UNIVERSITY OF LIVERPOOL

Spring 2020 | Issue 3

Insider Imprint

The life sciences journal for undergraduate and masters students at the University of Liverpool

CRISPR vs Prime Editing

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Insider Imprint

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INSPIRE

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The Academy of Medical Sciences

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Foreword

Professor Sónia Rocha

Executive Dean

Institute of Systems, Molecular and Integrative Biology

Welcome to the third issue of Insider Imprint, the first journal of its kind in the University - run by students for students. I am so proud of being part of such vibrant and enthusiastic student and research community. As the new Executive Dean for the Institute of Systems, Molecular and Integrative Biology, I am particularly excited to get to know you all better in the coming months and years.

It is amazing and inspiring to see such a diverse range of content from across our university in this edition. Particularly important and relevant are the career and, given the current situation, the wellbeing sections of this issue.

Insider Imprint is a magnificent initiative that gives opportunities to our students to accrue skills and experience. I would like personally to thank everyone involved in its production. This truly is a great achievement and a testament to the dedication and immense passionate work of the editorial team and the valuable and unique contributions from our students. I hope that you enjoy reading this issue and congratulate again everyone involved in such an amazing asset for us all.

Issue 3 is here!

The world and how we live in it has changed a lot over the last few months, but one thing definitely stayed the same: we continued working hard behind the scenes to bring you a fantastic third issue of Insider Imprint. Publishing articles from students right here at UoL, issue 3 is full of content from insightful stories to hard core science. Congratulations to all our authors for possibly your first publication as you trail blaze to further success!

We are excited to have introduced a new section on wellbeing to Insider Imprint. In this issue we have focused on mental health, and we have given you our own wellbeing tips. We hope you like this addition.

We hope you enjoy reading issue 3, gaining new knowledge and perhaps getting inspired to try something new.

Stay safe and well.

Insider Imprint team

UNIVERSITY OF LIVERPOOL

Insider Imprint

STAFF Awards 2019 — Innovation of the Year

Meet the Team

Meet the team behind Insider Imprint! We are a dedicated team from across the Faculty coming together with a common goal of creating a space for undergraduate and masters students to showcase their achievements, to inspire the upcoming generation of students and to support students in becoming confident and competent presenting their work, ideas and experiences.

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Animal Biology

What are we all about?

Insider Imprint is Liverpool's Life Sciences journal, publishing work from undergraduate and masters students at the University of Liverpool. The journal goals are to:

- Provide a platform for undergraduate and master students to **showcase** their many activities and successes
- To **inspire** the upcoming generation of students through facilitation of transfer of knowledge & experiences between students
- To **support** students in becoming confident and competent presenting their work, ideas and experiences for publication
- **Communicate** the best Life Sciences research from the students of the University of Liverpool

Collaboration • Sharing • Supporting Success

Who can write for us?

If you are an **undergraduate or masters student** in the **Faculty of Health and Life Sciences**, you can write for us. If you have something you want to write about, we want to hear from you!

What do we publish?

Research articles

E.g. based on honours, masters projects or internships

Literature reviews

Commentaries such as:

Reflections on work/internship experience

Hot topics and debate articles

Book/seminar/summer school reviews

Reflections on topics of interest related to your studies

Creative pieces such as:

Artwork

Photography

….and more!

Got an idea not listed above?

Great, contact us!

Why publish with us?

Publishing with Insider Imprint is an excellent opportunity to **showcase your achievements** and **enhance your CV**. As an open access online journal, you will gain **visibility for your accomplishments**. Whether you are interested in staying in academia or have alternative career goals, having a published article or two is a sure way to **increase your employability** and impress potential employers.

Publishing with us will give you experience on how you go from an idea to print. We will guide you through the typical publication roadmap, from following author guidelines, submitting your work & receiving feedback to reviewing proofs before publication. You will also gain an insight into the peer-review process that is widely used to validate scholarly work.

For those of you interested in staying within academia, getting your first publication can **be a big step forwards for your career** and will help break down barriers early researchers often face when contemplating their first article submission.

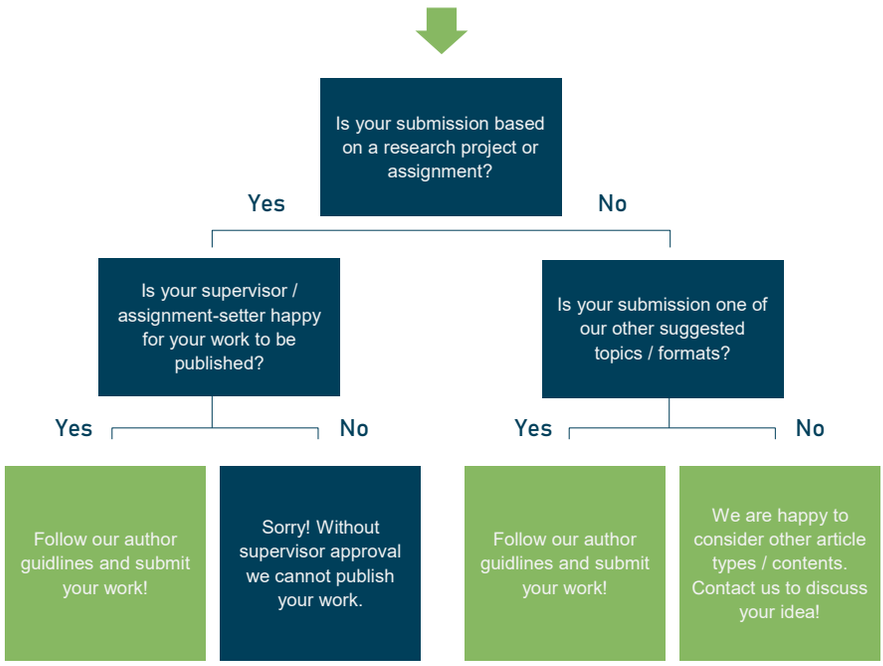
Getting started...

At *Insider Imprint* we accept a wide range of article types. Here are some things to think about to get started:

* If your submission is based on work set as an assignment, or on research you have taken part in, you will need **permission** from the member of staff involved before we can publish your work. So make sure you check this before you put in a lot of effort!

* If you are preparing an article for us, make sure you have a read over our **author guidelines** on our website. Just like other journals, we have certain content and formatting rules we ask you to follow - if you are unsure about anything, get in touch so we can guide you.

* If you have an idea for an article but are not sure if it would be considered for publication, just send us an email so we can advise you.



Motivations to publish

Showcase your achievements

Boost your CV

Inspire other students

Wow your future employer

Your work viewable to everyone online

High quality and peer-reviewed

Gain real-life experience working with a journal to publish your work

Opportunities for PhDs

Editorial Board:

We are a small team of PhD students, and will be periodically recruiting new members; keep an eye out for our calls or drop us a line via our email to express your interest.

Peer Review:

We are looking for enthusiastic and committed individuals to join our team of peer-reviewers. No prior experience is needed; an optional online training course is provided. Please find our application form at insiderimprint.com and send it over to us.

For both of the above, you must be a PhD student in the Faculty of Health and Life Sciences at the University of Liverpool. You must have permission from your supervisor and at least 1 year of your studies left.

The Fine Print

Work involving others, such as a supervisor, needs approval from them before it can be published. Any work previously submitted as an assignment should be re-written to align to our format, and appropriate permissions given from the staff member who set the work. You can find the rest of our journal policies on our website.

Connect with us on:

Email us at: insiderimprint@liverpool.ac.uk

More information at www.insiderimprint.com

Reflections

What are your fellow students up to?

**Interested in travelling, volunteering, conservation?
Read on to discover what your fellow students have been doing and their advice to you.**

Volunteering in Africa

Education, conservation and construction

Megan Kerridge
2nd year Zoology BSc

Volunteering in South Africa with VESA (Volunteer Eco Student Abroad) was wonderful and life changing. Going to the other side of the world without my parents was nerve wrecking and came with new anxieties and worries I had to overcome. Thankfully with the support of my friends and family I got there and had the best two weeks of my life filled with excitement, hard work and life lessons. I hope this article provides a good insight into what a volunteering trip might entail and may encourage others to do something similar.

I had been considering going travelling in the summer. I thought a good place to start would be volunteering trips as most of the trip is already organised by the company. I did research into volunteering abroad and peoples experiences, but could never come to a decision. However, one day a friend told me about a talk she had been to at University that was done by a company called VESA. After looking into VESA and finding brilliant reviews I felt comfortable to apply for one of the places on their Africa trip. Their quick responses to questions and knowledge of the trip made it hassle free. I would recommend them to anyone looking for an amazing experience volunteering.

My volunteering started with a few days at a local children's crèche, where I was introduced to an inspiring woman known as Mama Doris. Every day she looks after between 60 – 140 children of all ages, providing them with food, shelter and a safe place to grow up and learn. One of the jobs was to help hand out food to the children that turned up at the crèche each day. This was when I witnessed first-hand the terrible poverty that these children face every day, as there was not enough food to go around. Luckily, we brought out food that was left over from our lunch and handed it to the children who had nothing. This was a hard-hitting wakeup call for us volunteers who had come from more developed countries. If it wasn't for the food we had left from our lunch, there would have been children who did not eat that day.

I spent time with the children and taught them some maths and English. This was incredibly rewarding to do, not only was I helping these children better understand their school work, it was giving them a chance of a better future. Whilst doing this, I became very aware that the chairs and tables were old and broken and books/school equipment were in short supply. This led to our volunteering group raising almost 7000 rand (£383) for the crèche to help them afford new school supplies for the kids, to make sure that our short time there would still make a difference months after we left.

> "I left the crèche with a new outlook on life, and a very heavy but full heart."

One of my favourite parts of my time doing education in Africa was free time with the children. You could not move without being swarmed by joyful kids all wanting you to play with them. The older children were having dance battles, some were playing football, others doing flips and tricks off of volunteers. The atmosphere was so full of joy and excitement and every child was so happy and grateful even when they had so little. It was during this moment that I was filled with gratitude, thinking back to moments in my life where I took things for granted, and how these children as young as 3 years old taught me, an 18-year-old, the lesson of being grateful for all that I have. There is never a day that goes by that I don't think about those children, who inspire me to work my hardest to make the world a better place for those who don't have the same opportunities as me. I left the crèche with a new outlook on life, and a very heavy but full heart.

The second part of my volunteering in South Africa led me to something I aim to do in the future, work with animals. I spent a day at the Emdoneni Cheetah project, where I got up close to cheetahs, servals, caracals and African wildcats. This project aims to breed and release these cats

back into protected areas as well as educating the visitors and local communities on the problems African cats face such as poaching and the pet trade. This led to discussions highlighting the importance of researching animal attractions and the role of social media and how it impacts these animals.

Meeting the cats was incredible, as I got to get close to beautiful creatures such as Cassidy, a serval (a medium sized African cat with stilt-like legs and large ears) hand reared after its mother died of pneumonia and Dusk, a cheetah with birth defects leading to an overbite and a kink in his tail. Seeing them up close was breath-taking and definitely a highlight of the trip. After marvelling at the cats, I helped clean out enclosures and do maintenance around the centre, giving me an insight into the hard work that goes on in the background.

"Seeing them up close was breath-taking and definitely a highlight of the trip."

I got to spend time at a research centre and breeding facility for crocodiles in St Lucia. I helped build four new enclosures by mixing concrete by hand for making the pools; digging trenches for piping and securing fences and all whilst a very chilled crocodile watched from the other side of the pool. This was one of the hardest days for me physically as we worked tirelessly to try and get these enclosures finished before the end of the season.
It was very rewarding to see that my hard work had paid off as the enclosures were almost completed by the time we left, allowing the centre to continue on rescuing and rehabilitating crocodiles. Another very rewarding aspect of the trip was the construction days, where I worked on building a new house for a family of seven in St Lucia who were at the time living in just two rooms. Working alongside builders, I mixed sand and cement, rendered and painted walls, moved bricks and shovelled concrete. This was another very physically challenging day, especially in midday African sun, but the outcome was worth it.
Being able to see the progress of the house each day to its completion was amazing and I am incredibly thankful to have made a positive impact in the family's life.

I had the amazing opportunity to go on three safaris during my time in Africa. One was a sunset safari in iSimangaliso Wetland Park, just outside the town of St Lucia. Here I came face to face with a bull elephant, buffalos, zebra and even two rhinos, all whilst the sun set on the horizon. This was my first experience of seeing African animals in the wild, you cannot put into words just how incredible it is to see them with your own eyes. I went on another safari in Swaziland, home to a game drive famous for its population of roan antelope. Not only did I see the roan antelopes, which in itself was extremely amazing, I got to see wildebeest, warthogs, blue cranes, zebras and crocodiles, all while the knowledgeable driver told us about each one and how they impact on each other in the ecosystem.
The biggest safari was a 6-hour drive through Hluhluwe Imfolozi Park, one of South Africa's biggest game reserves. The guides said we definitely had 'beginners' luck' as the animals we saw have extremely rare sightings. One of these was a huge herd of over 40 elephants with calves. This was breath-taking, and possibly my favourite sighting of my time in Africa. The whole forest seemed to come alive because of these gentle giants, moving freely across the land as little calves held onto their mothers' tails.
Another extremely lucky sighting was a pack of lions with cubs. Arriving to the area where the lions were spotted, we noticed that the females were showing hunting behaviour, this got our guide very excited as he had never seen the lions hunt before. It wasn't until we saw what they were hunting that the mood changed from excitement to horror. On the floor lay the carcass of a female rhino, poached by humans a few days prior to our visit, and by its side, the rhino's calf, defending its lifeless mother from the incoming lion attack. We held our breaths as the lions tried to take down the young rhino, who was doing a very good job at defending itself and its mother. Eventually the lions retreated, leaving the rhino to mourn over its mother alone once again. Unfortunately, a few hours later, we came across another poached rhino being swarmed by vultures. These upsetting sights showed the devastating impact that poaching is having on Africa's wildlife, and brought on very

Figure 1. One of the hand reared cheetahs with the keeper at the Emdoneni Cheetah project.

Figure 2. An African elephant eating a bush close to the jeep.

Figure 3. Zebras drinking at a watering hole at Mlilwane game reserve in Swaziland.

Figure 4. One wall of the house volunteers helped to build.

useful discussions of what we can do as individuals to help combat poaching. The biggest one being educating those who are less informed to raise awareness of poaching, as the more people who know and contribute to petitions and donations, the more chance we have of stopping it and saving thousands of animals lives all across the world.

"The whole trip was extraordinary and went far beyond my expectations."

Arriving home after such a life changing experience was bittersweet, as I realised that my wonderful time in Africa was over (along with the beautiful sunny weather) but it was great to see my family and friends again and to relive the trip with them. I also made friends from all around the world and learnt about their lives and cultures which was really interesting, made even better by the confusion and explanations of various colloquialisms.

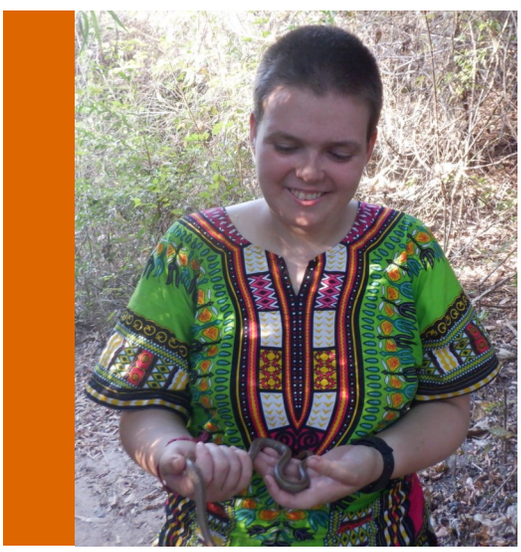
The whole trip was extraordinary and went far beyond my expectations. The accommodation in St Lucia and Swaziland were clean, safe and provided everything us as travellers needed and more (including some delicious meals). The VESA staff were friendly, approachable and made the trip smooth and free of stress from day one.
I have definitely changed for the better as a result of my volunteering and experiences in Africa. I have become even more determined to make an impact in the world and try to improve the lives of those less fortunate than me, as well as raising awareness of the issues of poaching, pet trade and ecotourism. I have become much more confident in myself and I am looking forward to my next adventure, knowing that if I can do it once, I can do it again. Finally, I hope to encourage more people to volunteer, whether that is locally, or abroad, as one person really can make a difference and improve the lives of others.

To find out more about VESA please visit www.vesabroad.org

Operation Madagascar

Louise Buchan

2nd year Bioveterinary Sciences BSc



Last summer I volunteered for six weeks in Madagascar as a research assistant with a conservation organisation called Operation Wallacea. I first saw the advertisement for a lecture about their expeditions in the University of Liverpool Guild of Students. I attended to learn more about their trips to conduct research in other countries and decided it was something I was keen to take part in. The only hurdle I could think of was how much money it would cost; however, I had some savings from working over summer and decided to leap at the opportunity to travel to Madagascar since the benefits far outweighed the costs.

Operation Wallacea gave me the opportunity to get a head start in the world of field research and reminded me of the reason why I turn up to my 9am lectures (or try to). Learning about the biodiversity and conservation of various species in the Mahamavo forest and Nosy Be, an island off the northwest of Madagascar, wasn't always easy but was worth every second.

My flight departed from Birmingham on the 13th June. On arrival in Antananarivo, Madagascar, I travelled by taxi to my hotel. The following day we began our journey as a convoy of minibuses to Mahajanga and then transferred to 4x4s for the rough terrain to base camp in Mariarano.

At base camp there was a long house with wooden tables and benches and a generator for electricity, which was only switched on for a few hours a day. Sleeping arrangements were two to a tent beneath the Sifaka (endemic lemurs to the region) inhabited trees. We showered with a bucket of cold water, our bathrooms were long drops, and we ate rice and beans for lunch every day. In spite of these circumstances, it was 100% worth it.

Mist netting in Mariarano was the most interactive survey which studied the distribution of bird species at specific sites in the forest. Huge nets were strung up from bamboo poles in the forest while bird calls were played from speakers in an effort to capture birds of the particular species that the researchers were studying. The nets were checked regularly, and the birds were carefully removed. Taking birds out of the net was a delicate task so we were trained beforehand by Malagasy research staff and observed closely. Often the staff would step in to help if the bird was particularly feisty in order to minimise the stress by making the process quicker as they were very experienced. Measurements such as weight, wing and tail length, as well as tarsus length and diameter were taken and recorded. Certain species had their blood taken for genome sequencing and were given a numbered silver ring. Every captured bird was fitted with a combination of three coloured rings later used to distinguish the individual if recaptured.

Antafiameva was the hottest camp so I took every opportunity available to sleep, in order to avoid the uncomfortable heat in the afternoon. We only spent up to two days there at a time because it was the most off-grid camp. A local boatman, known to us as "the Captain", took us on his boat for several crocodile and wetland bird surveys. During the first crocodile survey, which took place at night, we watched fireflies light up the trees. We hadn't seen anything, and we were on our way back to camp when Randy (an American herpetologist) spotted shining red eyes amongst the mangrove trees. On closer inspection we determined that it was a four-metre-long crocodile, which is pretty huge!

Matsedroy was everyone's favourite campsite. There were numerous reasons for this and to list a few, this included: the beautiful lake, the pancakes for breakfast, scorpion surveys, frog surveys, ghost spiders, and of course the late nights traditional Malagasy dancing around the

Figure 1. Mist netting in Mariarano.

campfire with bottles of Three Horse Beer (a local lager). The most memorable survey was a ghost spider study at Lake 2. Julie (one of the entomologists) picked up a large vibrant red, yellow and blue locust, which crawled from her shoulder onto her face! On the same survey I saw two beautiful Sifakas in the trees as well as tried raw honey from abandoned honeycomb.

Nosy Be was like a different world compared to the forest. It was a tourist island with hotels, resorts and roads. We stayed in a youth hostel with bunk houses, running water and flushing toilets – it was luxury compared to the forest!

"It was the most magical experience."

Learning to dive involved two sessions in a swimming pool then training sessions in the Indian Ocean. On the boat we set up our buoyancy aids by attaching them to our bottles of air and our regulators. Exercises we were tested on included removing and then putting our weight belts back on underwater, practicing hand symbols that mean things such as "Ok? Ok", "Out of air", "Breathe", "End of dive", "Share air", and "Ears not clearing". We all became qualified open water divers within the first week. I found it very disorientating feeling weightless and being able to spend 50 minutes up to 14 metres underwater. At times I was so excited at the marine life we saw I tried to talk to my dive buddy… then realised no one could hear me as the bubbles erupted out of the regulator in my mouth. The deeper you go below sea level the more light is absorbed so even brightly coloured things seem blue which is why many of the photos I took underwater appear to have a blue filter over the lens.

Although we had real beds, we didn't have as many opportunities to get involved in research surveys as in the forest. Most of our time was spent with dive masters training to gain our open water qualification. After we were qualified, we weren't able to lay transects as it takes experienced divers able to maintain neutral buoyancy otherwise the coral could be damaged. However disappointing as this may sound, we learnt a lot about marine life in our lectures including how to identify types of coral and tropical fish which we practiced on our following dives.

Even though Nosy Be was a tougher environment because diving was very tiring, it was the most magical experience. We saw beautiful coral reefs and wonderful

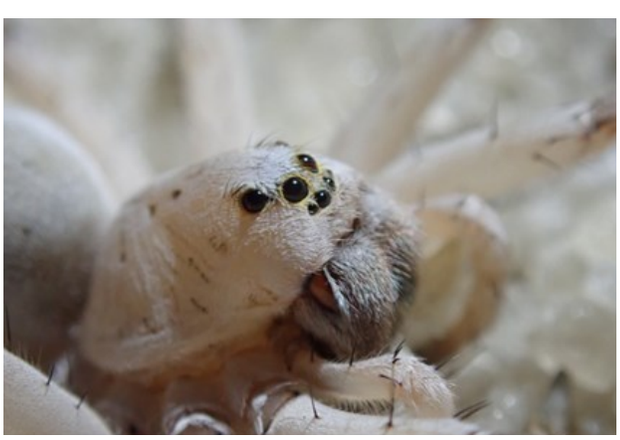


Figure 2. Rare ghost spiders found at Lake 2.

Figure 3. Underwater views at Nosy Tanihely.

creatures including mantis shrimp and lionfish. We went snorkelling at Nosy Tanihely Marine Park and encountered Hawksbill turtles and Moorish idols. Luckily, I had my waterproof camera and was able to capture lots of photos of the marine life. We also got to spend more time relaxing and socialising with sunset walks on the beach, swimming in the sea and a well-deserved pizza night at a small restaurant.

"I felt like I had not only gone on a journey of scientific research, but also one of personal growth and discovery."

At the end of the trip, I felt like I had not only gone on a journey of scientific research, but also one of personal growth and discovery. I overcame many fears, such as travelling alone internationally. Escaping the routine of my usual life and settling down into a new one gave me a renewed sense of maturity. The social media purge that resulted from absolutely no internet access and no phone signal was refreshing, and the simple lifestyle in Madagascar inspired me to rethink my priorities.

I made so many lasting memories while in Madagascar and hope to embark on many more research expeditions in the future. I am looking forward to the LIFE 222 Tropical Field Ecology trip to Uganda, an optional module aimed at Zoology students. Personally, I recommend as much travel and new experiences as possible especially when you're young. I also hope to gain my Advanced Diver qualification on another expedition with Operation Wallacea to Indonesia.

The message I would like to leave you with is a quote from Eleanor Roosevelt: "The future belongs to those who believe in the beauty of their dreams."

To find out more about Operation Wallacea please visit www.opwall.com

Thailand, the land of smiles

Victoria Drozdz

2nd year Biological Sciences BSc

Thailand is quite literally the land of smiles. I will always remember the warm, friendly faces of Thai people and the beauty of their serene country. The temples sparkled in gold just as the sun coated the tropical beaches. The hustle of Bangkok radiated through the city, just as the smell of street food grabbed the attention of people passing by.

I never imagined what a great experience travelling alone would be. After two weeks of travelling with Camp Thailand, I decided to venture off by myself. It was the most confidence boosting adventure and I learned to rely on myself more than ever. Not just in terms of planning and organisation, but also in terms of learning to enjoy my time in solitude. I had never felt so comfortable in my own company. After fighting off the jet lag, I began my trip with visiting the Grand Palace, which dazzled in the Bangkok heat. I was impressed with the golden structures and I marvelled at the intricate details of the Temple of the Emerald Buddha, within which Buddhist worship was occurring. I felt lucky to experience the way monks worship Buddha and the peaceful atmosphere in the temple. Khao San Road bustled with tourists and locals selling an interesting array of delicacies such as insects and crocodile. I had to accept the challenge to savour a scrumptious scorpion.

Another highlight of my trip was visiting the Wildlife Friends Foundation where I got the opportunity to bathe and feed rescued elephants who were previously abused and exploited. A tour around the centre involved seeing rescued gibbons, macaques, lorises, bears and other wild animals. This visit educated me about the importance of fighting against animal cruelty by spreading awareness about the maltreatment of animals. If you wish to find out more about the work of the organisation, please visit www.wfft.org.

In Kanchanaburi, I visited the bridge over the River Kwai, the location of the Death Railway. The bridge's construction came at the expense of the lives of thousands of prisoners of war. I remember taking the

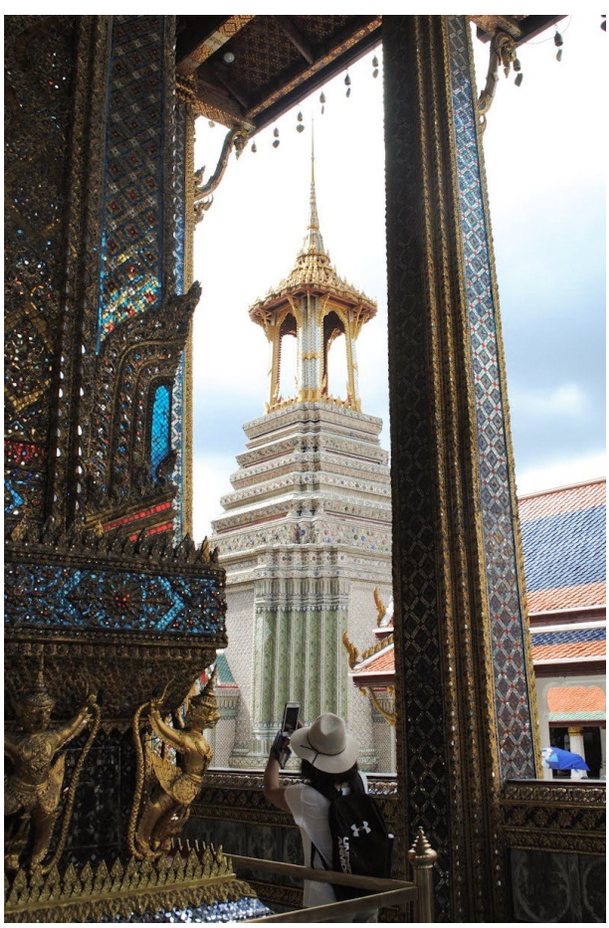


Figure 1. Temple of the Emerald Buddha.

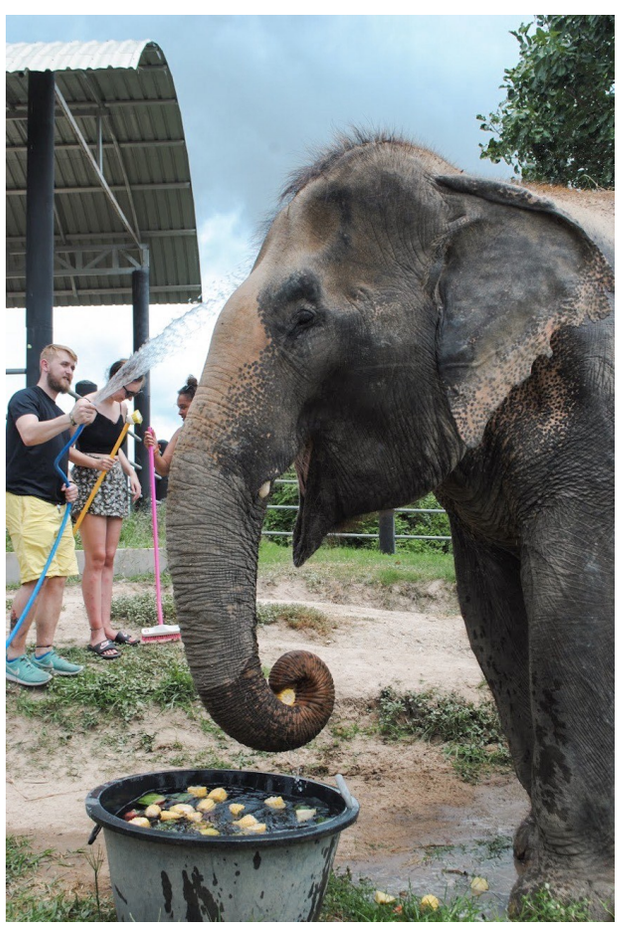


Figure 2. Rescue elephant at the Wildlife Friends Foundation.

Figure 3. Detail of a plant growing in an old train at the Death Railway.

photo of a plant growing out of a crack in an old train; it made me appreciate the perseverance of nature and the ways in which it adapts to survive.

Following a journey on Thailand's overnight train I arrived in Chiang Mai where I visited the umbrella making centre and Sankampaeng hot springs, where I was mesmerised by many butterflies surrounding a group of pink flowers. I managed to capture one of these butterflies before it flew onto another flower in a hasty fashion. My next stop was Phuket and the Phi Phi islands where I concluded my trip basking on the pristine beaches.

"Travelling has taught me transferable skills which I can use at university and in work."

This experience helped me to transition into the second year of my studies and gain a new found confidence to academic work. Travelling has taught me transferable skills which I can use at university and in work. Of course, I am still apprehensive about exams and assignments however, this apprehension does not rule over me anymore. If you are considering taking a trip by yourself one summer, no matter the scale, I advise you to go and learn a little bit more about yourself!

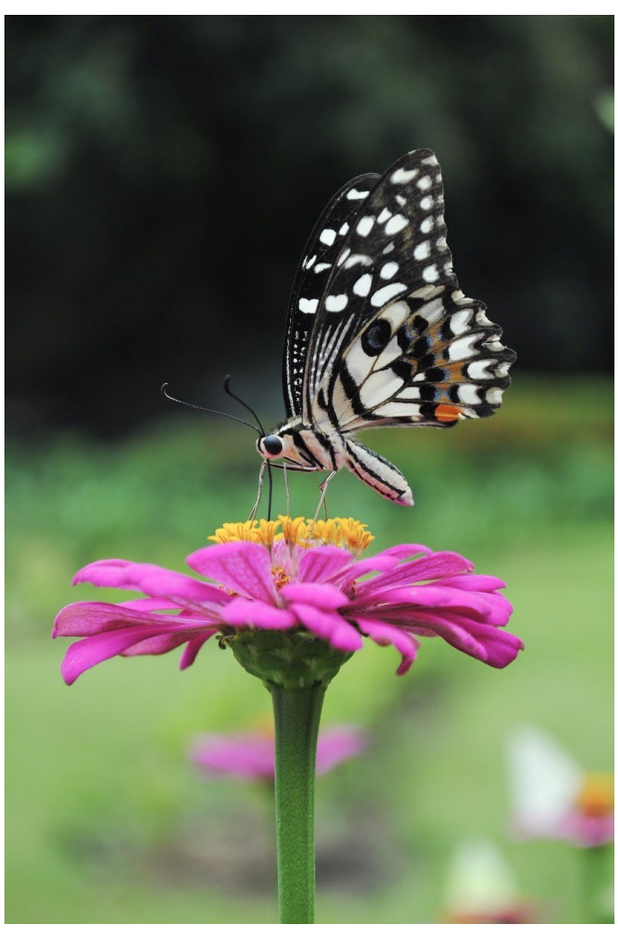


Figure 4. Butterfly at the Sankampaeng hot springs.

Figure 5. Huay Pla Kang Temple in Chiang Rai.

Research

Read about research from students at Liverpool

Students at the University of Liverpool have the chance to get involved in exciting research on many different topics. In this chapter you can read about a wide range of research being undertaken by both undergraduate and masters students.

Investigating the effect of butyrate treatment on resistance to *Toxoplasma gondii* infection using 3D model cultures

Bethan Timms[1], Nadine Randle[2], Catherine Hartley[2], Stuart Armstrong[3]

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This study investigated whether treatment with butyrate, a breakdown product from dietary materials in the large intestine, could protect the small intestine from toxoplasmosis; a disease in humans and animals that is caused by a parasite that invades the gut. In pigs, the disease can cause inflammation of the heart and brain. Disease spreads from animals to humans after eating undercooked infected meat or consuming contaminated food and water. Infection causes inflammation of the brain in people with a weakened immune system. If infection occurs during pregnancy, the disease is passed to the baby and causes problems with the nervous system and vision. Pig intestine models were established and either treated with butyrate or left untreated. The protein quantities that were present in butyrate treated and untreated cultures were analysed. Following butyrate treatment, cultures were infected with the parasite that causes toxoplasmosis and the percentage of invaded intestinal cells was calculated. Treatment with butyrate increased the production of proteins associated with joining cells in the small intestine closer together and the immune system. On the other hand, proteins that are important in the gut barrier and immunity were decreased in response to butyrate. The results showed that butyrate treatment reduced the number of cells in the small intestine that were invaded by the parasite. In conclusion, further studies are needed to understand if farmers should add butyrate to their pig feed to prevent toxoplasmosis.

Abstract

This study investigated whether treatment with butyrate, a short-chain fatty acid that increases protein abundance within tight junctions, could increase resistance to *Toxoplasma gondii* infection by altering the porcine intestinal epithelium. Toxoplasmosis is a disease caused by *T. gondii*, an intracellular protozoan parasite, which induces severe health implications on livestock such as encephalitis and myocarditis in sows. Zoonotic transfer occurs following ingestion of undercooked infected meat or food and water contaminated with oocysts. Infection causes encephalitis in immunocompromised individuals, as well as ocular and neurological defects via congenital transmission. Here we established 3D *in vitro* cultures of porcine intestinal epithelium, named organoids, that were treated with butyrate at concentrations between 0.5-1.0 mM for 22-24 hours, or left untreated. Quantitative label-free proteomics were then used to determine any differences in the protein abundance of butyrate treated and untreated organoids. Following butyrate treatment, cultures were infected with *T. gondii* (Pru-GFP) and flow cytometry analysis was conducted to assess parasite invasion of epithelial cells. Butyrate treatment upregulated protein expression of galectin-1 and selenium-binding protein 1 which are involved in cell-cell adhesion and the immune response. Contrarily, polymeric immunoglobulin receptor, serine protease 8, nectin-2, heat shock protein family A (Hsp70) member 4 and complement component 3 were downregulated in response to butyrate treatment and are important in immune functioning and the gastrointestinal barrier. We observed decreased invasion of intestinal epithelial cells by *T. gondii* following butyrate treatment. In conclusion, further studies are needed to determine whether butyrate supplements would be beneficial in preventing porcine toxoplasmosis.

Introduction

The ability to study and thoroughly understand enteric infections of domestic livestock is becoming of increased importance as they are accountable for outbreaks of abortion (1), worldwide economic losses (2) and the zoonotic transfer of harmful pathogens, resulting in major impacts on human health (3).

Toxoplasma gondii is an intracellular protozoan parasite which infects livestock (4) (Fig. 1). Toxoplasmosis usually develops via environmental transmission, where food or water contaminated with the infective stage oocysts are ingested (5). Oocysts are resistant to disinfecting practices (6), resulting in difficulty of disease eradication.

Toxoplasmosis induces severe health implications in livestock, such as encephalitis and myocarditis in sows, alongside piglet abortion (7). *T. gondii* is a zoonotic pathogen which causes toxoplasmic encephalitis in immunocompromised individuals (8), as well as ocular and neurological defects if infection occurs during pregnancy (9). The annual foodborne illness cost of *T. gondii* in the United States is estimated around $3 billion (10), highlighting the need to target livestock to reduce zoonotic transfer and thus prevalence of human disease. There are currently limited treatments for toxoplasmosis (11), therefore improved *in vitro* models are required to better understand host-pathogen interactions and enable the development of novel therapeutics.

The intestinal epithelium uses multiple mechanisms as a defence against invading enteric pathogens. Goblet cells secrete mucus which contains sticky binding sites to capture microbes (12) and paneth cells secrete large granules containing protective molecules which control microbial populations (13). Tight junction complexes contain proteins which enclose spaces between adjacent intestinal epithelial cells to increase the integrity of the intestinal barrier (14) and prevent paracellular entry of microbes (15). Understanding how different factors affect gastrointestinal barrier function has the potential to drive the development of specific methods that could promote the defensive mechanisms of the intestinal epithelium (16). For example, probiotic bacteria induce an anti-inflammatory effect which could reduce protozoal disease (17).

Butyrate is a short-chain fatty acid by-product of microbial fermentation in the large intestine (18). It can be used as a feed supplement in poultry and livestock to control enteric pathogens (19) because it increases the abundance of the main constituents of tight junctions (20). Butyrate also upregulates the expression of antimicrobial peptides which prevents pathogen colonisation in the gastrointestinal tract (21). Currently, intestinal epithelial cell monolayers (22) and epithelial cell lines (23) are used to study host-pathogen interactions. However, these model systems lack the cellular diversity found within the intestinal epithelium and are therefore not representative. Whole intestinal crypts or intestinal stem cells can be grown in a Matrigel matrix to produce 3D cultures of the intestinal epithelium, named organoids. 3D models allow cells to grow and interact in all directions unlike 2D models where growth is limited to flat surfaces (24). These models are advantageous because they contain cell lineages that would be observed *in vivo* and host-pathogen interactions can be examined in a physiologically relevant context (Fig. 2) (25).

There is currently no research on the effects of butyrate on toxoplasmosis using porcine organoids. The aim of this study was to understand how application of butyrate alters the porcine intestinal epithelium using quantitative label-free proteomics and assess the effects of butyrate on intestinal epithelial resistance to *T. gondii* using flow cytometry to investigate if butyrate supplements have the potential to reduce prevalence of porcine toxoplasmosis (Fig. 3).

Methods

Resuscitation of porcine organoids

Cryopreserved porcine organoids were transferred from storage in liquid nitrogen to a waterbath at 37°C. The solution was pipetted into a centrifuge tube and 5 ml IntestiCult (Stem Cell Technologies) was added. The organoid suspension was centrifuged (300 x g, 10 minutes, 4°C) to form a pellet. Supernatant was removed and the organoid pellet was resuspended in a mixture containing a 70:30 ratio of Matrigel (Corning) to IntestiCult. The organoid mixture was pipetted into a 24-well plate and incubated (37°C, 5% CO_2, 20 minutes). IntestiCult was added to each well and Dulbecco's phosphate buffered saline (PBS) was added to surrounding wells to prevent evaporation.

Passage of porcine organoids

Organoids were resuspended in PBS and transferred to a centrifuge tube. The organoid suspension was centrifuged (200 x g, 5 minutes, 4°C). Supernatant was removed and the pellet was resuspended in 70:30 Matrigel/IntestiCult solution. The mixture containing passaged organoids was pipetted into a 24-well plate and incubated (37°C, 5% CO_2, 20 minutes). IntestiCult was added to each well and PBS was added to surrounding wells before incubation (37°C, 5% CO_2). Organoids were passaged every three to seven days.

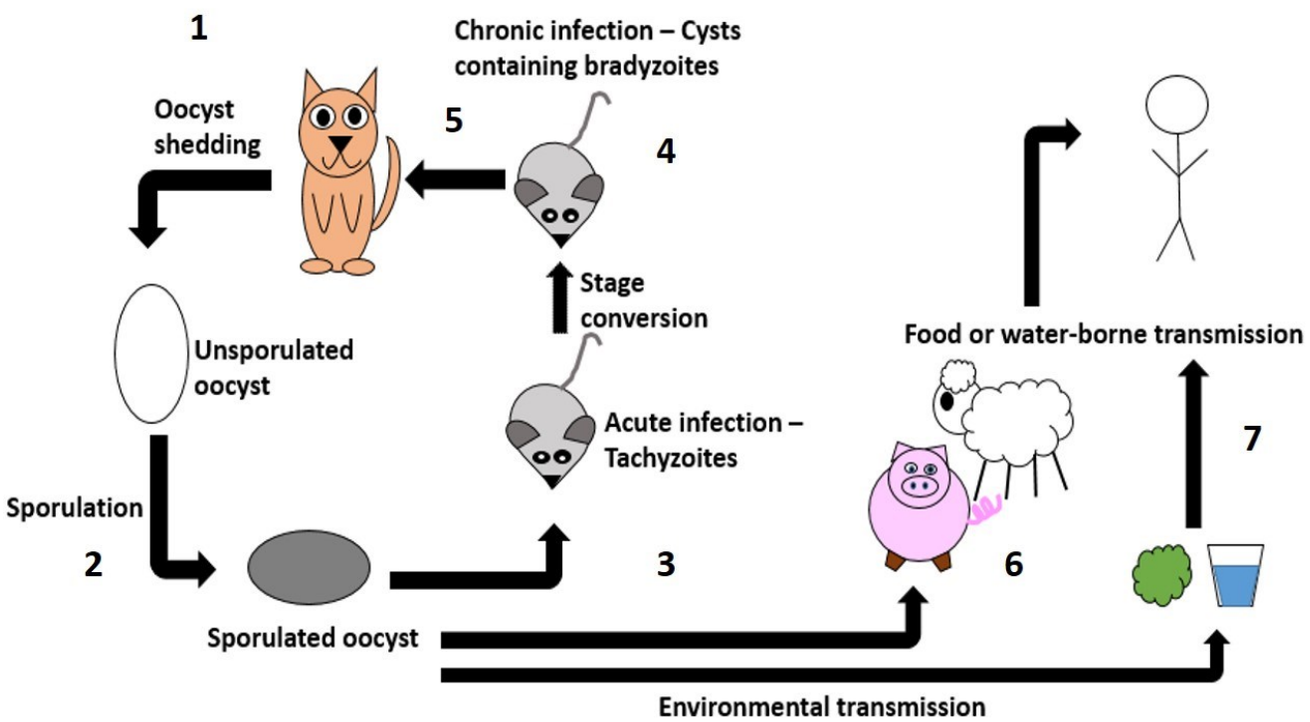

Figure 1. *Toxoplasma gondii* life cycle. (1) Sexual replication of *T. gondii* occurs inside the small intestine of members of the Felidae family which are the definitive hosts of the life cycle. Unsporulated oocysts are released into the environment in cat faeces; (2) Unsporulated oocysts undergo sporulation to become infective stage sporulated oocysts; (3) Rodents ingest soil, food or water containing sporulated oocysts and tachyzoites disseminate throughout the rodent, causing acute infection; (4) Tachyzoites differentiate into bradyzoites which form cysts within the tissues, resulting in chronic infection; (5) Sexual replication of the parasite within the cat begins again following the ingestion of rodents with encysted muscle tissue; (6) Sheep and pigs are intermediate hosts of the life cycle and become infected via environmental transmission; (7) Ingestion of fruit and vegetables or water contaminated with sporulated oocysts causes cysts to form in their tissues. Humans become infected by ingesting food or water contaminated with infective stage oocysts or by eating undercooked meat comprising tissue cysts. *T. gondii* can cause defects to the brain, eye and the heart of immunocompromised individuals and can also cause damage to a foetus via congenital transmission. Figure adapted from (43).

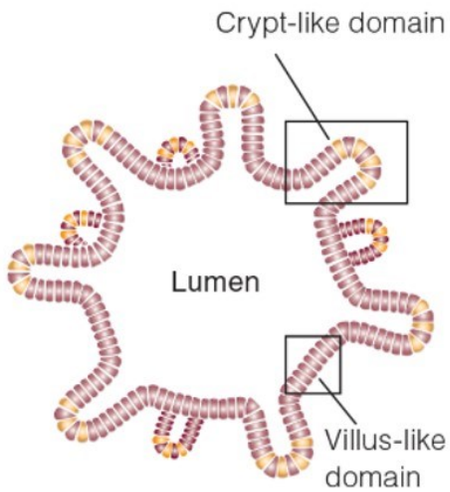

Figure 2. Organoid structure. Small intestinal organoid cultures have a central lumen and distinct crypt-villus domain that contain all the differentiated intestinal epithelial cell lineages. Figure adapted from (46).

Treating porcine organoids with butyrate

Sodium butyrate (Millipore Speciality Media) was added to IntestiCult to produce a solution with a butyrate concentration between 0.5 - 1.0 mM. Cultures were treated with butyrate or left untreated. Butyrate treatment lasted 22 - 24 hours.

Harvesting butyrate treated and untreated organoids

Butyrate treated and untreated organoids were harvested 22 hours post-treatment. Organoids were resuspended in PBS. Treated and untreated resuspended organoids were added to separate centrifuge tubes. Organoid suspensions were centrifuged three times (300 x g, 5 minutes, 4°C). After centrifugation, supernatant was removed and both tubes were placed in a -80°C freezer. This was repeated three more times to produce four replicates of butyrate treated and untreated organoids.

Harvesting *T. gondii* for porcine organoid infection

Vero cells infected with *T. gondii* (Pru-GFP) tachyzoites were scraped from flasks and centrifuged (2000 rpm, 10 minutes, room temperature [RT]). Supernatant was removed before PBS was added and the parasite mixture was centrifuged (2000 rpm, 5 minutes, RT). A blunt 5ml needle was used to release intracellular *T. gondii* and moved through a PD-10 desalting column. 10µl parasite suspension was added to a haemocytometer and the total number of *T. gondii* parasites were calculated. The remaining solution was centrifuged (2000 rpm, 10 minutes, RT), resuspended in the volume of IntestiCult required for passage and put on ice.

Infecting porcine organoids with *T.gondii*

Butyrate treated and untreated organoids were resuspended in PBS and separately transferred to two centrifuge tubes, filled with PBS and centrifuged (200 x g, 5 minutes, 4°C). Supernatant was removed and the organoid pellets were resuspended in the Toxoplasma/ IntestiCult suspension before incubation (37°C, 5% CO_2, 1hr). Following incubation, the volume of Matrigel needed for passage was added to the treated and untreated organoid/Toxoplasma suspensions and pipetted into a 24-well plate. 1 x 10^7 *T. gondii* (Pru-GFP) infected each well. The plate was incubated (37°C, 5% CO_2) for 20 minutes. IntestiCult was added to each well and PBS was added to surrounding wells. Infected and uninfected organoids were incubated (37°C, 5% CO_2) for three days.

Fixing butyrate treated and untreated organoids infected with *T. gondii* and uninfected untreated organoids

T. gondii infected organoids treated with butyrate were resuspended and transferred into two centrifuge tubes. This was repeated for the infected organoids with standard IntestiCult and the uninfected untreated organoids. PBS was added to each tube and centrifuged (100 x g, 3 minutes, 4°C). Supernatant was discarded to remove any extracellular *T. gondii*. Gentle Cell Disassociation Reagent (Stem Cell Technologies) was added to create a single cell suspension. Suspensions were centrifuged (1400 rpm, 5 minutes, 4°C) and supernatant removed. 4% paraformaldehyde was added and organoids were fixed for three hours in a refrigerator.

Proteomic analysis

Four experiments involved treating cultures with butyrate or leaving cultures untreated. These organoids were washed before RapiGest (Waters) was added. Cultures were sonicated on ice and then heated (10 minutes, 80ºC). Dithiothreitol (Sigma) was added (10 minutes, 60ºC) for protein reduction and iodoacetimde (Sigma) was added (30 minutes in the dark at RT) for protein alkylation. Trypsin (Sigma) was added at a 1:50 ratio of trypsin:peptide and incubated (37ºC, 5% CO_2) overnight. Tricholoroacetic acid was added to total 1% of the overall concentration, incubated for 2 hours (37ºC, 5% CO_2) and centrifuged (12,000 g, 4ºC, 1hr). Peptides were desalted using C18 spin tips and resuspended in acetonitrile with 5% trifluoroacetic acid.

Proteins were analysed using NanoLC-MS/MS. Samples were transferred to a trap column attached to a nano-electrospray emitter and connected to a Q-Exactive mass spectrometer. Thermo RAW files were uploaded to Progenesis LC-MS proteomics data analysis software. Peptide intensities for treated and untreated cultures were compared to determine differences in protein expression. Spectral data were exported using PEAKS Studio for protein identification. Tandem mass spectrometry data were searched against the Sus scrofa predicted proteome (Uniprot release Aug 2018).

Flow cytometry

After organoids were fixed, PBS was added to each of the six centrifuge tubes – two containing uninfected untreated organoids, two containing *T. gondii* infected organoids with butyrate treatment and two containing *T. gondii* infected untreated organoids. Each single cell organoid suspension was strained and transferred into a fluorescence-activated cell sorting tube. Flow cytometry analysis was performed using a MACSQuant Analyser to detect parasite GFP inside host epithelial cells. Gates were used to determine whether invading parasites were single or replicating. Data was analysed using FlowJo software.

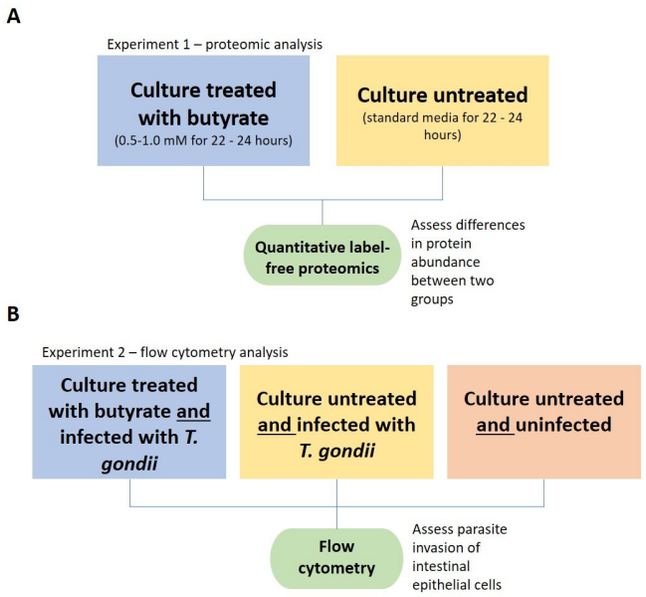

Figure 3. Experimental overview flowchart.

Results

Butyrate treatment significantly upregulates and downregulates protein expression

Exclusion criteria of *P*<0.1 and max fold change >1.5 were used to identify 7 upregulated proteins and 33 downregulated proteins following butyrate treatment. The low *P*-value and max fold change cut off points that were used reflects the small proportion of significantly up- or down-regulated proteins that were observed in the cultures.

Modulation of proteins associated with intestinal epithelial barrier function and immune function occur in butyrate treated and untreated cultures

Butyrate treatment upregulated three proteins that are of importance in this research (Table 1). Galectin-1 had the highest increased protein expression following application of butyrate and plays an important role in cell-cell adhesion and T cell homeostasis. Similarly, selenium-binding protein 1 is associated with the immune response and was upregulated in the butyrate treated cultures. 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1 expression was also increased in organoids treated with butyrate and is an intermediate of cholesterol synthesis. Butyrate treatment led to the decreased expression of five proteins (polymeric immunoglobulin receptor, serine protease 8, nectin-2, heat shock protein family A (Hsp70) member 4 and complement component 3) with functions relating to gastrointestinal barrier integrity and the immune response.

Butyrate treatment induces slight intestinal epithelial resistance to *T. gondii*

This study aimed to assess if butyrate treatment had an effect on resistance to *T. gondii* infection. Organoids were treated with 1.0 mM butyrate for 24 hours or left untreated. Cultures were then infected with *T. gondii* parasites expressing green fluorescent protein and flow cytometry was used to detect invaded intestinal epithelial cells.

Flow cytometry analysis demonstrated that cultures that were treated with butyrate showed slightly higher intestinal epithelial resistance to *T. gondii* than cultures that were untreated (Fig. 4). The total percentage of cells invaded with *T. gondii* (Pru-GFP) in the control cultures on average was 3.62%, which is slightly higher than that shown in the butyrate treated cultures which on average was 2.35%. Similarly, the percentage of cells that were invaded with replicating and single parasites was generally higher in the untreated organoids compared to organoids treated with butyrate. Comparing the ratio of replicating parasites to single parasites, the butyrate treated organoids had a slightly lower ratio on average (0.70) compared to that of the untreated organoids (0.74), however the range was very large for the control cultures.

Conclusions

Butyrate is a short-chain fatty acid that can be used as a feed additive in an attempt to control enteric pathogens (19) because it increases the abundance of the main constituents of tight junctions (20) and prevents colonisation of pathogens within the gastrointestinal tract (21). However, there is currently limited in-depth research on the effect of butyrate on the porcine intestinal epithelium and whether this could be implemented as a potential treatment to reduce prevalence of porcine toxoplasmosis and therefore prevent the zoonotic transmission of *T. gondii* into human populations. In this study, 3D cultures of porcine intestinal organoids were effectively established and treated with butyrate to investigate molecular changes within the intestinal epithelium. In addition, this study has

Gene name	Protein name	Function/Importance	Highest mean treatment	Max fold change	Anova (p)	Unique peptides
LGALS1	Galectin-1	Cell-cell adhesion, T cell homeostasis	Butyrate treated	4.9	0.0005	2
SELENBP1	Selenium-binding protein 1	Immune response	Butyrate treated	1.7	0.0416	2
HMGCS1	3-Hydroxy-3-Methylglutaryl-CoA Synthase 1	Intermediate in cholesterol synthesis	Butyrate treated	1.7	0.0801	4
PIGR	Polymeric immunoglobulin receptor	Facilitates IgA transport	Control	2.3	0.0248	29
PRSS8	Protease serine S1 family member 8	Intestinal barrier function	Control	1.6	0.0519	2
PRR2	Nectin-2	Cell-cell adhesion	Control	1.5	0.0731	2
HSPA4L	Heat shock protein family A	Immune response	Control	2.0	0.0865	2
C3	Complement C3	Component of the complement system	Control	1.5	0.0884	65

Table 1. Modulation of proteins associated with intestinal epithelial barrier function and immune function occur in butyrate treated and untreated organoids. Cultures were treated with 0.5 mM Sodium butyrate for 22 hours or left untreated and used as controls. This was repeated to produce four replicates of butyrate treated and untreated organoids. Cultures were analysed using nanoflow liquid chromatography-tandem mass spectrometry and proteomic analysis was performed to show the highest mean treatment, max fold change and *P*-value of all proteins present within the cultures. Exclusion criteria of *P*<0.1 and max fold change >1.5 were used to identify significant changes in protein expression. The function of each protein was determined using the PubMed database.

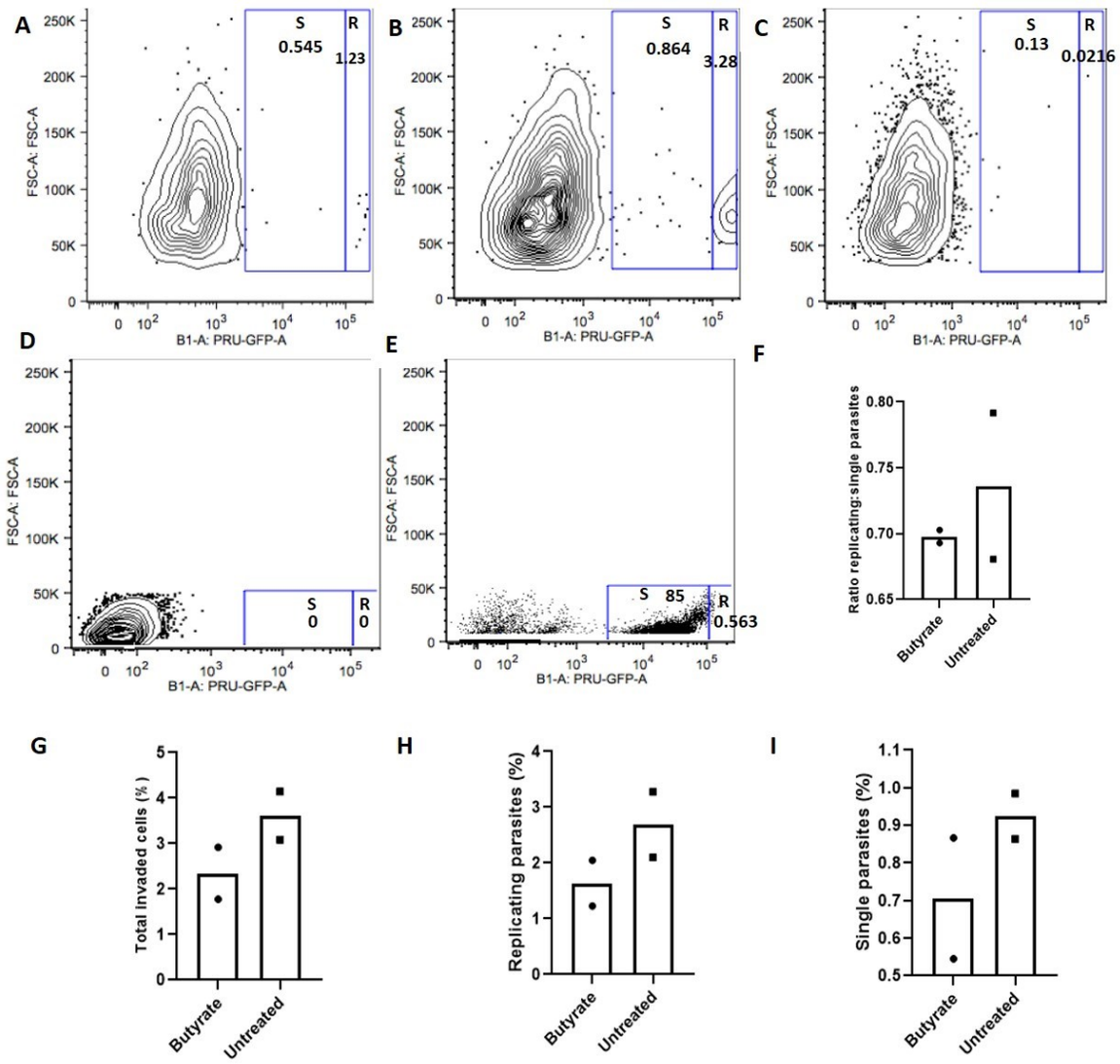

Figure 4. Butyrate treatment induces slight intestinal epithelial resistance to *T. gondii*. Cultures were treated with 1.0 mM Sodium butyrate (Millipore Speciality Media) for 24 hours or left untreated and used as controls. Butyrate treated and untreated cultures were infected with *T. gondii* (Pru-GFP) for three days. Additional cultures remained untreated and uninfected. Flow cytometry analysis was performed using FlowJo software to detect the proportion of green fluorescence parasites that had invaded intestinal epithelial cells. Gating was used to determine whether invaded *T. gondii* were single or replicating parasites. Comparison of parasite invasion was made between butyrate treated cultures infected with *T. gondii* (A), untreated cultures infected with *T. gondii* (B) and untreated uninfected cultures (C). Flow cytometry analysed the amount of extracellular *T. gondii* debris in uninfected cultures (D) and butyrate treated *T. gondii* infected cultures (E). Butyrate treated and untreated cultures infected with T. gondii were compared on the ratio of replicating parasites to single parasites (F), the total % of invaded cells (G), % of invaded cells with replicating parasites (H) and the % invaded cells with single parasites (I). S represents the percentage of invaded intestinal epithelial cells that contained single parasites and R represents the percentage of invaded intestinal epithelial cells that contained replicating parasites.

also shown that *in vitro* porcine organoids can be successfully infected with *T. gondii* (Pru-GFP) in order to determine whether application of butyrate has the ability to significantly reduce the invasion *of T. gondii* into porcine intestinal epithelial cells.

The results have demonstrated that abundance of galectin-1 is drastically increased in porcine organoid cultures following treatment with butyrate. Galectin-1 is a member of β-galactoside binding lectins which promotes cell-cell and cell-matrix adhesions (26) by directly binding to or increasing protein expression of cell-adhesion molecules (27). In addition to its importance in barrier function, galectin-1 plays a key role in regulation of enterocyte turnover within the villus of the small intestine which regulates homeostasis of the gastrointestinal tract during physiological stress (28). The upregulation of galectin-1 expression as a result of butyrate treatment is suggested to increase the integrity of the intestinal epithelial barrier and modulate gut homeostasis which could explain the slight increase of resistance to *T. gondii* following application of butyrate to organoid cultures.

Porcine organoids that were treated with butyrate also had increased expression of a protein important in the synthesis of cholesterol named 3-hydroxy-3-methyglutaryl coenzyme A synthase 1 (29). *T. gondii* exploits cholesterol from its host and transports it into its parasitophorous vacuole which allows the parasite to survive (30). Luu *et al.* (31) demonstrated that treatment of organoids with statins that blocked enzymes important in the synthesis of cholesterol could effectively inhibit

T. gondii replication, this further suggests that cholesterol is essential for parasitic infection. Therefore, butyrate treatment may be counterproductive as it encourages upregulation of the cholesterol biosynthesis pathway which may subsequently promote *T. gondii* infection as the parasite has a larger source of cholesterol to scavenge from driving its survival within the host.

Application of butyrate led to the decreased protein expression of protease serine S1 family member 8, a serine protease that inserts a protein named occludin, one of the main constituents of tight junctions, into the intestinal epithelium. Previous literature demonstrates that transepithelial electrical resistance of intestinal epithelial cell lines increases following application of serine proteases which indicates increased integrity of the gastrointestinal barrier (32). Due to the fact that this serine protease is associated with proper functioning of tight junction complexes and its expression was decreased in cultures that were treated with butyrate, it is suggested that butyrate does not have a significant effect on intestinal epithelial resistance to *T. gondii* due to the downregulation of protease serine S1 family member 8. Therefore, it is possible that treatment with butyrate may increase the ability of the parasite to transmigrate between adjacent intestinal epithelial cells which may cause injury to surrounding tissue (33).

Another suggestion as to why butyrate treatment did not significantly increase resistance of porcine organoids to *T. gondii* is that the parasite burden was too intense for application of butyrate to have a large impact. The proposed increased integrity of the gastrointestinal barrier due to the elevated abundance of galectin-1 may not have been strong enough to overcome the $1x10^7$ *T. gondii* (Pru-GFP) parasites that each well were infected with. In order to distinguish the difference between whether butyrate is ineffective at preventing infection or whether the parasite burden was too intense, future research should involve compounds from existing literature that have already been demonstrated to significantly reduce *T. gondii* invasion, such as atorvastatin (31).

Although organoid models of the intestinal epithelium are advantageous, a main weakness is the absence of an immune system. As discussed previously, this study showed that the expression of galectin-1 was upregulated in porcine organoid cultures following butyrate treatment. Alongside the increased intestinal epithelial integrity that galectin-1 induces on the gastrointestinal tract, it also plays a specific role in regulation of immune responses (34). Galectin-1 binds to complement receptor CR3 which is significant in the innate immune response (35). Zúñiga *et al.* (36) demonstrated that high concentrations of galectin-1 drives apoptosis of murine macrophages infected with *Trypanosoma cruzi*, suggesting that this protein is capable of instructing macrophages to control intracellular parasitic infections. Galectin-1 also has importance in T cell homeostasis by promoting apoptosis of neutrophils in order to inhibit the release of toxic contents which may destroy neighbouring tissue (37).

Butyrate treatment also upregulates the expression of another protein that has key roles in immune function called selenium-binding protein 1. Suitable levels of selenium are required to promote the immune system, as well as control inflammation and modulate excessive immune responses (38). Low levels of selenium can induce immunosuppression which subsequently results in increased susceptibility to disease (39). Previous literature demonstrates that organic selenium fed to pigs enhanced their immune response (40). In relation to parasitic infection, addition of selenium to rats that were subject to *T. cruzi* infection displayed reduced parasitic burden (41), suggesting that increased selenium as a result of butyrate treatment may have similar effects on *T. gondii* infection. Due to the fact that galectin-1 and selenium-binding protein 1 both drive multiple immune response mechanisms that are important in controlling parasitic infections, if butyrate was applied *in vivo* where an immune system was present, then a more significant increase in resistance to infection may have been observed.

On the other hand, the proteomic analysis from this study may suggest that the application of butyrate *in vivo* will not increase intestinal epithelial resistance to *T. gondii*, even though an immune system is present. This is because the expression of several proteins that are important in the immune response were downregulated in treated cultures. Our results showed that polymeric immunoglobulin receptor had decreased protein expression following butyrate application. Polymeric immunoglobulin receptor is an Fc receptor which interacts with IgA to reduce the immunoglobulin's susceptibility to digestion, before transporting it into the lumen of the intestine to reduce parasitic burden (42). If butyrate treatment was conducted in a model where immune cells were present, the downregulation of polymeric immunoglobulin receptor would reduce the transport of IgA into the intestinal lumen to control *T. gondii* infection.

In conclusion, this study demonstrates the potential of butyrate to protect the intestinal epithelium against *T. gondii* invasion, however further research is required to determine whether butyrate supplements should be recommended as a preventative measure for porcine toxoplasmosis. Future experiments should trial different concentrations and treatment periods of butyrate to thoroughly understand the molecular and structural changes that it induces on the porcine intestinal epithelium to determine whether treatment with butyrate has the ability to increase resistance to infection.

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Population Genetics of the Eurasian Bat *(Myotis brandtii)*

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Population structure defines relatedness between individuals that live in the same place or in different geographical locations. Individuals may be more related when they live in small groups or are isolated from other populations, due to inbreeding. Individuals from populations that are less related tend to be separated by long distances or geographical barriers such as mountains. An understanding of a species' population structure can help conserve species and understand how diseases spread between populations. This is particularly important in bats as they carry a lot of viruses that can infect humans, such as rabies. This study investigated population structure within the Eurasian Brandt's bat (*Myotis brandtii*) and subspecies found in Mongolia (*Myotis brandtii gracilis*). This study found a lack of population structure in the European populations but found the Mongolian population to be isolated from the others. Populations with increased distances between them were less related. However, the European populations still appear to be breeding with one another, suggesting a lack of geographical barriers. Male bats tend to travel further distances than female bats and how this affects population structure is an interesting focus for further study.

Abstract

Genetic studies of bat populations are important due to the wider implications within conservation and the control of transmissible diseases. Brandt's bats (*Myotis brandtii*) are widely distributed throughout Europe and Asia and exhibit swarming; a promiscuous mating system that reduces the risk of inbreeding. The aim of this study is to use seven microsatellite *loci*, polymorphic in *Myotis brandtii*, to determine the level of large-scale population structure across eight populations in Europe and Asia, including a population of the cryptic subspecies *Myotis brandtii gracilis*. The distance between sites is large and highly variable with multiple potential dispersal barriers. Genetic diversity between and within populations was measured using Hardy-Weinberg exact tests, F-statistics and the Mantel test assessed isolation by distance (IBD). The results of this study indicated a low level of population structure between the individuals sampled in Europe, with high levels of heterozygosity. The Mongolian population of suspected subspecies *M. b. gracilis*, was the most genetically differentiated from the other populations. Mantel tests found significant IBD between each population, with a gradual increase in genetic differentiation with geographical distance. Despite high IBD, evidence of high gene flow and heterozygosity suggests IBD is not considered a conservation issue in the Brandt's bat. This study stimulates the need for further investigation into sex-biased dispersal.

Introduction

Data obtained through population studies has implications within conservation biology. Knowledge of migration between sub-populations can help to predict the likely impacts of habitat destruction and segmentation. *M. brandtii* is currently listed as 'least concern' on the IUCN red list due to high abundance (1) and widespread distribution throughout Europe and Asia (2). However, in the event that a species becomes threatened or endangered, population studies can help make informed decisions regarding breeding programmes and reintroductions (3-4). Identifying the lack of population structure in the closely related Natterer's bats (*Myotis nattereri*) highlighted the importance of the identification and conservation of swarming sites, as they are invaluable in reducing inbreeding by increasing gene flow between sub-populations (5).

Population genetics also give an insight into movement patterns, aiding the development of accurate analytical models of the spread of disease (7). This is particularly important within bats as they are major natural reservoirs of several multi-host viruses (SARS-Like Coronaviruses, MERS-Like Coronaviruses, Nipah virus, Ebola virus, Rabies virus and Hendra viruses) (8). European Bat Lyssaviruses type 1 (EBLV-1), type 2 (EBLV-2) and Bokeloh Bat Lyssavirus (BBLV), are all causative agents of rabies in European bats, and have been isolated from several Myotis bat species (*M. daubentonii*, *M. dasycneme* and *M. nattereri*) (9). As a result of habitat disturbance, bats are living in closer proximity to humans and the infection of new host species is becoming more frequent, posing a higher risk for human infection (10). Studies also provide an insight into the epidemiology of diseases affecting bats. White-nose syndrome (WNS) is a fungal disease of North American bats and causes death in susceptible species (11). Population studies of little brown bats (*Myotis lucifugus*) suggested that population structure, derived from female migration patterns, influenced the spread of WNS across Pennsylvania and West Virginia (12).

In this study, seven microsatellite *loci*, polymorphic in *M. brandtii* (13-15) are analysed to identify genetic variation between and within sub-populations of *M. brandtii* and subspecies *M. brandtii gracilis*. This study will provide evidence to assess the level of population structure and isolation by distance in *M. brandtii* and discuss the implications. Ecological data of the Brandt's bat is limited due to cryptic morphology, nocturnality and ability of flight. *M. brandtii* morphologically resembles three other species (*Myotis alcathoe*, *Myotis mystacinus* and *Myotis ikonnikovi*) (16). *M. brandtii* is considered a small bat species with an adult body weight of 4-8 g and can live upwards of 40 years (2). They inhabit temperate broadleaf, mixed or coniferous woodland, typically near water (17), and feed on non-aquatic small insects and spiders (18). Summer roosts are being found more frequently near human habitation; in roofs and bird boxes, but more commonly in hollow trees. Hibernation locations tend to be in caves, cellars, mines and tunnels (19).

The Brandt's bat could show population substructure. A previous study found that Schreibers' long-fingered bat,

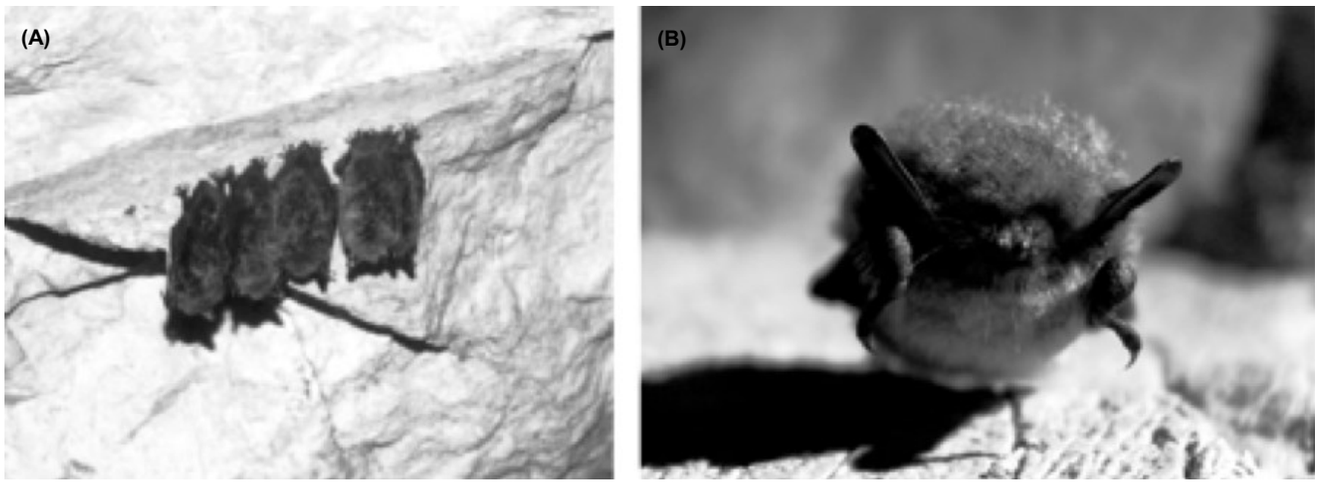

Figure 1. A small group of Brandt's bats roosting in a cave (A) and ventral photograph of a single Brandt's bat (B); reproduced from (55).

Minipterus schreibersii natalensis, demonstrated strong population substructure in the South, West and North-East regions of South Africa. Without obvious geographical barriers, the study concluded that the genetic diversity between the three sub-populations resulted from morphological differences and local biomes (20). However, due to the bats' flight ability, promiscuity and presence at swarming events, we predict *M.brandtii* will show little to no population structure, similar to that of Daubenton's bats (*Myotis daubentonii*) (21). Many studies have found a lack of population structure to be universal in seasonally migratory bats (22).

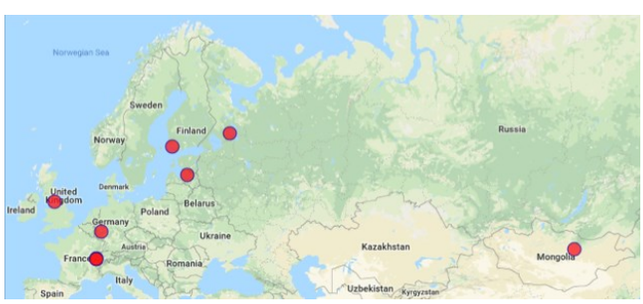

Figure 2. Red dots represent sampling locations across Europe. Map created for this study using mapping software [online] Available from: https://www.darrinward.com/lat-long/

Methods

A total of 135 bats were sampled from eight locations including England (Easegill Caverns), Germany (Mayener Grubenfeld), Switzerland (Gouffre de la Pleine Lune and Couffre Cathy within Parc Jurassien Vaudois), Finland (Turku), Latvia (Sikspārņu Cave), Russia (Petrozavodsk) and Mongolia (Ulan Bator) (Fig. 2). DNA was extracted and purified from 3 mm wing punches (stored in 70% ethanol at -80ºC) using the Qiagen DNeasy Blood and Tissue Kit. Wing punches (3 mm) do not disrupt flight and heal completely in 2-3 weeks (23). The yield of genomic DNA was measured using the Thermo Fisher Invitrogen Qubit Fluorometer. Single template multiplex PCR was carried out with a final volume of 10 µl, containing: Qiagen Multiplex PCR Master Mix, forward and reverse primers (fluorescently labelled) (Table 1), Genomic DNA, and dH_2O. Thermal cycling programme: 95 °C for 5 minutes followed by 28 cycles (95 °C for 30 seconds, 59 °C for 90 seconds and 72 °C for 30 seconds and lastly, 60 °C for 30 minutes). The PCR products were diluted with water (1:100) and amplicons were separated by capillary electrophoresis on the Applied Biosciences 3500xL Genetic Analyzer. Genemapper V software (24) was used to size and assign alleles.

For each sample site, observed heterozygosity (Ho), expected heterozygosity (He), allelic richness and effective number of alleles (Ae) was calculated using GENALEX 6.5 (25) add-in for Microsoft Excel. Guo and Thompson's (26) exact Hardy-Weinberg test was used to assess compliance with Hardy-Weinberg Equilibrium (HWE) for each locus in each population using the population genetics program GENEPOP 4.6 (27). Hardy-Weinberg exact tests are performed frequently in population genetics as deviations from HWE may highlight problems such as selection bias (28) and genotyping errors (29). The Markov Chain Method is used to estimate the exact *P*-values for each locus in individual populations and Fisher's method was used to calculate the overall *P*-value of all *loci* in each population. Pairwise F_{ST} values were calculated using GENEPOP 4.6 (27). F_{ST} (the fixation index) is the value for genetic distance. It is proportional to the level of inbreeding within subpopulations relative to the metapopulation (30). The inbreeding coefficient (F_{IS}) of an individual relative to the subpopulation were calculated using GENALEX using the Weir & Cockerham (31) method. A negative F_{IS} value suggests individuals are less related to each other and a positive F_{IS} value suggests individuals are less related. Isolation by distance is assessed on GENEALEX using the Mantel test, which compares genetic distance and geographical distance. Statistical significance of R (mantel coefficient) was attained by forming 999 permutations.

Ethical approval for this study was obtained by my supervisor, Professor Steve Paterson.

Results

The yield of genomic DNA, extracted from 3 mm wing punches, ranged from 1.9–220 ng/µl, with a mean final concentration of 20.2 ng/µl.

Locus	Sequences (5'-3') Forward (F) and Reverse (R) Primer	Array	Fluorescent Tag	Allele Size Range (bp)	Marker concentration (mM)
A24-Mluc [13]	F: GTGGTATGAAATAACCAGTTCACTTTG R: GTTTCAGACTGCATTACTGAAGAAATTATGG	(AC)n	FAM	473-491	0.2
Clone A2-Mluc [13]	F: TGGCCCATGCTCATCATC R: GTTTCTGGTCTCAACTGGGTGCTC	(CA)n	VIC	91-135	0.05
D9[14]	F: GTTTCTTTCCTCCCCTGTGCTC R: TCTGGACCCAAAATGCAGG	(CT)n	NED	120-150	0.2
E24[14]	F: GTTTGCAGGTTCAATCCCTGACC R: AAAGCCAGACTCCAAATTCTG	(TC)n	FAM	215-253	0.2
ES43-Mluc[13]	F:GTTTAAGGGGGAGAGGAGTGG R:GCTGCGTGTCCAGAGG	(AC)n	FAM	377-403	0.2
H29[15]	F: GTTTCAGGTGAGGATTGAAAACAC R: GCTTTATTTAGCATTGGAGAGC	(CA)n	FAM	170-202	0.4
Paur 6[16]	F: GATCAGATTTCCAAACAGAG R: GTTTAGGTTCTTTCTTCAGCTATG	(AC)n(AG)n	PET	156-186	0.2

Table 1. Adapted from (22). Forward and reverse primer sequences, arrays, fluorescent tag, allele size range (bp) and marker concentration (mM) for the microsatellite *loci* used in this study. Allele sizes adapted for *M.brandtii*.

Sample site	n	Observed heterozygosity	Expected heterozygosity	Allelic Richness	Effective Number of Alleles	HWE *p*
England	21	0.649	0.703	7.14	4.67	0.3638
Germany	33	0.686	0.694	10.00	5.05	0.0540
Switzerland	23	0.621	0.641	7.71	4.73	0.2198
Finland	25	0.584	0.661	9.14	4.42	< 0.001
Latvia	20	0.634	0.652	8.57	4.96	0.2613
Russia	13	0.702	0.670	6.29	4.41	0.4213
Mongolia	29	0.842	0.803	10.00	5.96	0.9192

Table 2. Genetic diversity indices averaged across microsatellite *loci* for each population and the HWE *P*-values for exact tests. n= sample size.

Sample Site	A24-Mluc	Clone A2-Mluc	D9	E24	ES43-Mluc	H29	Paur6
England	0.4286	0.3708	0.9683	0.2280	**0.0226**	1.0000	0.6281
Germany	**0.0388**	0.1466	0.1358	0.0656	0.4036	0.6770	0.5965
Switzerland	-	**0.0386**	0.5689	0.0945	0.5664	0.5725	0.6693
Finland	**0.0005**	**0.0035**	0.0608	0.1211	0.1792	**0.0116**	0.7078
Latvia	1.0000	0.1308	0.8831	0.6416	**0.0051**	1.0000	0.5691
Russia	0.5046	**0.0060**	0.8908	0.3585	0.7923	1.0000	0.9758
Mongolia	0.1413	0.8094	0.7102	**0.0000**	**0.04041**	0.8657	0.8804

Table 3. HWE p values for each locus in each individual population calculated using Markov chain method. The significant values are underlined ($P < 0.01$) and in boldface ($P < 0.05$).

Population Diversity

The observed heterozygosity in each population ranged from 0.584-0.842 (Table 2). One population (Finnish) out of six deviated significantly from HWE for $P<0.05$. Three *loci* deviated significantly from HWE in the Finland population ($P<0.05$) as opposed to a maximum of two *loci* in other populations (Table 3). Russia and Mongolia had higher observed heterozygosity (0.702 and 0.842, respectively) than expected (0.670 and 0.803) whilst the other populations had lower observed heterozygosity than expected (Table 3). Mongolia and Russia also had the least number of positive F_{IS} values (two *loci*) inferring higher levels of inbreeding than expected as opposed to a minimum of three *loci* in the other populations. Positive inbreeding coefficients (F_{IS}) were found in all populations at a maximum of six *loci* (Finland). The highest inbreeding coefficient (1.000) was found at the Finnish site for the locus A24-Mluc (Table 4), where all individuals are homozygous. The lowest inbreeding coefficient (-0.3333) was found at the Russian site (Table 4).

Population Differentiation

Pairwise genetic distance (F_{ST}) and geographical distance are presented in Table 5. F_{ST} values range from 0.0055 (Latvia and Germany) to 0.1662 (Finland and Mongolia). The largest linear geographical distance (7018 km) exists between Switzerland and Mongolia. The Mantel test found a positive correlation between genetic distance and geographical distance (Mantel test: R= 0.416, $P< 0.001$) showing significant isolation by distance.

Discussion

Population Diversity

Each population had high heterozygosity across *loci*. One of the possible explanations for high individual population heterozygosity values in *M. brandtii* is that they are one of many bat species present during swarming (32-34). Swarming is a phenomenon that reduces the risk of inbreeding, taking place in late Summer and early Autumn, where large numbers of bats visit hibernacula (34). Currently, there is no consensus as to why bats swarm but there are multiple hypotheses. Three principal theories include opportunities to mate (35), information transfer from parental bats to offspring and, to assess suitable hibernacula (34). Opportunity to mate as a hypothesis has the most supporting evidence. Bats display significant social vocalisation, chasing and copulatory behaviour during this period (36). There is significant evidence to suggest swarming events increase gene flow between sub-populations, increasing genetic diversity in progeny (32).

Bats also demonstrate reproductive strategies known to increase genetic diversity within other animal species. The behaviours demonstrated by *M. brandtii* during mating periods are described as indiscriminate and promiscuous

Sample Site	A24-Mluc	Clone A2-Mluc	D9	E24	ES43-Mluc	H29	Paur6
England	**0.5714**	**0.1765**	-0.0026	**0.2139**	**0.1504**	-0.1799	-0.0696
Germany	**0.1754**	**0.1384**	-0.0999	**0.1537**	-0.0613	-0.0604	**0.0000**
Switzerland	-	**0.2636**	-0.0649	**0.0686**	**0.1304**	**0.1493**	-0.1020
Finland	**1.0000**	**0.3070**	-0.0360	**0.1273**	**0.0523**	**0.2222**	**0.0213**
Latvia	-0.1053	**0.0788**	-0.1362	**0.0917**	**0.1268**	-0.1347	**0.0710**
Russia	-0.3333	**0.2809**	-0.0602	**0.0000**	-0.0614	-0.0588	-0.0254
Mongolia	-0.0162	-0.0859	-0.1027	**0.0403**	**0.0949**	-0.0897	-0.0335

Table 4. F_{IS} values at each *loci* for each population. Results that infer higher levels of inbreeding than expected are in boldface (31).

Sample Site	England	Germany	Switzerland	Finland	Latvia	Russia	Mongolia
England		790.3	1057	1628	1764	2292	6880
Germany	0.0214		436.4	1465	1413	2085	6678
Switzerland	0.0272	0.0204		1873	1773	2468	7018
Finland	0.0195	0.0153	0.0161		391.6	665.5	5276
Latvia	0.0123	0.0055	0.0139	0.0083		709.4	5265
Russia	0.0241	0.0200	0.0212	0.0198	0.0100		4616
Mongolia	0.1488	0.1622	0.1645	0.1662	0.1590	0.1554	

Table 5. Pairwise F_{ST} values and distance between populations (km). Distances (km) are above the diagonal. F_{ST} values are below the diagonal.

(35). After copulation, females store spermatozoa within their reproductive tract, delaying ovulation and fertilisation until Spring (35, 37). Studies on dissimilar species have demonstrated that sperm storage and promiscuity increase genetic diversity, for example, in the *Anolis sagrei* lizard (38) and passerine birds (39).

Despite populations showing high observed heterozygosity and not deviating significantly from HWE, each population had positive inbreeding coefficients (F_{IS}) at various *loci*, particularly in three *loci* (Clone A2-Mluc, E24 and ES43 Mluc). This contradiction could be attributed to sampling bias, small sample sizes and null alelles (40).

Population Differentiation

Pairwise European F_{ST} values indicated little genetic differentiation between populations, using guidelines for interpretation (41), indicating low population structure in the Brandt's bat. The results suggested there are no significant barriers to gene flow in Europe. These findings are in agreement with other population genetic studies on seasonally migratory bats such as the Daubenton's bat (21) and wide ranging species such as the Regent Honeyeater bird, *Anthochaera phrygia* (42). The Mongolian population had high pairwise F_{ST} values with all other populations, suggesting they are isolated from the others and not interbreeding.

Low F_{ST} values suggest populations are not significantly genetically distinct. However significant IBD presents a barrier to gene flow. As distance increases, populations become more genetically diverse. Geographical barriers of movement in the Brandt's bats may include mountain ranges and large expanses of different climates (deserts). Bright lights and disruption of vision can affect bat migration and tracking long-range movements (43) suggesting brightly lit cities and roads have the potential to restrict geneflow. However, migratory behaviour which can reach up to 618 km (43-45), and flight abilities may be the reason for the apparent lack of barriers to gene flow between sub-populations.

Similarly to the apparent lack of geographic barriers found in this study, Castella *et al*. (14) also identified a lack of population structure between two populations of *Myotis myotis* on either side of the Gibralter Strait (14 km wide stretch of water separating peninsular Spain and Gibralter from Morocco and Ceuta in Africa). However, further analysis of a mitochondrial gene (cytochrome b) confirmed the two populations were genetically distinct with no interbreeding over the Strait of Gibralter. This suggests other methods of molecular analysis on the European populations of *M. brandtii* are required to definitively state whether they are interbreeding. Myotis bats also demonstrate a lack of site fidelity, changing roosts frequently depending on their reproductive status (35, 46), encouraging interbreeding between sub-populations. Male bats are recorded to disperse further than females. Females tend to be more philopatric as they have to exploit resources and provide parental care to immobile progeny (21, 47). Population structures generally fall into three categories – populations divided by age, sex or social/geographic factors (48). This study analysed geographic factors suggesting further investigation into age and gender diversity indices is necessary to determine sex barriers on population structure.

Implications within conservation and Disease transmission

Low genetic distance between populations suggests a lack of geographical barriers within Europe which is promising for bat conservation. This study highlights the importance of protecting swarming sites as swarming species have higher gene flow (49). A major feature currently concerning the conservation of many species is climate change. Studies have indicated that European bats may face a serious threat with climate change (50) and may respond with a population shift northwards, breeding earlier (51) or changing migration times (52). This study confirms the widespread distribution of *M. brandtii* in temperate regions and tropical climates (Mongolia) suggesting they are able to adapt to changing climates, providing there is available habitat northwards.

Migratory bats are known to migrate over open sea as they are frequently found on remote islands, ships and oil rigs (53). This study indicates that British bat populations are interbreeding with continental European populations; presenting an interesting insight into the spread of diseases, such as the rabies virus. Many studies focus on the control of dogs and foxes to control the spread of rabies, and highlight rabies-free status in some European countries. However, rabies is a trans-boundary disease due to the migratory nature of bats, and the importation of infected animals and reinfections have occurred in Italy, Greece and Slovakia (54). This highlights the need for trans-boundary and species-wide approaches to controlling the spread of rabies.

Conclusions

In summary, European populations of *M. brandtii* show low population structure but significant isolation by distance. The high F_{ST} values between the Mongolian population of *M. b. gracilis* suggest they are isolated and not breeding with the other populations. Similar to the Gibralter Strait study (14), further molecular analyses could confirm or refute the lack of population structure in Europe. There are a number of possible reasons for high heterozygosity and low F_{ST} values in the European populations, despite significant isolation by distance, such as: a lack of physical boundaries, ability of flight, migratory behaviour, swarming, lack of site fidelity, sex-biased dispersal and promiscuity. Similar literature suggests a study on sex-biased dispersal would provide further insight into the population structure of Brandt's bats. These findings highlight the importance of conserving swarming sites to reduce inbreeding, and the possible consequences to climate change. They also support the idea of trans-boundary disease control strategies due to the migratory nature of bats.

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Does equine osteoarthritis have an inflammatory component?

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Osteoarthritis (OA) is a common age-related degenerative joint disease in humans, dogs and horses defined by deterioration of articular cartilage, in addition to changes in bone and soft tissues. Synovitis is a key contributor to joint degradation and plays a fundamental role in the pathophysiology of OA. A novel macroscopic synovitis grossing scoring system was devised for assessment of synovitis severity in the equine metacarpophalangeal joint using parameters such as degree of inflammation and spread of synovitis in different regions of interest (ROI). This was then correlated with associated scores found using the Kawcak Gross Osteoarthritis Scoring System, which quantifies the severity of OA, to determine if there is a correlation between the visible inflammatory component of synovitis and OA severity. Scoring was performed by two independent observers and conducted on images collected from an abattoir as part of the University of Liverpool Equine Musculoskeletal Biobank.

Abstract

OA is one of the most common and disabling skeletal chronic joint disorders affecting humans, horses and dogs causing lameness. Synovitis is observed in osteoarthritic joints and is a key contributor to the pathogenesis of OA through the formation of various catabolic, pro-inflammatory and anti-inflammatory mediators, which alter the balance between extracellular matrix (ECM) degradation and repair. There is no exact answer to the pathophysiology of OA; however, many studies have hinted at the role of synovial inflammation and chronic synovitis as either a primary or secondary change in the disease process of OA. The purpose of this study was to establish if there is a relationship between synovitis and OA. A novel macroscopic synovitis gross scoring system was devised for the purpose of this study for assessment of synovitis severity in the equine metacarpophalangeal joint which was correlation tested with associated scores found using the Kawcak Gross Scoring System. It was hypothesised there was a correlation between synovitis and OA severity. Scoring was performed by two independent observers conducted on images collected from an abattoir as part of the University of Liverpool Equine Musculoskeletal Biobank. Inter-rater reliability was calculated using Cohen's Kappa Score. No significant correlation was found between synovitis score and Kawcak Gross Score from both observers, r= 0.07 and r= 0.09. Kappa Cohen scoring measured a fair and moderate agreement; Kawcak Gross Scores = 0.38 and Synovitis Scores = 0.68.

Introduction

OA is a chronic age-related degenerative joint disease found in humans, horses and dogs characterized by loss of articular cartilage components, particularly type II collagen, and morphological changes in bone and soft tissues with fibrillation, fissures, ulceration and full thickness loss of joint surface (1). Trauma, sepsis, immobilisation, shoeing, conformation, age-related degeneration and osteochondrosis are some of the numerous aetiologies that may contribute to the development of the disease (2). Often the end result is pain, deformity and decreased function that contribute to the wastage of horses bringing huge economic losses to the equine industry (2).

OA most commonly affects synovial joints and is a key contributor to equine lameness. Synovial joints are composed of articular cartilage-covered articulating bone surfaces, secured by ligaments and a joint capsule filled with synovial fluid. In a healthy animal, remodeling of articular cartilage is balanced by anabolism; however, over time and/or after joint injury, breakdown of extracellular matrix (ECM) components outweighs new matrix synthesis leading to ECM degeneration (2). The disease processes affect the entire joint; synovial membrane, subchondral bone, capsule, ligaments and peri-articular tissues. It is an 'organ' disease of the joint. Rose *et al.* (3) survey found that 33% of all the equine patients had intra-articular lesions related to OA. Tew and Hackett reported that 35% of the 72 equine joints evaluated had evidence of visual cartilage damage (4). This demonstrates the severe impact of OA on synovial joints.

The exact role of the inflammatory component of synovitis in OA is undetermined; however, it has been demonstrated that synovitis is a major contributor to joint degradation and plays a role in the pathophysiology of OA in both humans and horses (5). Inflammation of the synovium is characterised by an infiltration of neutrophils, T lymphocytes, monocytes, in addition to vascularization and hyperplasia of the synovium (6). Blom *et al*. (7) reported that macrophage aggregation in synovium is essential for cartilage damage potentially leading to early OA in mice. Synoviocytes, as well as chondrocytes are the main cell types involved in the pathogenesis of OA implicating the role of synovitis in the disease process (6). Increased levels of pro-inflammatory cytokines produced by synoviocytes, IL-1β and TNF-α, have been found in synovial fluid of horses with OA when compared to unaffected joints supporting this theory (6).

The exact cause of OA is unknown; however, numerous studies have hinted at the possibility of synovitis being a primary or secondary process; linked to initiation and propagation of OA as it is induced by the innate immune system following the cartilage damage and may be essential for further OA development (6, 8). Sellam *et al*. (9)

	0 (healthy)	1 (mild)	2 (moderate)	3 (severe)
Degree of inflammation	Thin and transparent synovial membrane (SM), no inflammation seen	Light inflamed tissue with slight red hue	Darker inflamed tissue with a darker red hue	Bright red inflamed tissue
Degree of spread of synovitis	No synovitis seen in SM	Less than 50% seen with synovitis	Greater than 50% seen with synovitis	Synovial membrane completely covered and seen with synovitis

Table 1. Macroscopic Synovitis Scoring System. Scores were determined from the degree of inflammation and the degree of spread of synovitis with scores from 0; healthy to 3; severe synovitis.

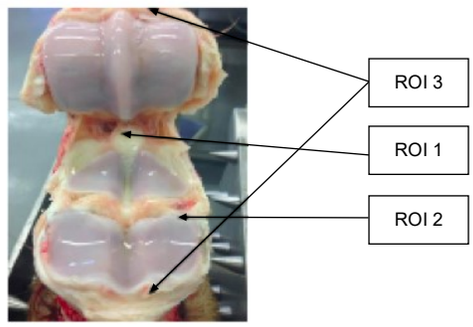

Figure 1. Equine metacarpophalangeal (MCP) joint with three regions of interest (ROI) labelled ROI 1, ROI 2 and ROI 3 on which synovitis scoring was conducted. This MCP joint enables flexion and extension articulating the third metacarpal, phalanx and sesamoid bones.

study on human osteoarthritic joints states that synovitis is a pivotal factor in the progression of the disease, despite not being a prerequisite for development of OA.

Several methods have been devised to quantify the severity of OA, both grossly and histologically (10). This study utilised the Kawcak Macroscopic Gross Scoring System using parameters such as wear lines, erosion and palmar arthrosis (10). Currently, there is no system to grade synovitis grossly from an image of a joint that is not taken following arthroscopy; therefore, a Synovitis Macroscopic Scoring System (Table 1) was developed for the purpose of this study.

This study's aim was to determine if there was a correlation between synovitis and gross OA score as measured by a Synovitis Scoring System created for this study and the Kawcak Macroscopic Scoring System applied to the equine MCP (fetlock) joint which is the articulation between the third metacarpal, proximal phalanx and proximal sesamoid bones enabling flexion and extension.

Methods

Sampling

Equine MCP joints were collected from an abattoir, as a by-product of the agricultural industry. The Animal (Scientific Procedures) Act 1986 Schedule 2 does not state collection from these sources as scientific procedures, thus ethical approval was not required for this research. These samples were whole MCP joints of 72 horses of varying breeds, gender and age. Ages of the horses were determined from their passport. The equine age average ± standard deviation was 12.94 ± 7.64 and the range was 3-35 years old.

Synovitis Scoring System

A Synovitis Scoring System was developed for the purpose of this research paper, due to the lack of current gross macroscopic synovitis scoring systems. McIlwraith *et al.* (1) microscopic synoviopathy grading system formed the basis of the grading criteria. This was then furthered through direct observation of differences in between the equine MCP joint photographs. Synovitis scoring was then chosen to be undertaken on three regions of interest (ROI) of the MCP joint (Fig. 1) (4). Grades ranging from healthy to severe were created for the following changes: degree of inflammation and degree of spread of synovitis. A cumulative score was then determined. For this scoring system, a higher score indicates a more severe synovitis.

Gross Scoring

Each horse's MCP joint was scored twice (on consecutive days) macroscopically using the Kawcak Gross Scoring System (10) and Synovitis Scoring System (Table 1). The average of the two scores of each of the system was used for data analysis. Each joint was scored twice by each rater to identify the reliability of the results.

Statistical Analysis

All statistical analysis was undertaken using GraphPad Prism Version 8 or Microsoft Excel. GraphPad Prism was used to create the scatter plots in Fig. 2 and 3. Microsoft Excel was used for data collection. Correlation analysis was performed using Pearson's Correlation Analysis. Cohen's Kappa score was calculated online which allows calculations of inter-rater reliability to be made (11). Cohen's Kappa scores are divided into degrees of agreement; 0.01-0.20 as none to slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as almost perfect agreement.

Results

Synovitis Scoring System

<u>Correlation analysis</u>

There was no significant correlation found between the Kawcak Gross Score and Synovitis Score by both raters; r= 0.09, *P*= 0.57 (Fig. 2A) and r= 0.07, *P*= 0.56 (Fig. 3A). A weak significant positive correlation between the Kawcak Gross Score and age was found; r= 0.32, *P*= 0.01 (Fig. 2B) and r= 0.35, *P*= 0.0004 (Fig. 3B). No significant correlation was found between Synovitis Score and age; r= -0.13, *P*= 0.35 (Fig. 2C) and r= -0.20, *P*= 0.14 (Fig. 3C).

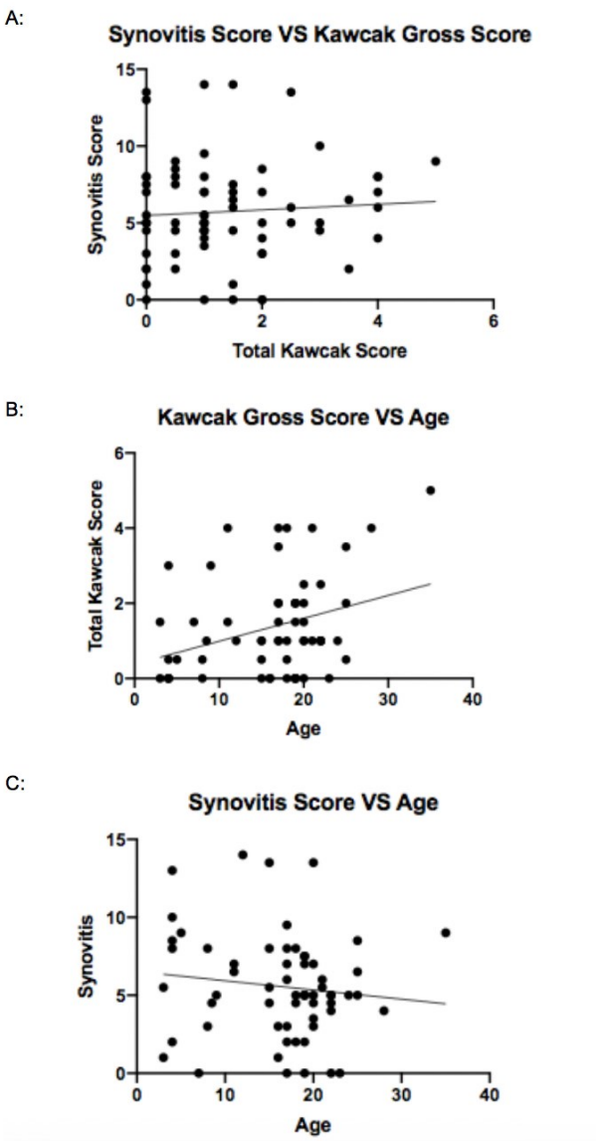

Figure 2. Scatter plots of scoring systems and age for rater 1. (A) Scatter plot of Synovitis and Kawcak Gross Scores; r= 0.094 and *P*= 0.57. (B) Scatter plot of Kawcak Gross Score and age (in years); r= 0.32 and *P*= 0.012. (C) Scatter plot of Synovitis Score and age; r= -0.13 and *P*= 0.35.

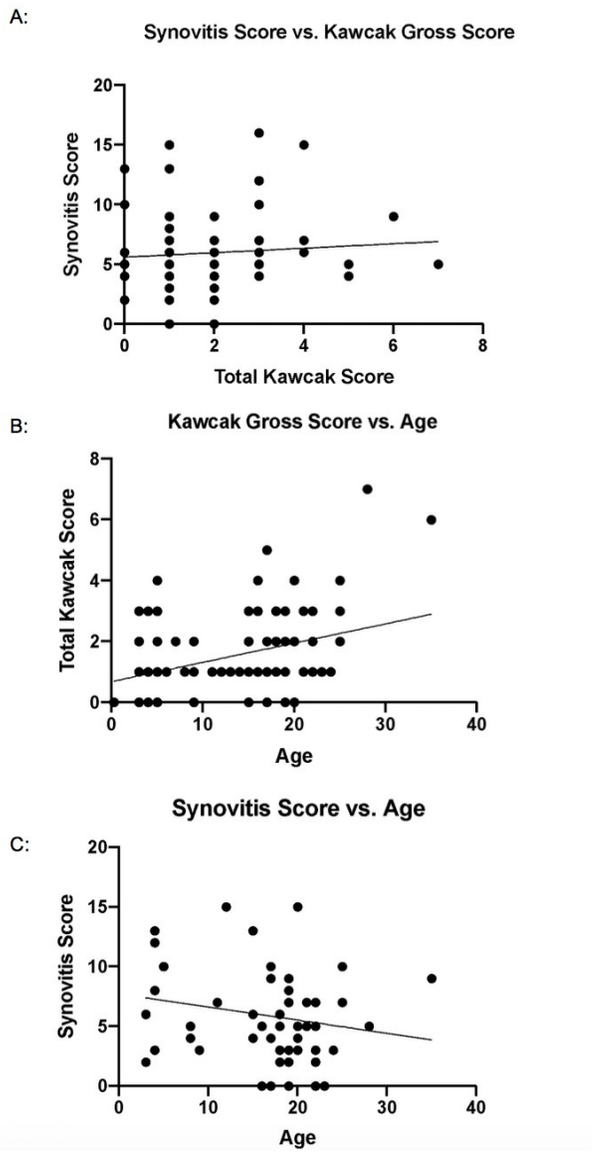

Figure 3. Scatter plots of scoring systems and age for rater 2. (A) Scatter plot of Synovitis and Kawcak Gross Scores; r= 0.07 and *P*= 0.56. (B) Scatter plot of Kawcak Gross Score and age (years); r= 0.35 and *P*= 0.00038. (C) Scatter plot of Synovitis Score and age; r= -0.20 and *P*= 0.14.

Cohen's Kappa Score

Calculation of the Cohen's Kappa Score was used to determine inter-rater scoring reliability. The Kappa values for inter-rater reliability of the scores provided fair (Kawcak Gross Scores = 0.38) and substantial agreement (Synovitis Scores = 0.68).

Discussion

This study was carried out to establish the relationship between gross synovitis and gross OA severity in the MCP joints of the equine biobanked samples. The severity of OA in these samples was principally low grade, early, age-related OA. Gross scoring was conducted on photographs of MCP joints from an abattoir, archived in the University of Liverpool Equine Musculoskeletal Biobank. Limited clinical and histological data was available on these horses, however for some horses details of age, gender and breed were known. It was hypothesised that there was an association between OA severity (as assessed by gross scoring) and synovitis.

There is a lack of studies within the literature investigating if there is an inflammatory component to equine OA. However, in human medicine, there have been several studies concluding that synovitis is an independent cause of OA; a higher synovitis score increases the risk of incidence of OA (13). In horses there are other potential reasons for synovial membrane inflammation such as navel ill in foals, sepsis and other infections. There have been many other studies researching the relationship between OA and increasing age that have found similar results (14).

The results demonstrated that there was no correlation (r-value less than 0.30) between both the Kawcak Gross Score and Synovitis Score and between Synovitis Score and age. However, there was a significant correlation (r-value more than 0.30) between Kawcak Gross Score and

age (15). This is often described as age-related OA and is a phenomenon that has been researched and observed previously in both human, canine and equine literature (14, 16).

There are a number of limitations to this study. There may have been potential errors introduced during data collection, as it was undertaken using digitalised photos. There are many variables that could have impacted on the quality of the photos, some of which include lighting and the angle at which the photos were taken. This may have inaccurately depicted the extent of macroscopic and synovitis changes in the MCP joint of horses. There was variability in the quality of the images, making it difficult to score some of them. This may have affected the credibility of the data, as unclear photo samples had to be discounted from the overall data analysis. Additionally, the quality of the photos displayed for each of the two raters may have varied depending on the device they have used to view the images. This may have contributed to reducing the agreement between the raters, especially that the scoring system was subjective.

A major limitation to this study is that all the samples were collected from an abattoir. This meant that there was a lack of clinical records for the populations of horses studied. It was unknown whether the donors were lame or had previous clinical complications affecting the state of their MCP joints, which may have created a bias towards the results collected. Since these were abattoir samples the OA changes were most likely to be low grade and predominantly age related potentially skewing the data. Carrying out another study with racehorses using the same methodology could potentially obtain data that is a better representation of a wider range of OA, as then the levels of OA should be more varied across the whole population.

For the future, it would be much more helpful if the photos were all taken using a set format and approach; making sure that the photos are in good quality and all ROIs are clearly visible. Additional studies such as histology and further parameters such as hypertrophy and hyperplasia could be included in the scoring systems making the results stronger in credibility.

Conclusion

In summary, this study demonstrated that there was no correlation between both the Kawcak Gross Score and Synovitis Score and between Synovitis Score and age. However, it was clear that the macroscopic changes of OA in the MCP joint increases with age. This study would benefit from clinical history, signs and larger variation in severity of OA.

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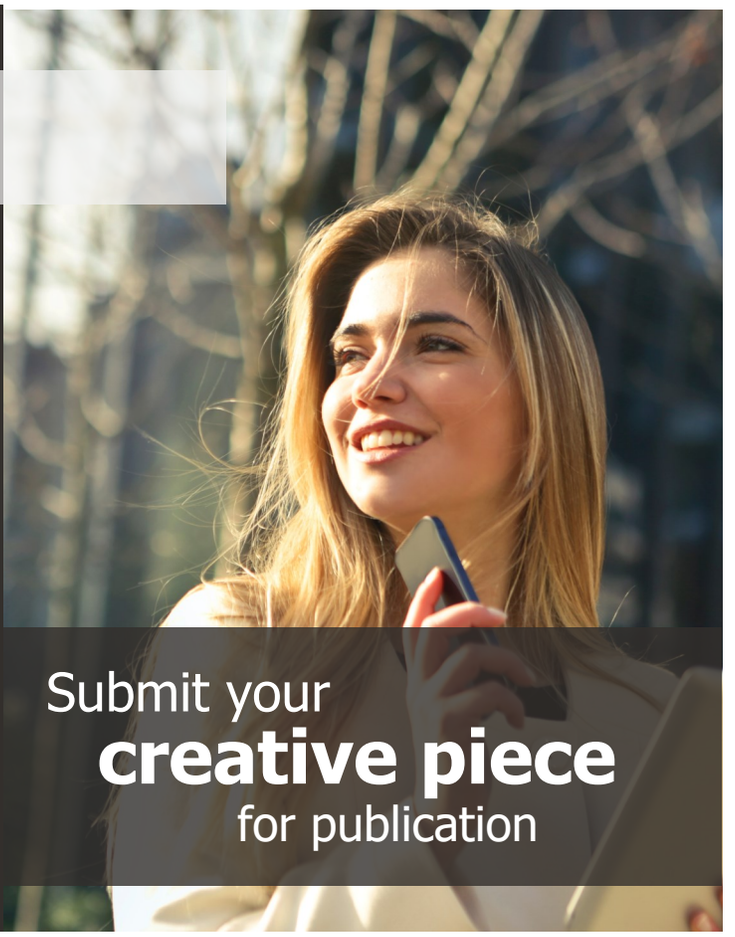

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Investigating genetic adaptations in pneumococcal bacteria which allow the bacterium to cause pneumonia and other invasive diseases

Isobel Maclean

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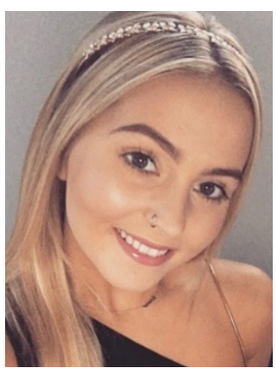
Pneumococcus is a bacterium which colonises the nose and throat of humans, mostly living harmlessly causing no symptoms of disease. Despite this, pneumococcus is the biggest cause of pneumonia worldwide, the most common cause of death in young children. Some strains of pneumococcus can infect sterile parts of the body, such as the blood or the brain, leading to serious diseases like sepsis and meningitis.

There are currently two vaccines available for pneumococcal infection, given to those most vulnerable; the elderly and young children. There are lots of types of pneumococcus, with great genetic diversity, due to a high mutation rate which allows the development of beneficial adaptations. Pneumococcus is also able to transfer small portions of DNA between bacteria in a process called horizontal gene transfer. Having lots of different types of pneumococcus means that neither of the vaccines protect against all pneumococcal types, current vaccines are not effective enough to prevent the burden of pneumonia.

The aim of this project is to identify what genetic adaptations allow certain strains of pneumococcus to cause pneumonia in the host. I will do this by growing pneumococcus in the lab, comparing harmless strains of the bacteria with disease-causing strains. This will allow identification of the genes that allow development of pneumonia in some hosts. This project will help us to understand what it is that allows pneumococcus to cause disease and could lead to developing a more effective vaccine, by targeting bacterial proteins that are essential for the pathogen to cause disease.



Understanding the mechanisms of cardiovascular diseases involving amyloid protein aggregation

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Proteins can enter an “amyloid state”, consisting of elongated fibres with β-pleated sheets; amyloid fibrils form due to the aggregation of misfolded proteins. When the cause of a disease involves amyloid proteins they are often, but not always, known as “amyloidosis” diseases; these can be systematic, affecting the whole body, or localised pathologies, affecting only specific tissues.

Research into the role of amyloid proteins in Prion disease and Alzheimer’s disease is in abundance and the mechanism is generally well understood. However, few discoveries have been made into the role of amyloid proteins in cardiac disease. Cardiac amyloidosis is most commonly characterised by the thickening of the walls of the heart, disrupting the architecture of the cardiac tissue. Ultimately, cardiac amyloidosis results in cardiac dysfunction and failure. Usually, multiple proteins are found to be aggregated in amyloidosis diseases which, based on the Prion hypothesis, is thought to reduce the time taken for amyloid fibril formation and therefore affect further aggregation of proteins.

This project will investigate four proteins found aggregated in the cardiovascular system - medin, apolipoprotein AI, transthyretin and light chains. To do this we will perform a cross-seeding experiment, which involves introducing a misfolded form of one protein to a different normally folded protein and determining if the time taken for amyloid fibrils to form has reduced. With this research, the mechanisms of cardiac amyloidosis will hopefully be elucidated allowing for the identification of potential therapeutic targets for the disease.

Current research

Snippets on research students are currently undertaking

Find out what some students are currently working on, and get inspiration for your own research project.

Potential new source of Antimicrobials

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The prevalence of bacterial resistance to antibiotics and antimicrobial compounds has increased dramatically in recent years. Ten million deaths are predicted to be attributed to infections with these un-treatable bacteria by 2050 (1). Yet, resistance development is a natural phenomenon as bacteria that produce antibiotic compounds must be able to survive their exposure. However, there are massive selective pressures applied to microorganisms through practices like industrial animal husbandry and over-prescription of antibiotics (2). This provides the impetus for these natural resistance genes to spread throughout microbial populations, resulting in multiple-drug resistant (MDR) pathogens, such as the MDR strains of Pseudomonas aeruginosa found causing infections in hospitals and in people with chronic lung diseases.

Current strategies are being rendered insufficient to treat these infections. Hence, there is a clear necessity to restrict the further spread of resistance and prevent the forecasted deaths. Change to global perceptions of the usage of these antimicrobials alongside the promotion of research and development into novel antimicrobial compounds is required. One emerging strategy is the use of potentiator compounds, which inhibit the cellular mechanisms that allow microorganisms to resist antimicrobials. Thus, rendering existing antibiotics more effective.

This research project aims to identify a novel class of antimicrobial drug or potentiator from a large drug compound library. Identification will involve screening compounds for activity against an epidemic strain of P. aeruginosa, in the presence or absence of antibiotics. If successful, compounds will be further tested to assess their toxicity and potential use as a therapeutic drug.

Acknowledgment

This work is based on a portion of Frédi Langendonk's PhD work.

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Yes we CAM:
The Crassulacean Acid Metabolism

Tracey Houghton

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The world's population is expanding rapidly and expected to exceed 9 billion by 2050. Agriculture is therefore under pressure to meet the growing demand for food, particularly in the light of global climate change. One proposed solution is to use crops that utilise Crassulacean acid metabolism (CAM). This photosynthetic adaption improves the efficiency of both CO_2 fixation and water use compared to C3 photosynthesis, which is the type most widely used by major food crops. Bioengineering CAM into C3 crops has the potential to protect crop yields in a changing world.

However, achieving this is impeded by incomplete understanding of the adaptations of primary metabolic pathways and stomatal control associated with CAM. Particular knowledge gaps centre on the interface between the core circadian clock and temporal optimisation of the CAM mechanism, and on the mechanisms underlying stomatal opening in the dark and closure in the light.

In my research project, bioinformatic techniques will be used to interrogate genome sequence and quantitative RNA-seq datasets obtained from the leaves of *K. fedtschenkoi*, a CAM plant. We hypothesise that the stomatal guard cell signalling genes that are important for the stomatal regulation during CAM will display temporal patterns of regulation over the 24 h cycle that are the opposite of the temporal cycles in the C3 model species *A. thaliana*. Many of these have been identified in *A. thaliana*, but the temporal regulation and functions of the orthologous genes in *K. fedtschenkoi* leaves during CAM remain largely unknown.

Insights

What are your peers thinking about?

In-depth reviews of some really interesting topics, learn about something new!

The Mechanism and Management of Carbamazepine-Induced Hepatotoxicity

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Carbamazepine (CBZ) is a frequently prescribed antiepileptic drug (AED), used in the treatment of epilepsy, neuropathic pain and psychiatric disorders. CBZ was the 176th most commonly prescribed medication in 2017 across the United States, with a total of 3,516,204 prescriptions written that year. CBZ is predominantly metabolised hepatically, subsequently increasing the risk of a CBZ-induced liver injury or CBZ-induced hepatotoxicity; with hepatotoxicity being defined as drug induced liver damage. Deviation beyond the therapeutic range of CBZ is consistent with toxicity, which combined with abnormal liver function tests, would be indicative of CBZ-induced hepatotoxicity. The liver is the leading organ for the maintenance of the body's internal environment, therefore obstruction of the liver's ability to conduct its regular function can carry a number of consequences. With a large number of patients receiving CBZ therapy worldwide, it is of absolute importance to understand the best clinical approach to the treatment of CBZ-induced hepatotoxicity. There have been a number of studies reviewing the type of liver damage that occurs in cases of hepatotoxicity, classified as either a hypersensitivity reaction or acute hepatitis, and how different methods of treatment specific to CBZ-induced hepatoxicity directly correlate with a successful outcome. Treatment of CBZ-induced hepatotoxicity can consist of recording serum levels of the drug whilst administering intravenous fluids and continuing CBZ therapy. A different approach would be that of primary gut decontamination with activated charcoal which has proven to be very effective, whilst various means of dialysis have been considered to have a limited ability to remove CBZ from the blood serum alone. This review will assess the mechanism of CBZ-induced hepatotoxicity alongside the most effective clinical management.

Introduction

Carbamazepine (CBZ) is an antiepileptic drug (AED) that is used in the treatment of several types of epilepsy and in the management of intermittent severe pain in trigeminal neuralgia. CBZ can also be used to stabilise mood in bipolar disorder, to reduce urine output in diabetes insipidus and to relieve pain in patients presenting with diabetic neuropathy (1). From a pharmacological perspective, CBZ is chemically related to tricyclic antidepressants, such as amitriptyline, and differs in its structure to other anticonvulsants. CBZ was the 176th most commonly prescribed medication in 2017 across the United States, with a total of 3,516,204 prescriptions written that year (2). The majority of patients receiving CBZ therapy will experience very few adverse drug reactions (ADRs), however the incidence of ADRs becomes more common with increased CBZ serum levels (1). CBZ hypersensitivity affects ~10% of patients receiving the therapy, whilst antiepileptic hypersensitivity syndrome affects ~1 in 5000 people taking CBZ or phenytoin (3). CBZ is almost entirely metabolised in the liver with only approximately 5% of the drug excreted in its original unchanged form. CBZ is a well-known cause of clinically apparent liver injury which can range in severity from acute to fatal (4). It is also an established cause of hepatotoxicity; the capability of a drug or chemical to produce toxic damage to the liver (5).

This review article will focus on the incidence of CBZ-induced hepatotoxicity in epileptic patients taking CBZ to manage focal seizures with and without secondary generalised seizures or to manage primary generalised tonic-clonic seizures (4). Various studies into CBZ-induced hepatotoxicity are indicative of a vast proportion of patients experiencing transient serum aminotransferase elevations (ranging from 1 to 22%), these elevations are typically benign and not correlated with any form of hepatic histological abnormality and as such will frequently resolve despite the continuation of CBZ therapy (7). Clinically apparent CBZ-induced hepatoxicity, although uncommon, is well described. CBZ hepatotoxicity typically occurs in the jurisdiction of anticonvulsant hypersensitivity syndrome with the onset of a fever, typically followed by a rash, facial oedema, lymphadenopathy, an elevated white blood cell (WBC) count as well as eosinophilia or atypical lymphocytosis; this frequently occurs 1 to 8 weeks after commencing CBZ therapy (7). This hypersensitivity syndrome is often referred to by the acronym DRESS (Drug Rash with Eosinophilia and Systemic Symptoms), with the most persistent form of systemic involvement being that of liver injury (7). The hepatic involvement varies from mild or transient elevations in the patient's serum enzymes, to the abrupt onset of an acute hepatitis-

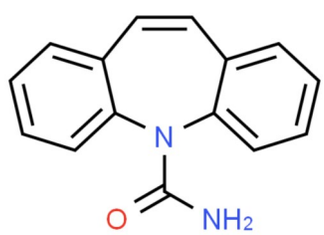

Figure 1. The molecular structure of CBZ, an aromatic anticonvulsant with a molecular formula of $C_{15}H_{12}N_{2O}$ and an average molecular mass of 236.269 Da (6).

like syndrome that can be severe and even fatal. As a result of this, CBZ is a commonly listed agent in cases of acute liver failure (8).

Mechanism of Drug Action

Antiepileptic drugs are pleiotropic; they have multiple effects on different ion channels (9). The mechanism of action of CBZ is not entirely understood. It is thought to inhibit neuronal sodium channels, stabilise resting membrane potentials and reduce neuronal excitability (3). CBZ prolongs the inactivated state of the sodium channel, halting action potential propagation. As a result, sodium channel inhibitors such as CBZ show a degree of specificity for the treatment of partial and secondary generalised seizure activity (9). Ultimately this can inhibit the spread of seizure activity within the brain of epileptic patients or block synaptic transmission in the trigeminal nucleus to control neuropathic pain, such as that present in patients with trigeminal neuralgia (3).

Mechanisms of Hepatic Injury

The mechanism of CBZ-induced hepatotoxicity typically occurs in two forms; a hypersensitivity reaction in the form of granulomatous hepatitis that presents clinically with a fever and abnormal liver function tests (LFT) or an acute hepatitis and hepatocellular necrosis accompanied by fever, rash, hepatitis and lymphadenopathy simulating a biliary tract infection (10). The association of liver injury with the human leukocyte antigen (HLA) haplotypes, a gene complex that encodes the major histocompatibility complex (HMC) proteins in humans, hasn't been as well demonstrated as the link between CBZ associated severe cutaneous ADRs, such as that of Stevens-Johnson Syndrome with HLA-B*1502 across Southeast Asian populations (5). Carbamazepine is highly bound to plasma proteins in patients. Metabolism is believed to play a fundamental role in the pathogenesis of CBZ hypersensitivity and hepatotoxicity. Although the mechanism of toxicity is poorly understood, it had been inferred that reactive metabolites are generally accepted as the causal agents of hepatic injury. The major route of metabolism is primarily via CYP450 oxidation, producing a pharmacologically active metabolite CBZ 10,11-epoxide. Following this, CBZ 10,11-epoxide is further metabolised by epoxide hydrolase 1; however, this is not the major metabolite. Several different variations of reactive metabolites have been postulated; the production of these reactive metabolites is dependent on oxidative metabolism by cytochrome P450 (CYP) enzymes (11). Similarly, minor metabolic pathways look at ring-hydroxylation producing 2-hydroxy-CBZ (2-OH-CBZ) as well as 3-hydroxy CBZ (3-OH -CBZ), with the production of each likely to be proceeded by an arene oxide (epoxide) intermediate.

Diagnosis of Carbamazepine-Induced Hepatotoxicity

The diagnosis of CBZ-induced hepatotoxicity is vastly a clinical diagnosis. As such, both the onset and offset of hepatic injury evidenced by the patient's liver function tests (LFTs), are fundamental factors to take into consideration when contemplating the diagnosis. Liver enzymes can serve as markers of hepatocellular injury e.g. aspartate aminotransferase (AST) with a reference range of 10 – 55 and alanine aminotransferase (ALT) with a reference range of 10 – 40 U/L (12).

Prospective studies are indicative of a sizeable proportion of patients taking CBZ displaying transient serum aminotransferase elevations (ranging from 1% to 22%); these are typically benign in nature and will resolve despite drug continuation (7). Additionally, most patients on CBZ therapy will develop mild-to-moderate elevations in gamma glutamyltranspeptidase (GGT) levels, that are likely indicative of hepatic enzyme induction as opposed to liver injury. However, a marked increase in aminotransferase (> 5-fold increase) occur less frequently and are indicative of hepatotoxicity or an alternative type of liver injury (7).

There are no specific diagnostic laboratory tests to identify CBZ-induced hepatotoxicity (8), however if CBZ toxicity is suspected, serum concentration will be measured, alongside the LFTs, and compared to that of the therapeutic reference range of CBZ which ranges from 4-12 mg/L. When doing so, blood should be taken immediately prior to the next dose when the serum level should be within the therapeutic range. Serial CBZ levels must be repeated every 4 hours. From a toxicokinetic viewpoint, the majority of CBZ will remain bound to plasma protein due to the high protein binding affinity, the drug will also enter the bloodstream from tissue stores. CBZ toxicity can be classified into three categories:

1. Disorientation and ataxia at serum levels of 11 - 15 mg/L;
2. Aggression and hallucinations at serum levels of 15 - 25 mg/L;
3. Seizures and coma at serum levels above 25 mg/L;
    a. A serum CBZ concentration of 40 mg/L is typically fatal even with clinical intervention.

CBZ is eliminated with a half-life of about 30 hours after the initial dosage; typically inducing the cytochrome P450 enzyme for subsequent doses, with increased elimination (13). Patients presenting with CBZ-induced hepatotoxicity may present with a variety of symptoms, subject to the severity of ingestion or toxicity. Mild toxicity may present with vomiting, ataxia, slurred speech, drowsiness, nystagmus, hallucinations and dystonic reactions or repetitive muscle contraction. Whilst severe toxicity may result in coma, seizures, hypotension and respiratory depression, potentially resulting in respiratory arrest (14). A number of biochemical indicators can be used in the diagnosis of hepatoxicity subject to whether the predicted level present is either abnormally high or low, indicating possible hepatotoxicity; these indicators are explored in Fig. 1.

Treatment of Carbamazepine-Induced Hepatotoxicity

There is no clear line of treatment for the management of CBZ-induced hepatotoxicity. The treatment protocol followed typically varies subject to the treating Trust's own protocols and the leading clinician's degree of experience or the country in which the patient is being treated. In

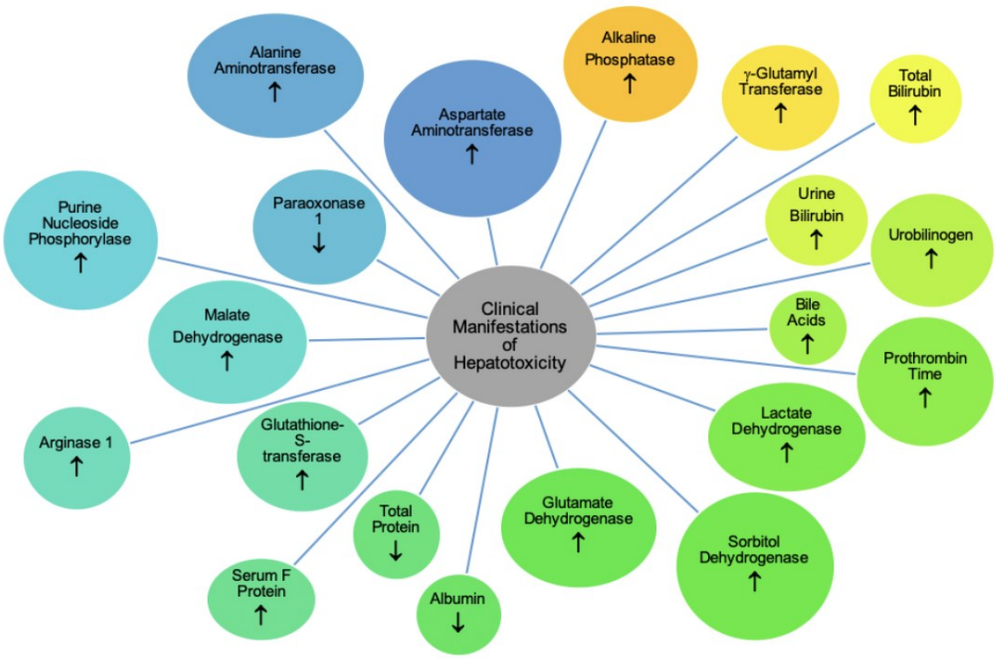

Figure 2. The clinical-biochemical indicators of hepatotoxicity in patients presenting with hepatotoxicity as a result of a drug overload or xenobiotics. Where ↑ indicates an increased value during hepatotoxicity whilst ↓ indicates a decreased value during hepatoxicity. Adapted from (15).

patient's presenting with CBZ toxicity, treatment can consist of monitoring serum levels of the drug alongside supportive care with the administration of intravenous fluids to manage hypotension whilst diluting the plasma concentration of the drug present in the blood. This line of treatment also typically incorporates the continuation of benzodiazepine treatment to manage epileptic seizure activity (16). Patients should have serial CBZ serum levels taken every 4 hours to monitor toxicity, with particular focus on a serum level taken immediately prior to the administration of the next dosage of CBZ; as this is when it is most likely for the levels to be back within the therapeutic range (14). An alternative line of treatment in CBZ-induced hepatotoxicity is that of primary gut decontamination with activated charcoal (17). There is a preference for this line of treatment as charcoal adsorption decreases CBZ enteral absorption subsequently reducing its plasma half-life. Gastrointestinal (GI) absorption of CBZ is unpredictable in nature and peak plasma concentration can be observed from 2-18 hours after a single dose has been administered. The plasma half-life ranges from 8-19 hours in continually treated, known epileptic patients (17). This half-life range is significantly lower than that of normal volunteers who possess a predicted half-life value of 21 to 55 hours (17). The increased plasma protein binding of CBZ is thought to be around 70-80%, and is fundamentally the reason as to why haemodialysis, peritoneal dialysis and alkaline diuresis are believed to have a very restricted ability in removing CBZ from the blood plasma, and subsequently treating the toxicity (18). In the case of an adult female presenting to medics having overdosed on CBZ; LFTs, routine bloods, GCS monitoring, ECG and routine observations were ordered. The leading clinicians on this case declared the management of the toxicity successful by combining intravenous fluid therapy, activated charcoal and haemodialysis with one another (19). A haemodialysis catheter was attached to the right femoral artery and two consecutive rounds of haemodialysis were conducted; due to the absence of carbon hemoperfusion. The CBZ serum levels were measured throughout, via high-pressure liquid chromatography. Prior to haemodialysis the serum level of CBZ measured at 57.7 µg/ml, following haemodialysis the serum level of CBZ was 28.9 µg/ml. By day three the patient was conscious and maintaining a GCS of 15, a significant improvement on the GCS 7 the patient were on presentation to the department; the patient's CBZ serum level had also dropped to 6.8 µg/ml (19). Fluid therapy earlier in the treatment protocol alongside activated charcoal and haemodialysis were administered in conjunction to encourage drug elimination. Due to the high mortality of CBZ toxicity, fluid therapy alongside activated charcoal treatment must be started as a matter of urgency, with haemodialysis being performed even in the absence of carbon hemoperfusion (19).

Discussion

Due to the frequency of prescription and subsequent administration of CBZ, an in-depth understanding of the pharmacokinetics and potential for hepatotoxicity is imperative for the improvement of the care of patients receiving CBZ therapy. Having reviewed the literature available around this subject, several fundamental conclusions can be drawn:

1. The routine monitoring of liver function in asymptomatic patients is unlikely to be of any merit as elevations in LFTs are to be expected whilst on

CBZ, but are typically not of concern until the patient is symptomatic;

2. Baseline testing of liver function and liver enzymes prior to the commencement of CBZ therapy would be of benefit to establish a comparative point, should the patient later become symptomatic;
3. CBZ should not be advised in the treatment of patients with a known history of liver disease or hepatic injury, unless it is the only viable course of treatment, as elevations in LFTs and liver enzymes are to be expected following the commencement of CBZ therapy.

CBZ hepatotoxicity is typically associated with elevated serum levels of CBZ in the blood. Elevated serum levels are those beyond the therapeutic range, which is 4-12 mg/L for CBZ. Symptoms of hepatotoxicity vary with the extent of CBZ toxicity in the blood plasma. Patients will present with predominantly CNS symptoms including ataxia, nystagmus, convulsions, myoclonus, coma and ophthalmoplegia. Other symptoms can include sinus tachycardia, hyperthermia, respiratory depression and even respiratory arrest. CBZ-induced hepatotoxicity is a known cause of death, with a 13% mortality rate observed in a study conducted on 307 patients presenting with CBZ toxicity (20). As a result of this relatively high incidence of mortality, it is vital that intravenous fluid therapy and activated charcoal treatment must be commenced urgently on admission by the clinicians responsible for the patient. Haemodialysis must then be subsequently implemented even in the absence of carbon hemoperfusion, in order to reduce the extent of toxicity present in the bloodstream and provide the patient with the best possible chance of survival (19).

Conclusion

With CBZ being the 176th most commonly prescribed medication in 2017 across the US, with a total of 3,516,204 prescriptions written that year (2), alongside the statistical knowledge that ~10% of patients receiving this therapy experience CBZ hypersensitivity, whilst antiepileptic hypersensitivity syndrome affects ~1 in 5000 people taking CBZ or phenytoin (3); the need for an awareness of identifying when and how to best treat patients experiencing CBZ-induced hepatotoxicity is truly emphasised. However, when it comes to CBZ-hepatotoxicity there is no specific antidote or designated treatment protocol; multiple dose activated charcoal or haemodialysis are the main lines of treatment but are often used in competition as opposed to in collaboration; which, as mentioned beforehand, is known to be an effective course of treatment for patients experiencing toxicity. Deaths as a result of CBZ hepatotoxicity have been reported, with a 13% mortality rate noted in a study of 307 patients presenting with CBZ hepatotoxicity; further emphasising the need for routine serum monitoring whilst being treated with CBZ therapy as well as the need for an established treatment protocol should toxicity occur (20).

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Anti-Angiogenic Therapy

A commentary on a promising cancer treatment

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The involvement of Vascular Endothelial Growth Factors (VEGFs) in tumour angiogenesis has been a well-known contributor to cancer metastasis (generally stage 4 or advanced stage) (1). As such, the development of anti-angiogenic therapies (AAT) has been of great interest to the many UK based pharmaceutical companies, notably Pfizer and AstraZeneca. This is due to the fact that, in essence, the inhibition of the VEGF pathway could provide treatment for various oncological cases by prevention of normoxic conditions around cancerous cells (2). This therapy can either be direct as a monotherapy, or in combination with traditional chemotherapy and radiotherapy.

VEGFR subtypes and mechanism of action

Angiogenesis is broadly split into two categories: hemangiogenic - which involves the generation of blood vessels, and lymphangiogenic - which concerns lymphatic vessel formation (3). VEGF is a collective term for a collection of ligands that bind to VEGF receptors (VEGFRs) and promote the production of new vasculature, around a tumour core. VEGFRs usually operate as a Receptor Tyrosine Kinases, of which VEGFRs have three types: VEGFR-1, 2 and 3- differing on the specific VEGF ligand that binds (Fig. 1). Fig.1A is involved only in lymphangiogenic, whereas B and C are essential for hemangiogenesis.

The specific signal transduction mechanism for VEGFR involves the auto phosphorylation of intracellular tyrosine domains, activation of phospholipase c-γ and the subsequent increased activity of Akt and ERK (second messengers involved in proliferative signalling) leading to cell proliferation and, ultimately, angiogenesis (5). Later intravasation of the carcinogenic mass into the new vasculature may lead to secondary deposits in other tissues and organs as the tumour metastasises (6).

An important factor to identify when considering cancer-associated angiogenesis is the greater permeability of the new vasculature due to the lack of smooth muscle surrounding the vessel (7). This allows for greater ease of intravasation of the cancerous deposit, allowing for the tumour to metastasise more readily.

Current drugs, research and novel therapies

Extensive research is underway in the area of VEGF/VEGF-R inhibition. One of the most promising involves the production of monoclonal antibodies against the VEGFR molecules, terminating downstream signalling events that are essential to angiogenesis (8).

As for drugs that are currently available on the market, the most well-known is 'Sunitinib' (Sutent™ produced by Pfizer) (9) which acts as an RTK inhibitor. This has shown to be a successful treatment against renal cell carcinoma, as well as imatinib (Gleevec) for resistant gastrointestinal stromal tumours (9). The humanised antibody bevacizumab monotherapy has generally been shown to have little effect on solid tumour regression (11).

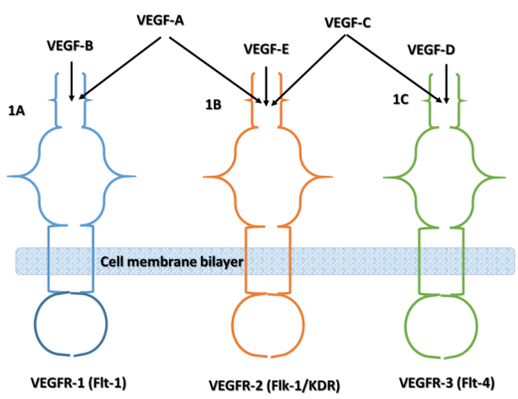

Figure 1. The range of VEGF subtypes and their associated receptors (4).

A novel targeted therapy undergoing research is against the NRP-1 (Neuropilin 1) molecule which has shown to increase vascularisation, when associated with VEGF-A, of prostate tumours. Antibodies against NRP-1 have been shown to decrease metastasis of gastric carcinogenic cells - the mode of action involved dephosphorylation of Akt (12).

The development of resistance to anti-VEGF agents

Despite the promising theoretical nature of anti-angiogenic treatment (AAT), this idealised view has been stunted by the rise of resistance (13). This is thought to be linked to activation of other signalling pathways - not pertaining to that of VEGF. These factors that are involved in escaping anti-VEGF agents include, but are not limited to, Ang2, Bv8, FGF and IL-1 (Fig. 2) (14).

A novel method providing a mechanism for anti-VEGF resistance in vascular mimicry (VM) (Fig. 2) when tumours acquire traits of endothelial cells, allowing them to adjust to increased metabolic demand of the neoplasia (16), due to the hypoxia that anti-VEGF agents induce. Despite this,

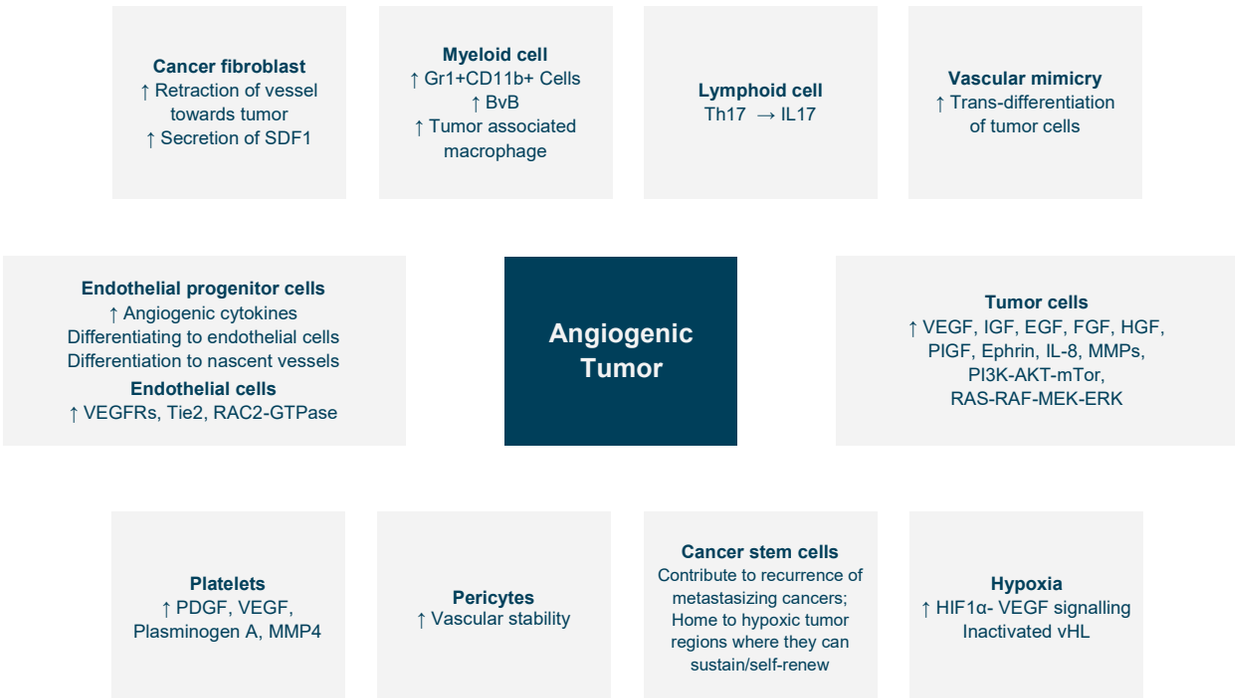

Figure 2. The multi-factorial nature of anti-angiogenic therapy resistance. Adapted from (15).

the discovery of VM, and the protein signalling cascade(s), associated with it provide potential novel targets for further chemotherapeutic development.

Other mechanisms of resistance come from complex signalling cascades, one ligand associated with angiogenic tumours is that of PDGF (Platelet Derived Growth Factor) (Fig. 2), which is upregulated in the presence of a tumour core (17). Thus, targeting this angiogenesis stimulating pathway could provide further areas to prevent tumour growth, migration and metastasis (18).

What does the future hold for VEGF targeting?

The most exciting future prospect for anti-VEGF agents is the potential for combination with immunotherapy. In this, the known immunosuppressive effects of VEGF can be counteracted by the presence of anti-angiogenic therapy (19). This implied synergy would overcome the issue of the low efficacy associated with monotherapy. Further implications of hypoxia on the VEGF cascade (Fig. 2) are also being studied in order to find even more novel therapies related to tumour migration and metastasis inhibition. For example, it is now understood that VEGF has an important role in stimulating Hypoxia Inducible Factor (HIF) (20), a class of transcription factors activated during hypoxia that are associated with transcription of cancer-associated genes for angiogenesis and proliferation (21).

Conclusion

In summary, the effectiveness of anti-angiogenic therapies through VEGFR targeting is a contentious argument. With strengths in various tumour types, but drawbacks when compared to other chemotherapeutic agents such as traditional Cisplatin and when AATs immunosuppressive side-effects are considered. Despite this, it is clear further research must be done, particularly into the pathway of VEGF and the signalling cascades associated with resistance to AAT. The combination of anti-VEGF agents with immunotherapy provides optimistic targets for cancer treatment in the future, especially when the synergistic effects and novelty are considered-,potentially providing a solution to the huge problems facing anti-cancer therapy.

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CRISPR vs Prime Editing

Is it the end of an era for CRISPR-mediated genetic manipulation?

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In the era of genomics, it is almost certain we will be editing our genomes in the near future, but the question is how? And what are the consequences? Knowledge of human genetics has expanded rapidly since the complete human genome was published in 2003 (1), leading to increased interest and investment in further research. One of the most astonishing achievements in genetic research was the understanding and use of the CRISPR/Cas9 system to edit DNA sequences. However, experiments using this technique encountered many downfalls including unpredicted changes to random, untargeted DNA sequence, including the introduction of stop codons (2). Several improvements have been made to CRISPR/Cas9 over time but a new candidate technique, prime editing, published in October 2019 (3) could steal the spotlight. Prime editing claims to be a more precise gene editing technique, improving on some of CRISPR/Cas9's limitations and having a safer mechanism of action. This review deciphers the mechanisms of both CRISPR/Cas9 and prime editing. The review aims to broaden the understanding of how these gene editing techniques work and why they can be subjected to off target effects. Upon review, prime editing is less error prone, most notably with the elimination of double-strand breaks (DSBs). Though, CRISPR/Cas9 has its limitations, it is still more reliable than prime editing, primarily due to the strong evidence-based research collected over time. With the same attention and experimentation, prime editing can certainly surpass the expectations of CRISPR/Cas9 in performing small, precise edits. In conclusion, CRISPR/Cas9 could be useful for larger, less precise edits to the genome. Therefore, although there are major implications on scientific advancement, the system could be adapted and utilised more in an experimental setting using model organisms. Whereas prime editing appears to be the most suitable genome editing method for use in the human population, surpassing the accuracy capabilities of CRISPR/Cas9.

Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) along with the enzyme Cas9 were originally found to be a natural defence mechanism in bacteria. The use of CRISPR and its associated Cas9 were found to be a powerful tool for editing genomes in 2012 (4). Since, CRISPR/Cas9 has been subject to a major upheaval in scientific research, particularly biomedical, where it has been shown to cure mice of genetic disorders (5). This included correcting common cystic fibrosis (CF) causing mutations leading to restored function of the CF transmembrane conductor receptor (5). Although more recently, it has been found that despite CRISPR/Cas9 being more precise than past gene editing techniques, so called 'off-target effects' are becoming an increasingly acknowledged issue (2). Other current limitations that have slowed clinical development of CRISPR/Cas9 in its tracks include; activation of the immune system, lack of targeting specific tissues or cells and the inability to enter cells in the first place (6). While the development of CRISPR/Cas9 has potential applications for eradicating genetic disease, it is questionable whether it can be approved for human use unless these impediments are addressed. These problems have led to research into alternative, more precise, gene editing techniques with more potential for human application. In October 2019, an article was published detailing a new kind of genome editing named 'prime editing' (3). The creators of prime editing claim that it could correct up to 89% of known genetic faults associated with human diseases, and can work similarly to CRISPR/Cas9, but with fewer detrimental effects (7). This review aims to evaluate both CRISPR/Cas9 and prime editing to determine which provides a safer and more accurate approach to gene editing. The discussion aims to reflect on which method has the most potential for continued development and research when considering biomedical applications.

The history of CRISPR/Cas9

Unexpected repetitive sequences later termed CRISPR identified in many bacteria and archaea were found to be strikingly similar to those in bacteriophages. First discovered in the 90's, the sequences were named CRISPR by the founder Francisco Mojica and colleagues in 2002, when CRISPR was first mentioned in scientific literature (8). The similarity to bacteriophage sequences led to the understanding that CRISPR were part of an immune response to past viral attacks and served to identify and destroy the DNA of similar invaders (9). After a viral attack, spacers (sections of DNA interspersed along repeat sequences) were found to be incorporated into the CRISPR region. When these spacers were manipulated to include specific viral DNA it was found that there was resistance upon infection with the same virus. These findings confirmed that CRISPR sequences were important for regulating bacterial immunity (4). The specific DNA sequences stored are transcribed into RNA, and when the CRISPR RNA (crRNA) binds to a complementary target

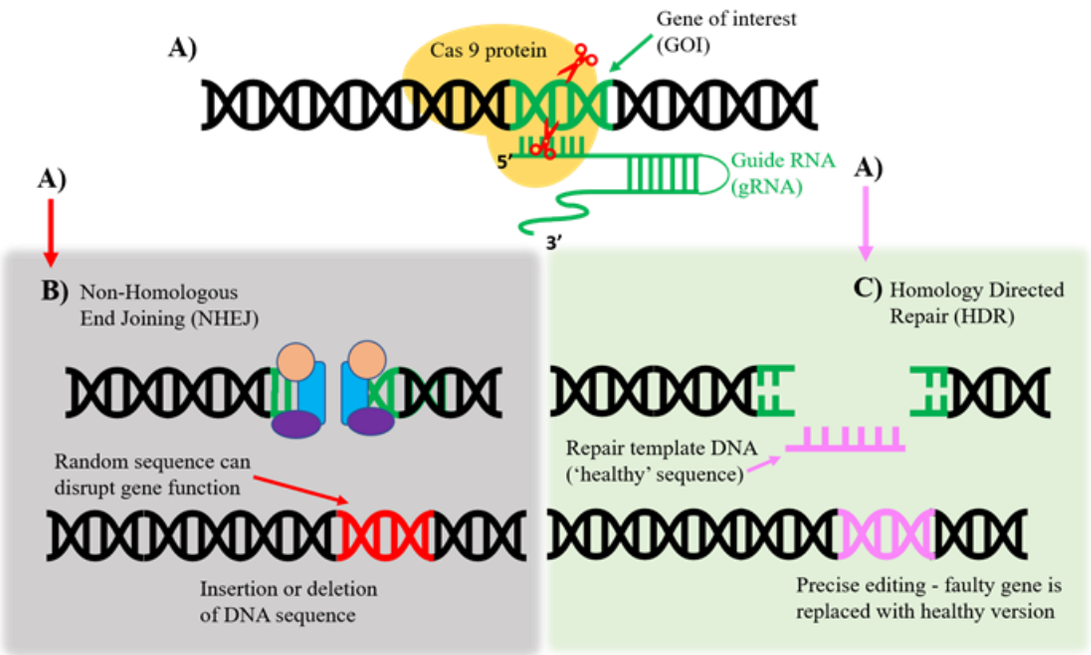

Figure 1. CRISPR/Cas9 mechanism of action. A) The Cas9 protein is guided to the gene of interest (GOI) with the gRNA which is complementary to the target gene. The Cas9 enzyme cuts both DNA strands in the GOI region; B) To disrupt a gene, the DNA is left to repair itself using NHEJ which is an error prone repair mechanism. This can lead to random DNA sequence being inserted or deletion of the DNA sequence, making the gene non-functional; C) If a repair template is added with a desired sequence to insert, HDR can take place. If the repair template DNA is ligated, the other strand can be filled in using this template, leading to a precise insertion of desired sequence.

sequence, the CRISPR system is activated. The system most commonly uses the endonuclease protein Cas9 to cleave the 'recognised invader' sequence (10) as shown in Fig. 1A. Studies in 2012 concluded that Cas9 could be guided to specific regions of DNA if the crRNA was designed to be complementary to the target of choice. The development of a guide RNA (gRNA) through fusing both crRNA and trans-activating crRNA (tracrRNA) could be used along with Cas9 as a two-component gene editing system (Fig. 1) (4).

When a target site is cut by Cas9, two different DNA repair pathways can be initiated, non-homologous end joining (NHEJ) or homology directed repair (HDR). Depending on the desired outcome of the gene edit, both can be utilised (Fig. 1B, 1C) (11). To correct a mutation, a DNA repair template which contains the corrected code can be added along with CRISPR/Cas9 and subsequently utilised in HDR.

Whilst this technique appears infallible, the organisation and function of the mammalian genome is still largely undetermined. Published research using CRISPR/Cas9 has highlighted this gap of knowledge in more ways than one. Schaufer *et al.* (2) compared the genomes of identical mice treated with CRISPR/Cas9 and those untreated (controls). A concerning number of single nucleotide variants (SNVs) across the whole genome were found upon analysis. In particular, 60 SNVs in coding regions of genes induced by CRISPR/Cas9, including one deleterious SNV in the form of a premature stop codon (2). This means that targeting one gene can lead to the knock-out of another gene outside of the targeted region. To reduce this effect the use of bioinformatics tools has proven to be useful. These computational tools use homology of sequences to predict the number off-target sites CRISPR/Cas9 gRNA could potentially interact with (6), essentially acting as a quality control procedure before confirmation and use of the designed gRNA sequence in an experiment. Although this reduces the harmful effects of CRISPR, it does not eliminate them, bringing into question whether there are other off-target effects of CRISPR/Cas9. Later in 2018, Simhadri *et al.* (12) conducted a different study using CRISPR coupled with Cas9 and found that humans could have an immune response to the Cas9 protein. If Cas9 proteins are displayed on the cell's surface and recognised as 'foreign' by CD8+ killer T cells, any Cas9 containing cell would be destroyed by the body as part of a natural defence mechanism (12). Additionally, CRISPR/Cas9 containing cells are traditionally engineered *in vitro* and reintroduced into model organisms due to the complex mechanism of action. Many components need to be intertwined into the DNA sequence of target cells before CRISPR/Cas9 can carry out its function (6). The development of these concerning problems over the years since the introduction of CRISPR has instigated a search for a CRISPR like tool, the most recent of which recycles CRISPR technology.

The New Prime Editing

Introduced by Anzalone *et al.* (3) in October 2019, 'prime editing' serves to edit genomes without the creation of DSBs or use of donor templates. The system uses the Cas9 protein which is altered to 'nick' only a single strand of the helix and is coupled with a new prime editing guide (pegRNA). PegRNA contains a specific RNA template and has a reverse transcriptase (RT) enzyme attached (Fig. 2A) (7). PegRNA is multi-functional, acting as a guide with

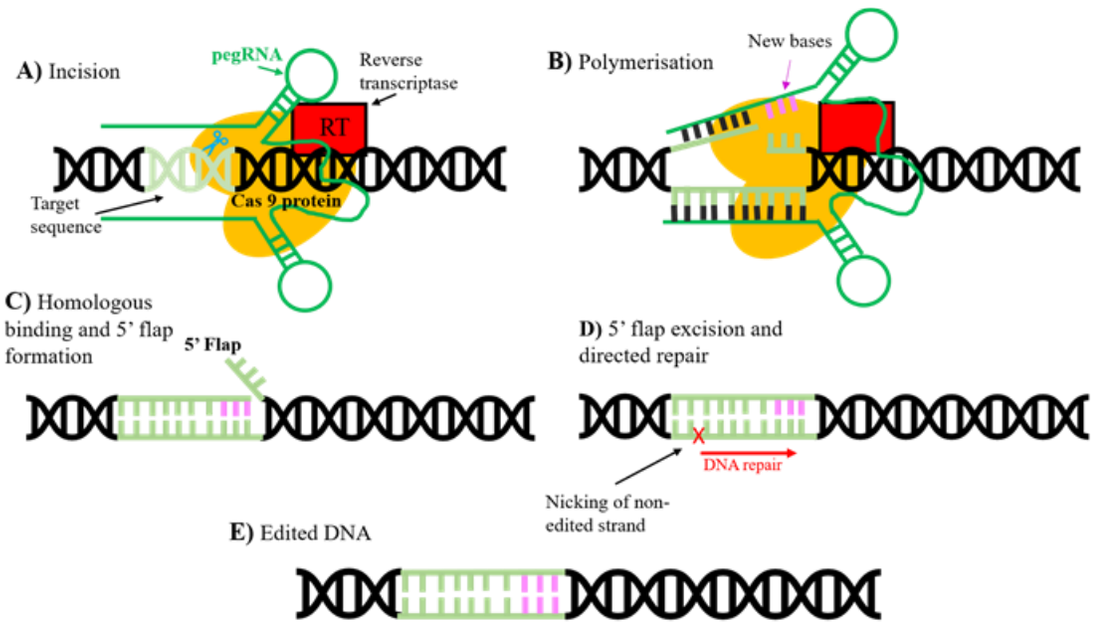

Figure 2. Prime editing mechanism of action. A) The complementary pegRNA with attached reverse transcriptase (RT) guides the Cas9 protein to the target sequence. The Cas9 protein has been adapted to only cut one strand of the DNA; B) Once cut, the broken strand hybridises to the complementary pegRNA and primes reverse transcriptase into action, inserting new desired bases; C) The newly transcribed strand re-anneals to the uncut strand, most frequently leaving a 5' flap as it is preferred during DNA repair using structure specific endonucleases and exonucleases; D) A second nick is introduced into the unedited strand to cause mismatch repair which incorporates the new edit; E) This process results in precise DNA edits being incorporated into both strands of the DNA sequence.

a target DNA sequence and containing an edit-encoding extension to be utilised RT. Cas9 nicks the DNA once at the target site exposing the 3' hydroxyl group which primes RT into action (Fig. 2A, 2B). As 5' flaps are the preferred substrate for structure specific endonucleases, they tend to be created upon hybridization of the strands after synthesis by RT (Fig. 2C) (3). Then a single nick of the non-edited strand biases DNA repair to this strand, favourably incorporating the new bases changes (Fig. 2D, 2E) (3). Using human and mouse cells, the creators of prime editing introduced mutations that cause sickle cell anaemia and Tay-Sachs disease and then corrected them. This resulted in few off-target effects and a high efficacy of successful editing (7). They utilised multiple human cell lines and additionally introduced a mutant allele conferring resistance to prion disease in human and mouse cells. They found that their most efficient variant of prime editor was successful in 53% of cells, with 1.7% indels (3). Indels are random insertions and deletions which are also referred to as 'off target effects'. Prime editing is precise and less error prone due to the accuracy of inserting small numbers of bases, although this methodology does not allow for large insertions and deletions comparable to those that CRISPR/Cas9 can achieve (13). Different pegRNAs and sgRNAs with varying 'nick' locations, along with many RT template lengths can be chosen to increase editing efficiency and specificity. This is an advantage that prime editing has over many other precision gene editing methods (3).

As prime editing is such a novel addition to the scientific community, there is a requirement for validation protocols. This novelty means a lack of research and therefore results to analyse in comparison to CRISPR/Cas9. It has been noted that similarly to CRISPR, delivery into living cells will prove to be difficult due to the complex nature of the machinery (7). Nonetheless, the vigorous analysis carried out by Liu and colleagues suggests that in theory prime editing could correct up to around 89% of the approximately 75,000 known human disease-causing variants (3).

Mechanisms	CRISPR/Cas9	Prime Editing
Targeting apparatus	Guide RNA	PegRNA
Cas9 cutting style	Double strand break	Single strand break
New template source	Homologous repair template DNA free in cell	PegRNA – Edit encoding extension
DNA repair induced	NHEJ/HDR	Mismatch repair with DNA polymerase
DNA repair error class	NHEJ = Error prone HDR = Error free	Error free
Approximate length of edits	300-1000bp	< 100bp

Table 1. Comparison of CRISPR/Cas9 and prime editing mechanisms (3, 15).

How will Prime Editing overcome CRISPR's limitations?

The most significant triumph for prime editing over CRISPR/Cas9 is the elimination of Cas9 induced DSBs. These DSBs are detrimental to cells and can lead to undesirable outcomes including insertions, deletions and more deleterious translocations (3). The resulting mixture of edits between cells determines CRISPR/Cas9 as a very non-precise editing technology. Even with a repair template it is much more likely that the DSB will incorrectly repair itself than incorporate the new sequence (13). Prime editing overcomes this issue using RT and an edit encoding extension held in place at the site of interest as it is part of the pegRNA. This significantly reduces the chance of faulty repair, along with the resection of only a single strand of DNA. Both strands of DNA are nicked during prime editing, but this is controlled to occur at different times to avoid depending on the cells genome repair system which creates incorrect edits (13).

At this moment in time, a downfall for prime editing is the complexity when it comes to delivering the large pegRNA construct and enzymes into living cells. There is no evidence yet that proves it will be functional in an animal model (7). In comparison, CRISPR/Cas9 has had time on its side with the successful generation of live animals expressing CRISPR/Cas9 in brain tissues. In this case, functioning animal models were created by injecting Cas9 and guide RNA into zygotes to modify the early embryos (14). Though the clear wider application of using CRISPR/Cas9 is shown, it is only a matter of time before prime editing could progress in the same way.

CRISPR/Cas9 edits can be of variable length, allowing large deletions or insertions of template DNA (15). The problem here is the lack of specificity, which prime editing brings, mediating all 12 possible base-to-base conversions and combinations of bases in human cells with no DSBs. Most genetic diseases are caused by insertions, deletions and duplications up to and smaller than 30bp (3). This makes increased size of edit with CRISPR/Cas9 less advantageous in these applications.

What is the future for CRISPR and prime editing?

The comparison of CRISPR/Cas9 and prime editing highlights the journey towards improving gene editing technologies for human application. It can be argued there would be more safety in human gene editing with the use of precise, low off target effect technologies like prime editing. However, it may not be the end of an era for CRISPR/Cas9, which has progressed and improved in many ways since its beginning. Instead of one or the other, these technologies could take different directions with more suited paths determined by their differences. CRISPR/Cas9 is ideal for research into gene expression and disease with the ability to create 'knock-out' organisms, whereas prime editing is an ideal candidate to take over CRISPR's proposed 'human editing' role.

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Restless Leg Syndrome

The most common condition you have never heard of

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What is Restless Leg Syndrome?

Willis-Ekbom disease, also known as Restless Leg Syndrome (RLS), is a neurological disorder that causes an involuntary urge to move legs and feet, accompanied by a crawling sensation which worsens during times of rest and inactivity (1). When asking people who suffer with RLS, they make it clear that the discomfort they feel every day is far more torturous than the pins and needles sensation we have all experienced. An astonishing 10% of adults in the UK are affected by RLS, meaning you are likely to know someone who suffers from this illness (2). Yet the source of this disease is still idiopathic (3) and current pharmacological treatments are of limited therapeutic value. As someone whose mum battles with RLS I am aware of the immense impact this illness has on all aspects of life and therefore how vital more research and awareness could be for sufferers.

What causes RLS?

The mechanism behind RLS is still not fully understood, however current studies recognise a strong association between this disease and iron deficiency (3). This may explain why a quarter of pregnant women acquire RLS, as mild anaemia is typical during pregnancy. However, RLS is still prevalent between both sexes and amongst all ages. Furthermore, research has also shown that abnormalities in the neurotransmitter dopamine are involved in RLS pathogenesis (4). This study aimed to develop an understanding of the use of dopamine receptor agonist cabergoline in targeting D2 receptors and found that cabergoline has profound benefits in relieving symptoms and therefore suggests that these receptors are involved in the mechanism of RLS. Disruptions in the dopamine pathway results in involuntary movement of the lower limbs, and in some cases these symptoms can progress to the whole body.

Life with RLS

Life with RLS can be tiresome, disruptive and lonely. As this illness affects people during times of inactivity, it is classified as a sleep disorder (5) and periodic limb movements of sleep (PLMS) is a symptom expressed by more than 80% of RLS patients, with twitching or jerking limb movements occurring up to every 15 to 40 seconds.

RLS patients can suffer with significant sleep deprivation and therefore feel exhausted during the day. As a result of extreme tiredness, patients experience social isolation where activities such as sit-down meals, cinema trips, reading and even working are disrupted (6). As these everyday tasks become incredibly difficult, RLS patients can be left depressed and isolated. This is reflected by statistics that show a significantly higher risk of self-harm in RLS patients than those that do not have this disease (7).

Coping mechanisms

With limited pharmacological treatments, RLS patients experiment with different coping mechanisms that work for them. When experiencing an episode of irritation, a distraction is often used in an attempt to psychologically resist the urge to twitch. Some patients use extreme methods such as self-harm to numb an area of discomfort. Having asked an RLS patient what their personal coping mechanisms are, they stated that listening to loud music with earphones at night helps to relieve symptoms. There is currently no study that has researched this psychological phenomenon, but the loud music may work as a distraction to provide short-term relief. They have also found drinking tonic water helps to ease irritation. This may be due to the presence of quinine (an anti-malaria agent). Quinine tablets are available in the UK and for recent years have been used to treat nocturnal leg cramps (8). However, this particular RLS patient found that tonic water was only beneficial for a limited amount of time before its affects become futile. A recent channel 5 documentary followed RLS patients to deepen our understanding of how people cope with this disease. They found many patients attempt to gain a feeling of numbness by taking strong painkillers and sleeping tablets such as codeine (6). However, in doing so patients expose themselves to accidental overdose as taking more of a drug does not boost its therapeutic outcome, it only increases their adverse effects. Overall, current coping mechanisms have been discovered by patients through trial and error and do not have much scientific study to fully understand their method of action but have shown to be beneficial to individual RLS patients for short-term relief.

Pharmacological Treatment

With more research into the pathogenesis of RLS, the future may see more successful drug development in this

Figure 1. RLS affects 10% of adults in the UK.

area. Currently therapeutic treatment for regular RLS symptoms is to prescribe dopamine agonists, for example, ropinirole, pramipexole and rotigotine skin patches, that are able to boost levels of neurotransmitters in the brain (9). A study was conducted to determine if cabergoline (a dopamine receptor agonist) can be used as a long-term drug for RLS patients based on its safety and efficacy values (4). It found that patients had a beneficial response to cabergoline as their sleep satisfaction levels increased as well as relieving symptoms during the day. The study analysed 302 RLS patients by monitoring the effects of cabergoline over a period of 26 weeks. A titration period determined an optimal dose of 1.5 mg, which was taken for the subsequent 181 days and the beneficial effects of the drug was displayed consistently throughout this time. The study discovered mild adverse effects of cabergoline relating to the gastrointestinal system and central nervous system. These effects included nausea, dizziness, fatigue and headaches. 78.8% of participants experienced at least one of these adverse effects during the experiment but as these symptoms are not classed as severe, the drug was determined as safe to use and well-tolerated by most patients. Cabergoline was successful in improving sleep in 85.7% of people that took part in the study and more than 68.1% of participants stated that they were completely relieved of all RLS symptoms during day and night, therefore showing cabergoline to have a high efficacy. Although the study failed to identify the pathogenesis of RLS, it can be understood that there is some benefit in targeting dopamine receptors, D2, which may help to improve future understanding of RLS mechanisms and drug development. In total, this research concluded cabergoline to be a safe drug and beneficial in relieving symptoms experienced by patients with idiopathic RLS. However, this treatment is not currently licensed for use in RLS in the UK.

If symptoms of RLS are only felt occasionally, levodopa is rarely prescribed as it poses the risk of worsening symptoms (10). Gabapentin and pregabalin, which can also be used to treat epilepsy, are used to reduce the neuropathic pain associated with RLS, as it uses a similar mechanism to dopamine and can reduce pain sensations. However, these anticonvulsant drugs are less potent than dopamine agonists and also come with an array of adverse effects such as neuropathic pain, pruritus and can potentially worsen RLS (11).

Opioids, such as codeine, are prescribed as a final option when patients are unresponsive to all other painkillers. Opioids require careful observation to avoid addiction or accidental overdose but have been successful in providing short-term relief amongst patients (6). Finally, cannabidiol (CBP) and cannabis has been used by patients and has shown success in its ability to numb restless legs. It is difficult to say if the mechanism of cannabis works by targeting receptors that switch off RLS or if it works as a distraction. However, controversy over the legalisation and pharmacological benefits prevent the widespread use of cannabis. There is currently a lack of randomised control trials to investigate the benefit-risk ratio of medicinal cannabis. Despite this, cannabis based products are available for medical use in the UK, where specialists can prescribe these using their clinical judgement on a case-by case basis (12).

Overall, current pharmacological treatment offers no curative options and current medicines are contraindicated which limits the number of RLS patients who can benefit from them. Hence future research has potential to expand the availability of RLS drugs and increase their beneficial outcomes.

In conclusion, it is evident that future research is essential in order to gain a greater understanding of RLS and develop more effective medical treatments. This research will help improve the quality of life for many people in the UK. To support RLS patients, visit the RLS-UK charity website to help raise awareness and funding for future RLS discovery.

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School competition

Science that changed the world

Congratulations to our two winners of our school writing competition. Both students really impressed us with their essays on science that changed the world.

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Making the link between inactivity and heart disease

Emily Crighton
Year 10, Liverpool College

Heart and circulatory conditions kill 1 in 4 people in the UK. Current NHS guidelines suggest that 150 minutes of moderate exercise a week is needed to maintain good cardiovascular health. Today there is a huge body of evidence showing a correlation between low levels of activity and poor cardiovascular health. Consistent studies have shown that sedentary people have twice the risk of developing coronary heart disease as active people. This link was first shown in a study by Professor Jerry Morris whose original study lead to further investigation which proved the undeniable benefits of physical activity.

Professor Jerry Morris was born in Liverpool in 1910 after his parents had left Poland. The family moved to Glasgow where he was educated before he went to medical school in London. He qualified as a doctor in 1934. He joined the Royal Army Medical Corps in the 1940s and was based in India and Burma and was involved in early penicillin trials. After the war he worked for the Medical Research Council's social medicine unit and became involved in public health initiatives at the start of the National Health Service. Morris entered medicine with the purpose of finding out why inequalities in health impacted different types of people in different ways.

After the Second World War there was little money around for medical research but Morris saw a solution using London buses as a way to study heart disease. There was an increase in the number of heart attacks in working men. In 1949 he proposed the hypothesis that men in physically active jobs have a lower incidence of coronary heart disease than those in more sedentary occupations. He carried out his investigation on conductors and drivers of London double decker buses. One job was sedentary, sitting down driving the bus all day and one was active moving up and down the bus and climbing the stairs. Commonly bus drivers and conductors were men from similar a socio-economic backgrounds with similar diets and working environments, the only main difference was the level of physical activity involved in their jobs. Morris' experiment included around 31,000 men employed as drivers and conductors of buses, trolleybuses and trams.

The study examined the sickness and absence records of the employees involved aged between 35 and 64. Records were obtained from GPs and hospitals and any cases of heart disease were noted. Death certificates were examined to look for deaths due to the condition. It was found that there were 80 cases of heart disease among the drivers and 31 in the conductors. This difference was shown in the drivers and conductors of buses and of the drivers and conductors of trolleybuses and trams. Although this was a small study the findings were significant. The conclusion was that employees who were more physically active in their daily jobs had lower rates of coronary heart disease. Further research was done which examined uniform size and early health experiences. More studies were done with contrasting occupations in the population, a study of telephonists who sat for most of the day and postal workers who walked or cycled on their delivery rounds, which supported the original findings.

Morris' study was the first to prove a link between poor levels of activity and heart disease. This was significant as it showed that inactivity was a risk factor for the disease. There have been many studies since the 1950's looking at the population over longer periods which has contributed more data to prove Morris' hypothesis. The importance of his work is in the public health messages that have been developed to influence the lifestyle of the population to prevent coronary heart disease. We now understand that many other diseases are linked to lack of exercise such as obesity, diabetes, hypertension and poor mental health. Morris himself believed in the benefits of exercise for good health and this pioneering, simple study was significant in establishing the link.

Stem cells in Veterinary Medicine

Lydia Lock
Year 12, Deyes High School

Stem cells are unspecialised cells that have the ability to continuously divide and to differentiate into specialised cells in the body. There are different types of stem cells, those including embryonic stem cells and adult stem cells. Embryonic stem cells are derived from embryos and have the potential to differentiate into many different types of cell, meaning they are pluripotent. Adult stem cells can only differentiate into a few different types of cells, so they are multipotent (1). As well as the many potential uses stem cells have in human medicine, researchers believe that stem cells have potential to treat a wide variety of conditions which currently have limited options in veterinary medicine (1).

Research has already been carried out into the impact stem cell therapy could have on tendon injury in horses. This injury can cause pain and lameness which especially in racehorses, often results in euthanasia (2). In a small study, 14 horses which were already destined for euthanasia due to tendon injuries were divided into two groups, one group receiving stem cells in their injured tendon, and the other group receiving saline (as a control) in their tendon. This was studied for six months before they were euthanised, and their tendons were analysed. The study showed that the tendons of the horses treated with stem cells had an improved quality of tissue compared to the horses treated with saline (2). In a much larger study, 113 racehorses with tendon injury were treated with stem cells derived from bone marrow, and after treatment the re-injury rate decreased from 50/60% to 27%. Further experiments on show jumping horses with ligament or tendon damage showed that after 2 years of allogeneic (from another individual of the same species) stem cell therapy, more than 80% of the horses returned to their previous level of performance, with re-injury rates at 18% rather than 44% for conventional treatments (3). Experiments like these have led to stem cell therapy being used commercially in the treatment of horses with tendon and ligament injuries and joint disease, improving the quality of life for racehorses in particular, which would otherwise be euthanised (4).

There have also been experiments into the usage of stem cells for treating osteoarthritis, a painful chronic condition where the surfaces of the joints wear down in dogs (5). In a study from 2014 to 2017, 130 dogs with osteoarthritis were treated with a stem cell injection in the affected joint. The outcome of this study was determined using orthopaedic examinations and owners' scores on improvement for six months. After just one month, 78% of the dogs had an improvement in the orthopaedic score, and after sixth months 88% of the dogs had improved. The owners' scores after six months had 92% of the dogs significantly improving, and 6% improving slightly. The results are promising for the future of stem cell usage, as no major adverse effects were recorded and the treatment seemed to be effective in the majority of dogs [6]. Stem cell therapy may also be favoured in the future as it is less invasive than standard procedures. The process is carried out in one surgical step, and the patient is able to go home on the same day as treatment (6).

Despite the promising future potential of stem cell therapy, there are ethical issues involved in experimentations and treatments. Whether stem cell therapy will be beneficial to a pet could be dependent on a number of factors such as age, breed, severity of injury, and anaesthesia risk, and there is no current way to predict which pets will benefit. As well as this, treatment may have to be repeated in cases where no improvement is made, which could be expensive for the owners (7). This may leave owners wondering if the stem cell treatment is worth the cost. It is however rare for treatment to have to be repeated and the cost of stem cell therapy is often much less than the cost of surgery, and could provide a treatment for conditions which currently have no permanent solutions such joint pain (8). In spite of the ethical issues stem cells have the potential to cure diseases such as spinal cord injury, heart diseases, and tissue regeneration and in researching stem cell uses in animals, researchers can learn more about how these treatments could be used in humans therefore research benefitting both veterinary and human medicine (2, 9).

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Careers

Getting a head start

Thinking about your future career can seem daunting, we know. Find out about the support available to you at Liverpool, how you can get a head start when thinking about your future career and what starting a PhD is like.

Career support at Liverpool

At the heart of our careers support is the Career Studio. This is a space led by students, for students, empowered by career experts. Whether you have your heart set on a particular career, or don't know where to start, our dedicated and highly trained Career Coaches are here to guide you. We are here to support students from every degree subject and year of study – from first year undergraduates to postgraduate researchers. The Career Studio is structured into three zones: Explore; Connect; Apply.

Explore

If you're not sure what you want to do, or have an idea but are not sure where to start, then we can help you develop your career plan. We'll look at your skills and experience and how they relate to your career ambitions. You can research different sectors and roles, find out more about studying and working abroad, and discover what employers are looking for in graduate recruits. You can also learn about the latest internship, placement and graduate vacancies.

Connect

You are encouraged to take part in a wide range of careers events, employer-led talks, careers fairs, and skills workshops through our CareerConnect programme. They offer a chance to meet leading employers, professionals and recent graduates to build your professional network, discover opportunities, and pick up practical advice that will help with all stages of the recruitment process.

Apply

We offer focused support to help you perform well during all stages of the recruitment process for internships, placements and graduate jobs. Take advantage of advice on CVs and applications, practise psychometric tests, face-to-face and video interviews, and learn what happens in assessment centre tests.

Being employable is about attitudes and skills, confidence and drive – it means being able to choose a career, apply effectively and be successful. As the world of work changes and organisations evolve, employers are increasingly searching for graduates not just with a specific degree but with the right blend of skills, knowledge and experience – graduates who can make a difference in any situation.

By making the most of the support available in the Career Studio and engaging with employability activities in the curriculum you will gain the skills and experiences while you are studying that will prepare you for life after graduation.

The Career Studio is currently closed, but we are still here to help you. If you have any questions or concerns, or need support with anything career related, then make use of our online resources and get in touch with the team via CareerHub.All of the resources normally available in the Career Studio can be accessed online, and our Career Coaches are here to provide you with any support that you need.

Find out about our online and virtual support for students: https://careerhub.liv.ac.uk/students/news

How to become a Career Coach

Find out more from ***Helen Elsworth***

Insider Imprint: Hello Helen! Can you tell us a little bit about yourself?

Helen: My name is Helen Elsworth and I am from Yorkshire. I am studying Tropical Disease Biology here at UoL.

Why did you apply to become a career coach at the career studio?

I applied to be a career coach at the career studio because I was excited by the idea of being able to help students on a peer-to-peer level in a space that is more welcoming than a traditional careers model.

Could you describe a typical day in the career studio?

A typical day in the career studio involves running our drop in service, which allows students to attend without an appointment for help with careers related problems ranging from assistance with their CV to help searching for graduate jobs or internships. We also often help with departmental activities and regularly hold events that involve our partner employers.

What help and support is available to Life Sciences students in the career studio?

For Life Sciences students there is a great deal of support available at the careers studio; we can support you through application processes for anything from placements and internships to graduate jobs and schemes. We are able to help assist you from start to finish; from exploring where to look and in what sector, to application forms, to preparing for interviews and assessment centres.

What is the best part of the role?

The best part of the career coach role is seeing students grow in confidence as they progress through their career journey. When you see students start with no direction and end with achieving their goal is really great.

What is challenging about the role?

Sometimes my role as a career coach can be challenging when students have specific queries, but together with the student it is possible to co-explore and make sure they leave the studio with the help they needed.

How do you fit this role alongside your studies?

Career coaches work up to 10 hours per week during term time, and the role is designed to fit around our timetables. As coaches we are allocated slots based on our commitments, and so it doesn't get in the way of our studies. As well as our regular times in the career studio

there are other activities such as open days and evening careers events that we can get involved with if we want.

What are your career ambitions?

When I finish my undergraduate degree my aim is to apply for post graduate entry medicine here at the University of Liverpool in order to become a doctor within the armed forces.

How do you feel the role is preparing you for the graduate job market?

I think that being a career coach has given me opportunities and skills that will aid me greatly when entering the graduate job market. The commercial awareness I have gained has massively helped me in the way I would apply to and understand the companies I am applying to.

What would be your one piece of advice for Life Science students wanting to apply to be a career Coach for 2020?

The advice I would give to a Life Science student wanting to apply to be a career coach would be to do your research. The career studio is not a traditional model so by doing your research and understanding how we work and the events we put on you would put yourself in a stronger position when applying.

The Careers & Employability team are hoping to shortly be opening applications to recruit a new cohort of Career Coaches for the 2020-21 academic year – look out for an email from the team to let you know once the vacancy is live on CareerHub.

From Masters to PhD student within the Institute of Ageing and Chronic Disease

Phaedra Winstanley-Zarach

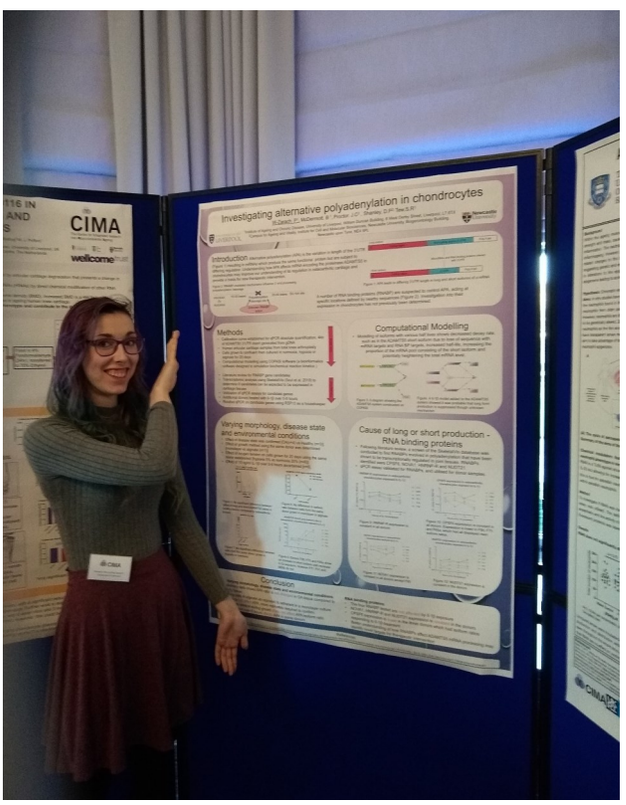
1st year PhD student in Musculoskeletal Biology

My overall experience transitioning from my masters (MRes Musculoskeletal Ageing) to a PhD student has been very favourable. I may have had a slightly more cohesive experience than many as my masters and PhD are both funded by the Centre for Integrated Research into Musculoskeletal Ageing (CIMA), and I have continued with the same primary supervisor for both. This meant when I started my PhD I was already used to the lab and the other lab users.

The main thing I think has made my experience positive is the people. I was lucky during my MRes that the post-doc in my group encouraged me to join in the social activities of the wider research group. I would encourage any masters or PhD students to do this. At first I was quite intimidated going along to coffee breaks in the morning, farewell parties and occasionally planned escape rooms with all these clever academics; but as time has gone by I now consider them to be my friends, which makes everything much less scary during bouts of imposter syndrome! I really benefit from this for example when I can't find something in the lab – I just need to ask a friendly face where the item is, as opposed to needing to ask someone I've never talked to before which can often make me a bit anxious.

Other extremely valuable resources are PhD students starting at the same time. I feel like it makes it much easier to transition when you have people going through the same thing as you and you can talk about it; or even having a chat with the PhD students further along their degree as they'll have been there to. Within my group of friends who started at the same time as me we've all made one or two silly lab mistakes, and it's good to have someone to laugh with.

Similar to the masters project, PhD project time is very self-motivated. This personally works quite well for me, however you do have to be strict with yourself on days you aren't keen to come in. One challenge during the PhD studies is the far away seeming deadline - whereas my masters only lasted a year, my new deadline is suddenly three years away now. It's taken me a little while to get used to the idea that I don't need to have everything done right away, and that I can't possibly have the whole three years planned in the way I could plan 7 months of lab work. This has lead to more emphasis on short term goals than during the masters, and more adaptability to see how experiments turn out and plan accordingly, as opposed to the rigid structure of the masters project experiments.



Becoming a PhD student means the project is being more shaped by my ideas and input, which can be a bit nerve racking. Whereas previously my supervisor was the driving force behind designing experiments, I am now more active in deciding which path we will take, which genes we will examine and which samples we should look at. It's starting to get quite exciting, however, seeing how genes I chose are responding. It was very rewarding when I presented a poster recently and was now able to explain confidently and in detail why those were the candidate genes.

Overall I really enjoyed my masters and I am really enjoying my PhD studies. I didn't find the gap between them too large of a jump so far, and look forward to obtaining more data.

What to expect at the start of a PhD

April Hayes

1st year PhD student in Antimicrobial Resistance

This time last year I was at the University of Liverpool, one term into my MSc. I am now beginning a PhD at the University of Exeter down in Cornwall on their Penryn campus. I'm specifically looking at whether non-antibiotic pharmaceuticals, things like painkillers and antihistamines, can select for antimicrobial resistance in freshwater bacteria.

With a PhD, you have a group of supervisors who are there to give you guidance and knowledge to help you complete your project. They can be academics from your department, but can also include non-academic partners. My supervisors are mainly academics, from Exeter and also from the University of Bath, but I also have a non-academic supervisor from AstraZeneca, who are the industrial partner for my PhD. In my opinion, having good supervisors is just as important as project choice. They're integral in helping direct your project, and until you become an expert in what you do, they will be the guiding force. You're going to be working with them for 3-4 years so it's an important relationship to get right.

I'm based in a lab group, composed of several principle investigators, several post-docs and around ten PhD students. I'm incredibly lucky to be a part of such a welcoming and supportive group. We go out to social events together, including heading to laser tag (you learn a lot about your co-workers at times like that), but, science-related, if you're not sure about something, there's always someone who will be happy to help or point you in the right direction.

There isn't a typical day in my 'PhD life'. I've mainly been reading up on the literature and starting to write a lit review, but I've also been working on deciding which compounds I'm going to screen, and starting to learn how to run my experiments. I've presented a poster at an internal symposium for microbiology researchers at Exeter, attended seminars from people both in my department and outside of my department and I've been all over the southwest for training for my doctoral training scheme (the people who fund me to do my project).

One of my friends in the lab recently asked me whether doing a PhD was how I expected it to be. I still don't know what I expected. Starting my PhD has been very different to anything else I've ever done, and it's very different to any taught university courses. The most important thing to understand, and something I came to realise pretty quickly, is that your project is just that, yours. Your supervisors are

there to guide and suggest things, but ultimately, it's your project, and you have the final say in every decision. It's disconcerting, but also exciting to be able to take your research in whichever direction you want to, depending on what the data looks like.

Engineering my way into Life Sciences

Joseph Barnes

1st year PhD in Tissue Engineering

It may seem strange to see an article written by an engineering student, in a life sciences journal; but that is precisely why it's been written. Before embarking on my PhD in Tissue Engineering, I completed a 4-year integrated MEng degree at the University of Liverpool. The purpose of this article is to highlight the links between biomedical engineering and life sciences research.

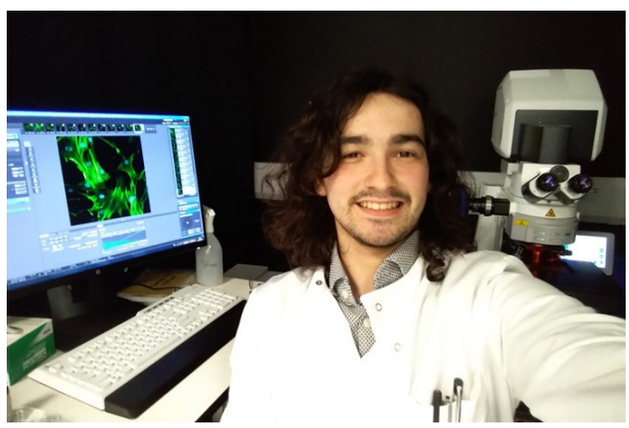
After realising that a career as an anaesthetist might not be for me, I ended up studying "Mechanical and Materials Engineering" at undergraduate level, not appreciating the subtle difference between materials 'science' and materials 'engineering' courses! The first year (and most of the second) of my degree was purely mechanical engineering – covering thermodynamic cycles, analysis of structures and fluid mechanics (all as exciting as it sounds…). Gradually, I specialised into materials topics; metallurgy, crystallography and a whole module on concrete (of all things). In my 3rd and 4th years, I discovered biomaterials and tissue engineering (TE), as well as a practical course on transmission electron microscopy which is usually unheard of at undergraduate or masters level!

For my undergraduate research project, I chose a project in TE, which at that point I knew little about – other than that biomedical materials was where I wanted to end up. I soon realised that my project was well removed from anything else I had done during my degree, although my GCSE in biology would prove to be more than enough to get me going! Fast forward through 18 months of undergraduate and masters level research, and I was applying for PhD studentships in TE and Biomaterials Development.

"But what is Tissue Engineering?"

Written simply, TE is an interdisciplinary field of research which applies both biological and engineering approaches to produce cost-effective solutions that improve the quality of life in our ageing society.

TE involves creating simulations of in vivo conditions in vitro, much like Life Sciences research. Specifically, TE involves combining isolated cells with acellular biomaterials, and perhaps drugs, genes and/or gene products delivered as therapeutic agents. The main targets of TE research are tissues which are exposed and therefore prone to injury such as skin, and those which often suffer disease or degeneration, including the nervous system, the skeleton and vital organs. Most TE solutions involve the use of primary stem cells derived from donor tissue. Providing these with a suitable cellular environment, in the form of an engineered scaffold which mimics the mechanical and surface properties of the native tissues, provides control over the cellular response and allows the cells to function as they would in the human body.



I decided to pursue TE research to PhD level, because it is a fast-moving field which aims to solve medical problems that simply will not go away of their own accord. Tissue engineered solutions will always be needed, and offer to make an outstanding contribution to our quality of life as we age.

Take home messages

Becoming a PhD researcher in the School of Engineering has given me the chance to immerse myself into a thriving research environment and combine the transferrable skills that comprise "The Liverpool Engineer". Nearly all of my lab-based skills and knowledge were acquired 'on-the-job' - so don't be afraid to venture outside of traditional Life Science fields. Although when I started, I had very little idea about biological research, I was fully supported all the while and at no point was I made to feel like an outsider because of my academic background. I'm sure that the non-engineers in our group would say the same too; working in an interdisciplinary field requires researchers from all backgrounds to collaborate, which is where our group excels.

Always keep your eyes open for unexpected opportunities. While it might not be a 'traditional' route into Life Science, having an engineering background gives me qualities that others cannot offer, simply because I 'side-stepped' into the interdisciplinary field of TE. As a Life Sciences graduate, you'll have more than the knowledge and skills needed to get started in a field such as TE (for example), even if you know very little about traditional "engineering" concepts. In fact, your biological background will mean you can offer a completely different set of skills to a research group, that an engineer like myself could not. Consider opening your horizons to next steps into biomedical engineering, by getting yourself involved with research that's beyond your original training.

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Going to a careers fair

A careers fair can be an invaluable opportunity for students. These fairs can provide information about potential jobs, postgraduate study and society memberships. They can help with general career information such as writing a CV, and introduce you to ideas and career paths you didn't even know about. Careers days can give you the chance to meet working scientists, to learn about what their daily routine is like and how they ended up in their particular job.

The Royal Society of Biology holds an annual Biosciences Careers Day in London for students all over the UK. Two students have written about their visit to last year's event; find out about the talks and workshops they attended and why they found the day beneficial.

We will have our very own Life Sciences Careers Conference in November so keep an eye out for that!

Royal Society of Biology's Annual Careers Day

Megan Hodge

3rd year Biological and Medical Sciences BSc

I am currently in my third year of studying a Biological and Medical sciences bachelor's degree at the University of Liverpool. I chose to attend the Royal Society of Biology's Annual Careers Day with the anticipation to expand my knowledge and comprehension on the various careers available within the industry. I initially found out about the event through the 'CareerHub' website run by the Careers Team. I found the event extremely helpful and inspiring, it has developed my understanding of the career pathways available to undergraduate students.

One of my favourite aspects of the Careers Day was attending a speed networking activity with various scientists in different fields. The scientists each had different backgrounds and interests, varying from conservation to medical sciences. It was inspirational to learn about how they achieved their current post and to see how they have grown within the industry. Additionally, it was interesting to learn about their current job roles, including their responsibilities and quirks of the job.

The event held an exhibition with representatives from various professional societies and top organisations. I found this extremely valuable because it gave me an insight into the various companies that are recruiting undergraduate students and the different career paths that are available. I also gained information about the various memberships that are available; the memberships allow you to follow/receive regular updates from societies or companies that interest you.

There were various talks from expert speakers across different sectors. I found the conservation science talk very memorable because the speaker opened up about their previous fears of finding a job in the conservation industry following graduation. Additionally, another talk from a speaker practicing medicine expressed how they went from a pharmaceutical scientist to a doctor specialising in pharmaceutical science. I found it fascinating how career pathways can be interchangeable and how you can convey your skills from one career and utilise them in a different career.

There was a talk on industry vs educational pathways, highlighting the advantages and disadvantages of each. This talk opened my eyes to an educational career pathway, one that I had never considered before. Additionally, it showed me that these two distinct career pathways can be interchangeable and how to utilise each of these career pathways.

I also attended a talk based on CV writing. The lead speaker who delivered this talk worked in recruitment and so had high levels of expertise on what makes a successful application. During this talk I learnt about 'transferable skills', this is the utilisation of previous skill sets to the job you're applying for. I found this beneficial, as it has given me the ability to transfer my previous skill set to the job I am wishing to apply for.

To conclude, I found the event very valuable, informative and would highly recommend it to future years.

Biosciences Careers Day – My Experience

Grace Boyling

2nd year Biological Sciences BSc

Mid-semester, I sat working through my coursework. There was the familiar "ding" of an email arriving in my mailbox. I read; "Funding for Liverpool students to attend the Royal Society of Biology Biosciences Careers Day" at Middlesex University.

I scrolled through the information sent out by our CareerHub and I saw who was exhibiting. I found a variety of companies, research universities and societies from a wide range of areas. These included; The BioChemical society and AstraZeneca, to name a few. As well as the fair itself which offered the chance to talk to these exhibitors, there was a selection of talks on career advice. An opportunity like this, I thought to myself, cannot be missed! I opened up the application form and filled out my details and a short paragraph on why I thought this would benefit me.

After several days, I was asked to attend a short briefing, hosted by Careers and Employability, on how to get the most out of this event. We were given a manual on the day's speakers, who was attending and some quick hints and tips. The Careers Team explained how the event would play out, the logistics of the travel down to Middlesex and how we could prepare to present our best self to these potential employers. Included in the manual was a preparation checklist. This detailed several steps that we could take to be as ready as we could be to impress.

It was advised to print copies of CVs to hand out, undertake practice interviews at the Careers Studio and research particular companies/societies that interested me. I found this especially important. Sometimes it is difficult to think of what is best to do before these types of events and I felt this outlined clearly what was recommended.

The day of the event finally arrives...and the 5am wake up call for the coach to Middlesex. I reminded myself that this early morning would definitely be worth it. After a pastry and a coffee at the service station, we arrived at the venue. Upon entering, we were met with a bustling scene of students exploring the stalls and exhibitors.
I approached several stalls that interested me and asked questions such as; "What would you be looking for in an application?" and "Is there anything I can do to make my application stand out?" This information gained would definitely help in a future internship application.

As the day continued, I entered my first talk, pad and pen in hand. The talk was "Careers in Plant Science". The panel consisted of a range of speakers from big companies, PhD students to research technicians. This allowed me to ask questions about their respective positions, how they got to their current position and what

they might recommend to current students looking to move into the field. The talk helped me to build a good picture of what a job in this field might be like. The talks continued on topics such as CV building, career planning and other topics like "Industry vs Academia". The first half of the day finished and I walked out to grab my packed lunch and speak to my fellow students on how their day was going.

The second part of the day began. As I wandered around the stalls, I noticed a sign detailing that there were practice interviews for students with some of the exhibitors who had come along to the event. I decided that this could be an unmissable opportunity to get helpful advice on how to improve my interview technique for the future. The interview was not stressful at all, rather the opposite. I had a good conversation with my interviewer who then walked me through my technique and gave me a leaflet showing my strengths and weaknesses. I liked the fact that I had a record on paper of where I should be improving, rather than relying on my memory. After a few more career based talks, it was time for the journey back.

Reflecting on this event, I am definitely very pleased I read the email about this and I am happy that I attended. Sometimes, it can be intimidating to attend these events and speak to potential employers but I felt the Careers Team prepared us well and calmed our nerves before sending us into the event. If someone asked me if I would recommend it for future students, I absolutely would. This gave me an unprecedented insight into a range of fields that I could enter with my degree and the information and advice on how to do this successfully.

I would also like to thank the Careers Team for organising everything and the School of Life Sciences for their continued support of their students.

Wellbeing

The importance of mental health

Emotional health can affect all aspects of life and can affect how you feel, think and behave. Being able to talk about any issues that concern you will make you feel empowered and help you find strategies to cope better.

In the next few pages you can find some advice, suggestions of activities to help you relax and contacts if you need someone to chat to.

From struggling student to first-class scholar

Simple steps for improving mental health at University

Jill Vevers

Applied Ecology and Conservation MRes

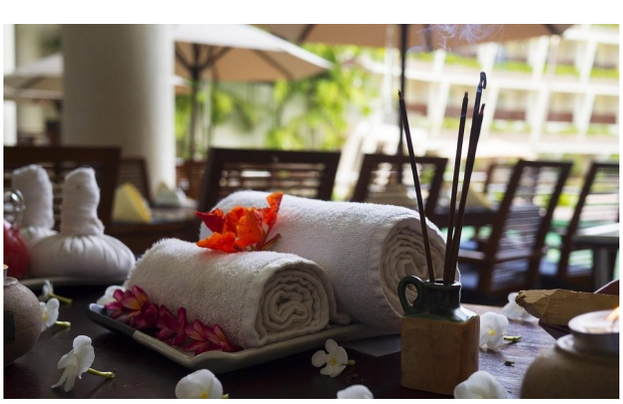
Let me set the scene; you're at university, you have friends and you're probably achieving average, if not good grades. Yet you still feel unworthy, lonely and overwhelmed – sound familiar? An alarming number of students balance mental health conditions and the demands of university daily and unfortunately, most of us don't feel comfortable enough to seek help. If this is you, it's okay, and you're not alone. There are a number of simple steps you can take at home to ease the stress.

Turn your phone off

The first, and honestly the most important, step you can take is detaching from your phone. We're all self-diagnosed social media addicts, myself included, but I cannot stress enough the importance of having at least one-hour quiet time a day, without being reminded of the stress of student life. So, grab your phone and let the 'do not disturb' setting become your best friend.

Self-care

Facemasks, bubble baths and books, that's all self-care is right? Wrong. I mean, for me it is, but self-care is anything you want it to be, as long as it makes you feel good inside or out. So why is self-care so important? One of the harsh truths many learn whilst at university is: no one is going to look after you (most of the time) apart from yourself.

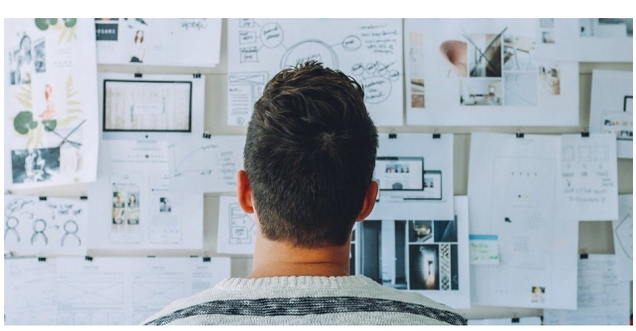


Socialise

To many, university is synonymous with parties, making friends and fun. However, after freshers finishes and you realise you're actually there to get a degree, not just to get drunk, university can be extremely isolating. Whether it's joining a society, just hanging out with flatmates, or even getting a part time job, socialising is ideal for helping forget your woes and help relieve stress and anxieties. (Side note: don't turn to alcohol and substance abuse as a coping mechanism to make friends, it's unhealthy and you know it).

Organisation

I can't say with complete certainty you fit into the stereotype of a disorganised student, but if you do, it may have a negative impact on your relationships and mental health. As someone who suffers from chronic disorganisation, I find keeping a diary is imperative to keeping my stress levels down. If keeping a written diary isn't for you, try visualising your schedule on a wall calendar or use an app to keep track of all your deadlines and meetings.



Disclaimer

I am aware mental health is far more complex than I will ever understand, and that this list is not necessarily applicable to or comprehensive enough for those with serious mental health conditions. If you ever feel like university is becoming too much, your grades are slipping or if you have any thoughts of self-harm, please seek professional guidance immediately. And remember, there is no right or wrong way to complete university, no such thing as a good or bad degree, and you can never take too long to complete it – **it's your degree**.

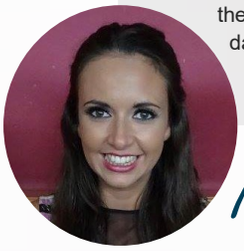
Study tips from your editors

"You can use apps like Trello or Google Calendar to help organise yourself. To relax, there are apps like Headspace, or you can find a YouTube meditation channel like Mindful Movement. YouTube channels can also be a great place to find workouts you can do from home."

Fabia

Heather

"I use the pomodoro technique to help with productivity and focus. You pick a task, set a timer for 25 mins (a pomodoro) and work only on that task until your timer rings. Then tick off this task on your list, take a short break and continue. After 4 pomodoros you take a longer break."



"I like to make lists of all the things I need to do then score them out once completed. Although a long list may seem daunting by scoring out activities, I also see how much I have accomplished which helps keep me motivated."

Natalie

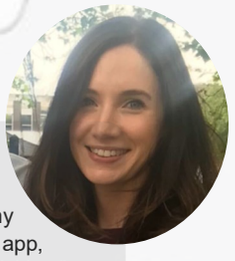
Juhi

"Find a quiet space for study and remember to take regular breaks. Breaks can involve getting a snack or simply getting some fresh air. Don't be afraid to ask questions! Ask a friend - or search the Internet for useful websites. There are many helpful resources out there."

"You can input key things on mobile phone notes and even use voice recorders to communicate your key messages. Listening to podcasts can be helpful. Use some meditative music to avoid distractions during your study time!"

Manohar

Kerry



"Technology can greatly enhance your organisation! I have the google calendar app on all my devices and use this to ensure I don't forget to do anything on my to-do list. I can also send myself reminders via the app, so that I am always prepared for upcoming activities."

"Make sure you get enough sleep, as this will greatly improve your concentration and memory! If you have an exam the next morning resist the temptation of pulling an all-nighter and get some proper rest."

Catarina

Insider Imprint

Tai Chi: ancient art, modern application

Mark Pountney
Tai Chi Instructor

People are exposed to more information than ever before; non-stop digital feeds from world news websites, social media platforms, manufacturers and on demand entertainment. The expectations placed on individuals as a result of all this can leave people feeling overwhelmed or burned out. Add to this pressure from exams, deadlines, balancing paid work with studying and finances, it is no surprise that students are at particular risk of stress related illnesses.

Imagine if there was an activity, which was easy to learn, required no special equipment and could be practiced anywhere at any time, by people of any age or fitness level that enhanced mental, physical and emotional health.

Such an activity exists, and it has been practiced since the eighth century BC. It is called Chi Kung (Qigong); the most well-known type of Chi Kung is called Tai Chi and like yoga its aim is to balance the mind, body and spirit. Tai Chi is a healing / martial art that combines movement, breathing and mindfulness to circulate energy (Chi/Qi) around the body. The movements (forms) incorporate the ancient Chinese theory of Yin and Yang (interdependent opposites) and the Five Element theory (Acupuncture is also based on these theories). There are several Tai Chi styles; the most prominent are Yang, Chen, Sun and Wu.

Chi is the life energy that sustains us. The development of the Chi was originally used to increase strength for combat, but the techniques have been found to improve health. This is the main reason why people practice Tai Chi today.

What benefits can I gain from Tai Chi?

- Better mood, with lower levels of depression, stress and anxiety
- More energy and stamina
- Enhanced flexibility, balance and agility
- Improved sleep quality

If you are interested in trying Tai Chi / Qi Gong classes, we offer a weekly class at the university. Foundation sessions last one hour and are suitable for all ages and abilities. Tai Chi can be performed in regular, comfortable clothing. Please contact **mark.pountney@liverpool.ac.uk** for more details.

Anyone who is facing new challenges as a student can benefit from counselling.

The counselling service

The Counselling Service provides confidential support if you are experiencing distressing psychological or emotional problems which affect your studies or general wellbeing.

Counselling offers an opportunity to talk freely about your concerns in an active, collaborative process. It can help you develop an increased understanding of yourself by exploring how you think, behave and feel about an issue and help you find appropriate ways to make changes in your life.

Our service provides a supportive pathway with a stepped care model; this means that you can access help quickly and get the most effective help for your needs:

- We offer something called **Single Session Therapy (SST);** the model is designed so that students can walk in, fill out two simple forms and be seen by a counsellor. Single Session Therapy is designed to address students presenting concerns within one session. This appointment is usually sufficient for many students.
- This is also the entry-point to our other counselling services, should a student present with more complex needs. We offer longer-term counselling over a few weeks or months, should this be appropriate.

To access counselling with one of our professionally qualified therapists, and to discuss any issues in confidence, Student Services runs a Wellbeing Drop-in for all students open Monday to Friday 11 am-3pm.

All you need to do is go to the Student Welfare Advice and Guidance Information Point in the Alsop Building, University Square, Brownlow Hill (building 759 on the campus map).

As the University is closed due to the COVID-19 lockdown, the counselling service is now offering online support through phone and email between 9 am and 5 pm each day. In addition, there are a number of online services that you can access via the counselling service webpages, including Big White Wall and SilverCloud.

You can find more information on
www.liverpool.ac.uk/counserv

Counselling Service

> *"The ambience was perfect. The counsellor was very careful, listened to me well, helped motivate me and gave good techniques"*
>
> *"The person I talked to was very understanding and willing to help"*
>
> *"I realised what the root of some of my problems/feelings was, and now have practical steps to take"*

Feedback from students who have accessed Single Session Therapy during June/July 2019

Shout: for support in a crisis

We all experience moments when life can feel overwhelming. This could be because of rising stress or anxiety, issues at work, school or at home, academic pressure or relationship breakdown. At Shout, we believe that no-one should face a crisis alone, and our 24/7 crisis text service is here for times when you need some immediate support. You can text **Shout** to **85258** any time day or night to be connected to a trained Crisis Volunteer who will chat with you by text. It is confidential, and for most mobile phone networks* is free, and won't show up on your phone bill.

It works by texting the word Shout to 85258 and after a few automated messages you will be connected to a trained Crisis Volunteer. They will introduce themselves, reflect on what you've said, and invite you to share at your own pace. You'll text back and forth, only sharing what you feel comfortable with. By asking questions, listening to you and responding with support, they will help you sort through your feelings until you both feel you are now in a calm, safe place. You might be provided with some signposting to other services that can provide you with further help, so that you can continue to get support.

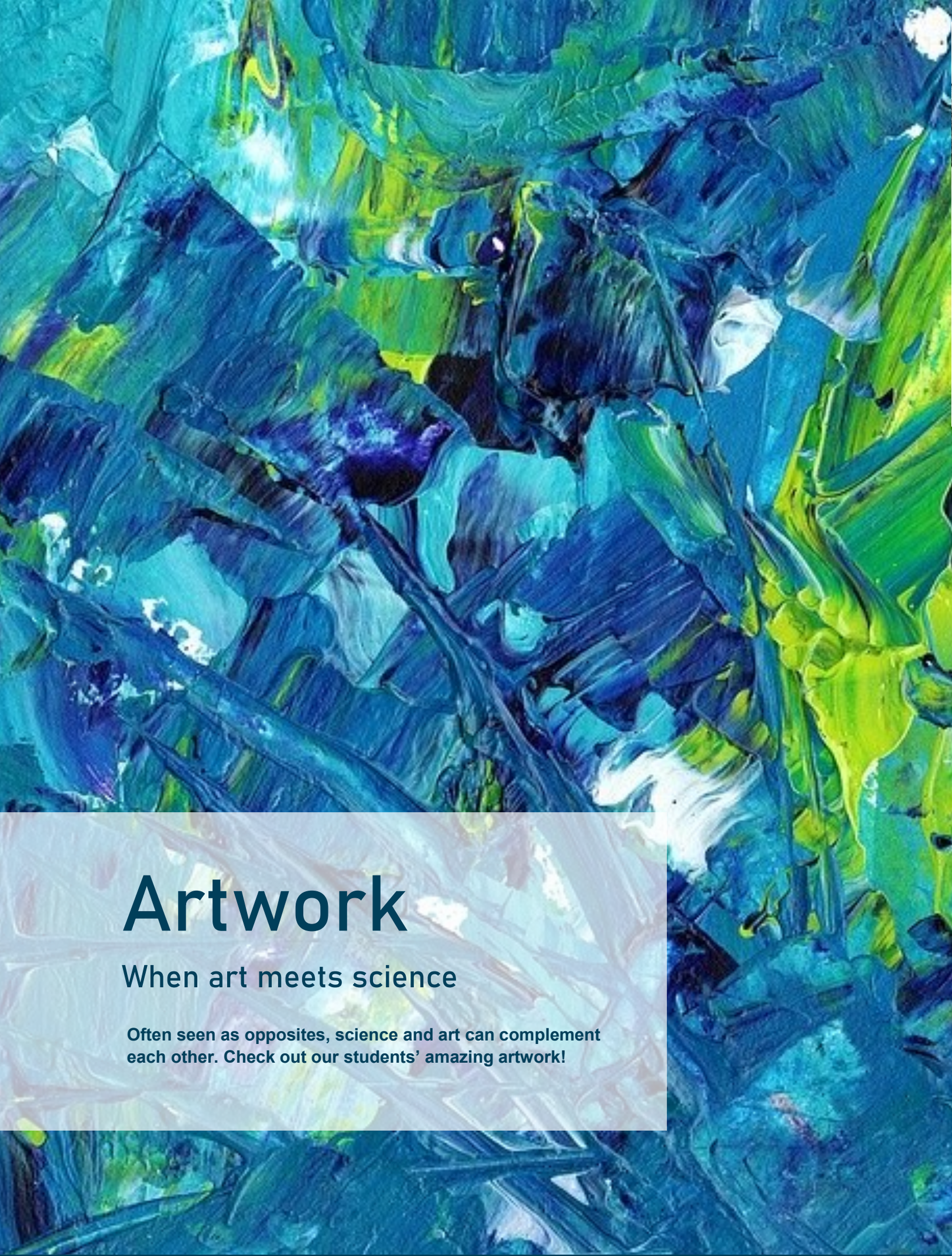
Shout can help with issues such as suicidal thoughts, abuse or assault, self-harm, bullying, relationship issues, academic stress, loneliness, sexuality and more. Our Crisis Volunteers operate under clinical supervision and create a safe space to talk about whatever is troubling you. It is not a one-way process, you won't be told what to do. You will need to work with the Crisis Volunteer to form your plan. If a life is at imminent risk call 999 for emergency help.

*It is free and confidential to text Shout from the major UK networks: EE, O2, Three and Vodafone. These include – BT Mobile, Tesco Mobile, Virgin Mobile, iD Mobile, Sky, Telecom Plus, Lebara and GiffGaff. This service is powered by Crisis Text Line.

For more information visit: **www.giveusashout.org**
Twitter: @giveusashout
Instagram: @giveusasshoutinsta
Text SHOUT to 85258 for immediate support 24/7

shout
for support in a crisis
CRISIS TEXT LINE

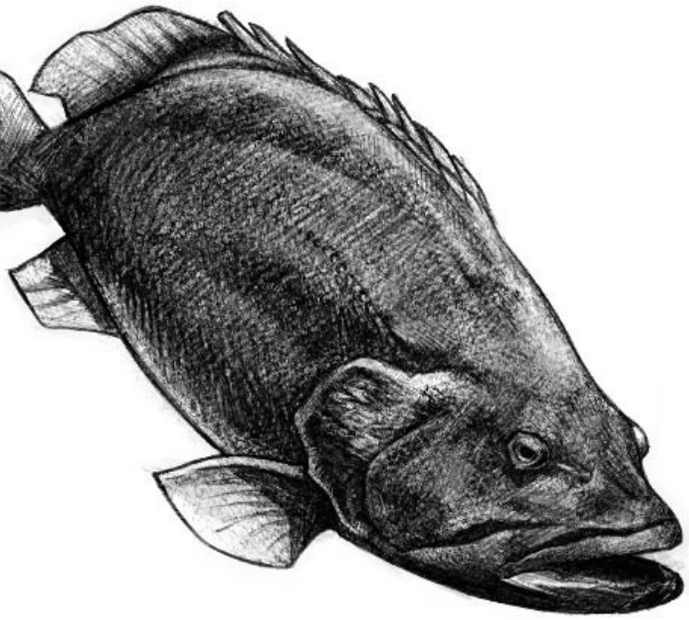
Caring for your mind is as important as caring for your body. **Nourish yourself.**



Artwork

When art meets science

Often seen as opposites, science and art can complement each other. Check out our students' amazing artwork!

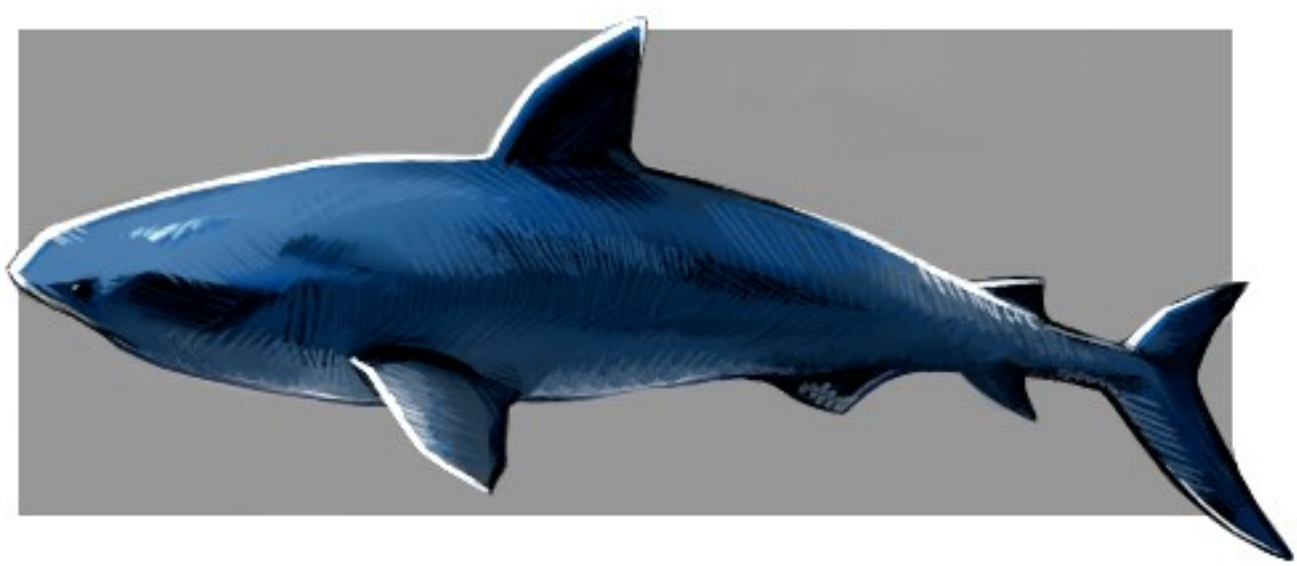


The Deep

"During my visit to the 'The Deep' aquarium in Hull, I took a lot of photographs of different types of fish and marine life. These included jellyfish, sharks, tropical fish and even the remains of prehistoric sea creatures such as the Megalodon and Dunkleosteus. However, the species of particular interest to me was the green sawfish, a bizarre looking animal that is currently endangered in all parts of the world. In fact, 'The Deep' housed the only pair of green sawfish in the UK. Observing these beautiful creatures through the glass I felt disheartened that so many species across the world face endangerment and extinction due to human interference. I wanted to combine my interests in art and biology and create drawings inspired by some of the photographs I had taken. The featured fish in these drawings are the potato grouper and a reef shark."

Paige Chapman

2nd year Anatomy and Human Biology BSc



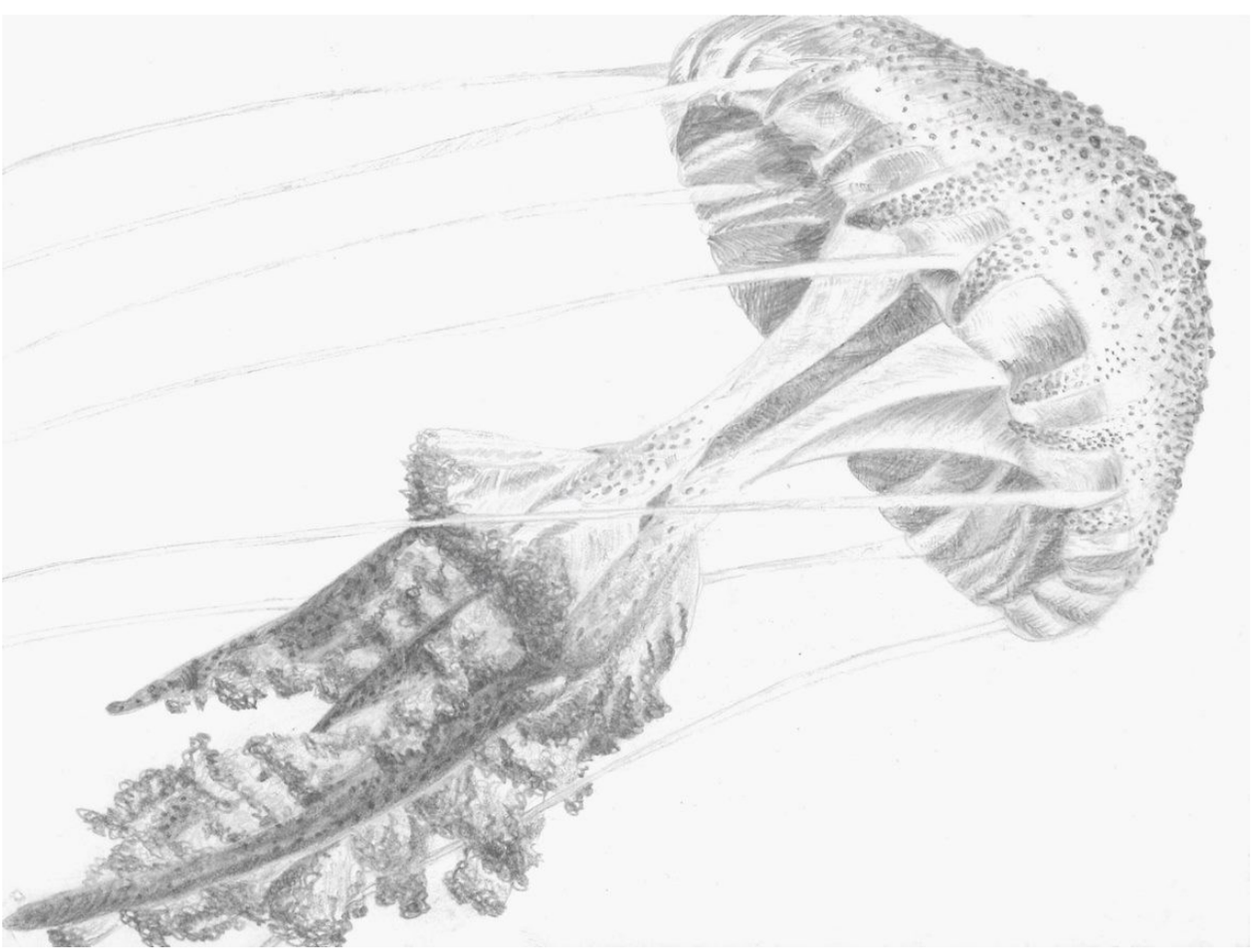
Purple-striped jellyfish

(*Chrysaora colorata*)

“Inspired by an exhibit at the Vienna zoo, this pencil study is an exploration of a Cnidarian’s anatomy and epidermis texture. Mesmerised by the flowing movement of the purple-striped jellyfish (*Chrysaora colorata*) and the gentle pulsations of its bell, I tried capturing this effortless motion on paper. At first glance seemingly delicate, this carnivorous jellyfish is equipped with rows of stinging nematocysts and is often met with prejudice and alarm when coming into contact with humans. What is often missed, however, is just how striking these organisms are when observed up close.“

Erika Kupyrova

2nd year Zoology BSc



In a While, Crocodile!

African dwarf crocodile (*Osteolaemus tetraspis*)

“The African dwarf crocodile is one of the smallest species of crocodile (subfamily: Crocodylinae) and currently classed as vulnerable on the International Union for Conservation of Nature (IUCN) red list of threatened species. Both habitat loss and overexploitation through hunting make this species of crocodile particularly at risk. African dwarf crocodiles are not considered to be aggressive and feed on fish, crustaceans and amphibians. I have always found crocodiles fascinating and through this piece I wanted to capture the vulnerability and beauty of this species, rather than the cunning predator they are often perceived as.“

Megan Parker

4th year MBiolSci

Syrian bear

“This commission piece depicts a portrait of a Syrian brown bear that was inspired by a colleague involved in setting up a national park in Armenia. Unfortunately, the project was called off, but the artwork remains as a reminder of the flagship species intended to promote the project. The establishment of the park was a controversial issue in the area as mining in the Syrian bears’ habitat was a major industry for local people. However, it was noted that the locals had a passion for artwork and it was hoped that by producing art that represented the beauty and vulnerability of the species and environment that their mindset would begin to shift. The expression on the bear’s face, particularly in the eyebrows, personifies the animal and its vulnerability to the mining industry. In addition, exposed palms are a common symbol of honesty in art and this has been represented by the exposed sole at the base of the work. All these elements were intended to capture the public’s imagination and hopefully aid the conservation project. The detail, exaggerated by the greyscale tones, is another element that brings the texture and detail associated with real life, but in a more abstract and emotive style. This illustration serves as an example of how art can be a valuable asset in the conservation industry as it connects otherwise disconnected people with the world around them. I hope that all these elements create an image in your mind in which the bear could simply turn its head and walk out the page.”

Jemima Western

4th year Zoology BSc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