding which Jocelyn hopes will help in the development of novel treatments. The next steps in this line of research will be to perform quantitative reverse-transcriptase PCR and next generation sequence analysis on tumour samples to study ectopic EPO production, as this would provide conclusive evidence that CTVT directly increases serum EPO levels. These findings could also be investigated in Tasmanian Devil Facial Tumour and there is scope for CTVT to be used as an animal model for paraneoplastic polycythaemia.
Lana Bordcosh from the Royal (Dick) School of Veterinary Studies used her Connect Bursary to fund her project which characterised and differentiated adipose derived and bone marrow derived mesenchymal stem cells.

The capability of canine mesenchymal stem cells (cMSCs) to be isolated from adult tissue, expanded and differentiated into mesenchymal lineages including bone, fat and cartilage, has sparked research in tissue regeneration and repair. In light of this, Lana’s study aimed to compare cMSCs from bone marrow (BM-cMSCs) and adipose tissue (AD-cMSCs) of the adult dog to better understand the reparative capacity of stem cells from different tissue sources.

BM-cMSCs and AD-cMSCs were characterised by morphology, expansion ability and differentiation potential down osteogenic and adipogenic lineages.

Lana found that BM-cMSCs and AD-cMSCs were similar in their morphology and in their ability to expand manifold in culture. Both also differentiated in adipogenic and osteogenic cells, although BM-cMSCs showed superior osteogenic potential and AD-cMSCs showed superior adipogenic potential. Neither cell population could be characterised via positive expression of pluripotency markers.

Osteogenic differentiation was confirmed in both cell populations by the detection of calcified matrix with Alizarin Red staining at 7 and 21 days post induction. The expression of RUNX2, an osteoblast specific marker, in BM-cMSCs 7 days post induction was further supportive of differentiation.

Adipogenesis was confirmed by the staining of accumulated fat droplets with Oil Red O and the expression of adipocyte specific markers LEPTIN and LPL in BM-cMSCs 21 days post induction.

Lana therefore surmised that both marrow and fat are potentially reliable sources for expanding and differentiating cMSCs for clinical application. She also concluded that while mesenchymal stem cells can be obtained from bone marrow and adipose tissue, the latter could prove to be a better source for harvesting these cells as adipose tissue is more abundant, easier to access and therefore a less invasive source of MSCs.

Further gene marker expression characterising cMSCs and their differentiation potential will have to be done to get a better understanding of how BM-cMSCs and AD-cMSCs compare in vitro. For completeness, qRT-PCR should be performed to quantitate gene expression and both tissue cMSC populations should be induced to undergo chondrogenic differentiation.

— Michaela Wegg, a fourth year student from the University of Liverpool, looked at the effect of local anaesthetics on cartilage by examining TRP channel activity in canine chondrocytes by calcium fluoroscopy.

Local anaesthetics (LAs) and corticosteroids are routinely injected at high concentrations into the joints of humans and other animals to relieve pain in osteoarthritis. It has been shown that LAs are toxic to chondrocytes, the cells responsible for the production and maintenance of cartilage, although the mechanisms of this are unknown.

Research previously carried out by Michaela showed that LAs cause an increase in the activity of calcium-selective transient receptor potential (TRP) ion channels. She hoped to expand on this work and to further studies which have shown that LAs increase intracellular calcium (which can lead to cell apoptosis) by identifying the specific TRP channel that is activated by LAs and to determine if this channel is responsible for the rise in intracellular calcium.

Michaela took cartilage scrapings from the elbow and stifle joints of skeletally mature canines and isolated cells. She then used calcium imaging to see how calcium channel activity changed with a range of pharmaceutical products and with the application of LAs.

She found that in both LA groups (1% mepivacaine and 1% prilocaine) there was a significant increase in intracellular calcium. 1% prilocaine had an increase in intracellular calcium which was not statistically significant from that of hypotonic solutions which cause the intake of calcium into chondrocytes. However, 1% mepivacaine had a significantly greater increase compared to hypotonic solution.

Michaela therefore concluded that LAs increase calcium channel activity in chondrocytes which leads to calcium influx in chondrocytes and that this is linked to apoptosis.

She also confirmed that the TRPC3 ion channel is activated by the LAs and that this is one possible pathway by which LAs are toxic to chondrocytes.

Michaela hopes that her research could eventually contribute to the creation of alternative therapies for the treatment of osteoarthritis – both before and after the administration of local anaesthetics – by targeting the TRPC3 channel to slow or halt cell apoptosis.
Rebecca Davenport, a final year student at the University of Nottingham, investigated host response to footrot in sheep.

Footrot is a highly contagious bacterial disease which infects the interdigital space between ovine claws. It results in varying degrees of lameness across flocks, with the most severely affected leading to separation at the skin-horn interface.

Ninety five percent of flocks in England have footrot, with a mean daily prevalence of c.10% and an estimated cost to the UK sheep industry of £24-£84 million per annum.

Footrot is a complex and multifactoral disease involving the co-infection of two bacteria: *Fusobacterium necrophorum* and *Dichelobacter nodosus*. The disease is exacerbated by wet conditions and physical damage to the ovine foot. Breeding resistant sheep and vaccination strategies are aiding in preventing the disease.

Whilst research into the bacterial causative agents and pathogenesis has been documented, there is little information regarding the interaction between bacteria and host, and more specifically the host’s immune response to infection.

Rebecca therefore sought to characterise the response of dermal fibroblasts to footrot bacteria in order to identify the pro-inflammatory factors expressed by those cells. She did this by a) determining levels of mRNA expression of TLR1, 2, 4 and 6; and b) determining levels of mRNA expression of genes encoding the pro-inflammatory cytokines IL-1β and TNFα.

Rebecca found that TLR2, IL-1β and TNFα expression increased in response to defined (LPS) and mixed bacterial ligands (heat-killed *D. nodosus* and *F. necrophorum*), and that dermal fibroblasts are able to mount a pro-inflammatory response.

There are a multitude of directions to carry this work forward. For instance, obtaining a greater understanding of the invasive nature of the bacterium into ovine foot samples using fluorescent *in situ* hybridisation or performing in vitro bacterial infections to try and determine the bacteria saturating point of disease and response curves.

Rebecca hopes that by following these future lines of investigation, the sheep industry will be able to develop improved target vaccines and their adjuvants to treat this debilitating disease.

Emma Peal, a fourth year student at the University of Nottingham, used her Equine Connect Bursary to fund her investigation into the factors affecting the immune response to equine influenza vaccination in donkeys.

The equine influenza vaccine has been available since the 1960s and is commonly used as equine influenza causes a contagious respiratory infection in horses – and up to 100% morbidity in naïve donkey populations. As in the human population, the prevalence of obesity in equids is increasing. Adipose tissue was traditionally thought of as inert, but today is recognised as a functional endocrine organ, and obesity is associated with chronic inflammatory state in people.

Although the chronic inflammatory state associated with obesity seen in humans has not been observed in horses, immuno-senescence and inflamm-aging have been documented. Decreased T cell proliferation and decreased length in lymphocyte telomeres characterise immuno-senescence, and inflamm-aging in horses is characterised by alterations in cell proliferation and levels of pro-inflammatory cytokines.

Increased level of inflammatory markers have also been found in fat old horses in comparison to thin old horses, suggesting that adiposity exacerbates the effect of inflamm-aging.

Emma therefore set out to test the hypothesis that a reduction in antibody titres in response to equine influenza vaccination would be seen in older donkeys with increased adiposity in comparison to younger, leaner equids.

The study was conducted on 55 serum samples from donkeys belonging to the Donkey Sanctuary which also provided background information for each animal including gender, age, body condition score (BCS) and vaccination history.

The serum samples were tested for a number of biochemical parameters which included antibody levels, triglyceride, cholesterol, non esterified fatty acids (NEFA) and adiponectin.

Emma’s investigations found no correlation between antibody levels and BCS, but did note a significant gender dimorphism: the females in the study had significantly higher antibody levels compared to the males (208.9 nm² versus 178.9 nm² on a single radial haemolysis assay) despite the fact that the the mean age of the female donkeys in the sample was significantly higher than the males (25 years compared to 19 years). Given that immuno-senescence is recognised in horses, this finding was unexpected, but showed that the effect of gender on the response to vaccination outweighed the effect of age.

Emma concluded that more research into gender dimorphism in response to vaccination and infection is needed, as currently little is known as to why it occurs. She also concluded that more research into the role of NEFA is needed as she found a significant correlation between NEFA and weight in male donkeys, but not in females.
Connect from MSD Animal Health is an information and support service for veterinary students which provides educational and sponsorship assistance.

The scheme incorporates a number of benefits including:

**Literature:**
MSD Animal Health has an extensive collection of literature on a variety of subjects in the companion animal, livestock and poultry sectors. For more information simply email our customer support service at vet-support.uk@merck.com

**Education Support:**
MSD Animal Health plays an active role in sponsoring clinical club meetings and welcomes requests for presentations and lectures to be made to these clubs.

**Bursary Awards:**
The annual Connect Bursary scheme provides financial assistance to students enabling them to undertake research in new and exciting areas of veterinary work.

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**How can I apply?**

The 2014 Connect bursaries will be launched in the spring with one student from each university eligible for £1,000 funding towards the cost of their research. An additional £1,000 will be available via the Equine Connect bursary.

The top three projects will also qualify for additional funding of the following amounts:

- **First place:** £3,000 (plus Connect trophy)
- **Second place:** £2,000
- **Third place:** £1,000

To submit your proposal for a 2014 Connect Bursary simply contact the staff representative at your university (see below) or Paul Jennings at ABC Limited on 01694 731777 or email paul@abccomms.co.uk

You will need to submit a synopsis of your project covering the aims, objectives and hypotheses of your research.

Successful candidates will be invited to the MSD Connect Bursary Award Day to present their research findings and to compete for the Connect Trophy.

**Your University Contact:**
- University of Bristol: Professor Ed Hall
- University of Cambridge: Katheryn Ayres
- University of Edinburgh: Antonia Robb or Dr Sally Argyle
- University of Glasgow: Lesley Nicholson
- University of Liverpool: Gina Pinchbeck or Elizabeth Laird
- Royal Veterinary College: Oliver Garden or Josh Slater
- University of Nottingham: Malcolm Cobb or Prof. James Leigh

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"The Connect Bursaries offer students a chance to be exposed directly to veterinary research. The prizes are an added bonus, but the experience students gained will ultimately be of greater value in their careers. I was very impressed by the quality of all the projects and the presentation of the results. However most inspiring was that they had all recognised that advancements in the care of animals depends on the profession embracing the research opportunities available."

**Professor EJ Hall - University of Bristol**

"The MSD Connect Bursary enables veterinary students to experience research and study a subject in much greater detail than usually possible during a veterinary degree. This can open the door to career paths in research which students might not previously be aware of. Likewise, for students who continue into clinical practice, having a deeper understanding of the research process behind many clinical decisions is equally valuable."

**Caroline Millins - Glasgow University**

"There are various bursaries available which enable students to gain research experience, but the Connect Bursaries stand out because they are totally student-centred, the students’ research is taken seriously, and the MSD team are so enthusiastically engaged. Not only was the overall standard high, the range of topics was very diverse, which must have presented the judging panel with a real dilemma in choosing the overall winners."

**Janet Daly and Sabine Tötemeyer - University of Nottingham**