



VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D5.4

VetBioNet 3Rs Training Event: Professional Training School in Experimental Design and Statistical Analysis

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Executive Summary

The aim of this work was to organize a professional 3Rs training event. This report outlines how this was successfully achieved, through effective collaboration between the VetBioNet partner (and the host institution) University of Nottingham and a scientific charity, FRAME (Fund for the Replacement of Animals in Medical Experiments).

The Training School in Experimental Design and Statistical Analysis of Bioscience and Biomedical Experiments held in Nottingham, UK, 9-11 January 2019 addresses the two of the VetBioNet objectives; to provide graduate and post-graduate training opportunities (WP5) and to define and disseminate best practice, particularly in relation to the 3Rs and animal wellbeing (WP4).

The report describes the objectives, structure and outcomes of the training event as well as highlighting some key recommendations relating to continuing and improving 3Rs education and training. The importance of this deliverable is discussed, how it supports the aims and objectives of the VetBioNet project and the consequences and impacts of conducting such a course are presented.

Further training opportunities and avenues of research that are compatible with VetBioNet and could benefit its members and the wider scientific community are also suggested. The lead VetBioNet partner, UNOTT, also recommends that a further Training School is organised, if resources permit, before the end of VetBioNet EC funded period (for example in 2021/22), as this will further strengthen the exchange of good practice and support the wider sustainability initiatives being delivered across the Network.

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1. Introduction

Within the VetBioNet project there are key objectives to provide graduate and post-graduate training opportunities (WP5) and to define and disseminate best practice, particularly in relation to the 3Rs and animal wellbeing (WP4). In order to meet these important objectives a 3Rs Training School in Experimental Design was organised and delivered between 9-11 January 2019 at the University of Nottingham, UK.

In 1959, Russell and Burch (1959) identified that reduction in laboratory animal use can most effectively be achieved by rigorous experimental design and appropriate statistical analysis of any results. This Training School provided an ideal opportunity to facilitate dialogue and enhance the application of experimental design and statistical analysis to infectious disease animal experimentation to improve: a) animal welfare; b) the amount of information from a given number of animals involved and c) the quality of biomedical research and testing.

The VetBioNet 3Rs Training School was hosted by the University of Nottingham and was a collaboration between FRAME and EU H2020 project VetBioNet.

This VetBioNet 3Rs Training School builds on a longstanding partnership between FRAME with the University of Nottingham, hence ensuring a high level of quality and professionalism in the delivery of the Training School. The University of Nottingham and FRAME collaborate and coordinate in developing alternatives to animal testing, through the FRAME Alternatives Laboratory based at the University's Medical School and in delivering training to scientists with colleagues in the Centre for Applied Bioethics in the School of Biosciences. FRAME has also previously been involved with the wider community of VetBioNet partners through NADIR in 2010. FRAME in partnership with universities, NGOs and projects delivers regular training schools to increase awareness among scientists about the need to reduce animal numbers in experiments and to refine procedures. FRAME is a UK medical research charity committed to replacing the use of animals in scientific experiments. It is dedicated to the development of new and valid methods that will replace the need for laboratory animals in medical and scientific research, education, and testing. Where the use of animals is currently necessary, FRAME supports the reduction of numbers involved to an unavoidable minimum and refinement of experimental procedures to minimise any suffering caused. The charity was founded 50 years ago with the main aim to promote the 3Rs (Replacement, Reduction & Refinement) as a way forward for animal experimentation.

The successful delivery of this training event facilitates and addresses several of the underlying aims and objectives of the VetBioNet project, these are described in more detail throughout this report but in summary the following are important consequences of conducting this deliverable.

The Training School coordinators and tutors (including VetBioNet team members Michelle Hudson-Shore and Kate Millar) have worked to achieve FELASA (Federation for Laboratory Animal Science Associations) accreditation for the course. This process began before the current event, but its completion was necessary during the audit of the present course. As described in section 6.4, this accreditation means that this course supports important aspects of VetBioNet in terms of the harmonisation of skills and best practice across the EU, and by providing transferable competency evidence it facilitates European researcher mobility through EU level accredited modules.





This Training School directly addresses the objective of Task 5.4, to provide training and education opportunities for postgraduates by attracting both early career and senior researchers and explicitly acts as a conduit to disseminate best practice in the 3Rs, particularly focusing on Reduction and to some extent Refinement. The residential and interactive nature of the course enables knowledge exchange and fosters new relationships between scientists from a wide variety of institutions, a diverse range of research fields and across national settings. In addition, the cooperative spirit of VetBioNet is embodied in the successful collaboration beyond the project partners, with FRAME, which is further exemplified by the attendance of the Coordinators from INRA at the event.

This report outlines how successful execution of this deliverable has been achieved by detailing the objectives, structure and outcomes of the Training School, concluding with the wider implications and giving recommendations for future activity in this area.

2. 3Rs Objectives for VetBioNet

2.1.3Rs Objectives in relation to VetBioNet

The key 3Rs objectives of the Training School are to:

- provide researchers with an understanding of basic design concepts that they do not seem to be gaining from other sources,
- give researchers the ability to use more efficient designs for their experiments, and
- stimulate engagement with the Three Rs and useful discussion between animal users in different sectors, such as industry and academia, on both refinement and reduction.

2.2. Importance of training and education

It is widely acknowledged that scientists that use animals in their research have an ethical responsibility to ensure that the animals that they unavoidably have to use must be treated with care and concern and that efforts are made to develop and apply the principles of the 3Rs (Replacement, Reduction and Replacement) to animal experiments (Mehlman *et al* 1989; Franco and Olsson 2014). There is also a long-standing consensus that an important means for improving the welfare of laboratory animals and promoting the 3Rs is education (Hau 1999; Forni 2007). The knowledge and competence obtained through continuous education and training for all related roles are fundamental to the responsible use of animals in research while maintaining high scientific standards (Hau 1999; Franco and Olsson 2014).

The importance of education, training and competency for professionals working with laboratory animals is reflected in the mandatory training and competency requirements laid down in the European regulations controlling animal experimentation, Directive 2010/63/EU (Article 23). Indeed, Franco and Olsson (2014) found that mandatory training in Laboratory Animal Science 'is a valuable means of educating and raising awareness about animal welfare' (p.59), because it makes participants more knowledgeable about the 3Rs and they report that it improves their actual implementation of the principles in their work. Of particular importance in the context of VetBioNet is the observation of Franco and Olsson (2014) who suggest that





this requirement for training may contribute to improving and harmonising practice across the EU.

A key area of competency is the ability to design and analyse animal experiments appropriately and effectively. In recent years several studies have highlighted that there are significant issues with poor design and incorrect analysis of animal studies which can have serious ethical, legal, scientific and indeed economic consequences (Festing *et al* 2016). It has also been recognised that while scientists normally receive an education, and achieve expertise, in their area of academic interest, they are often not as well informed about current thinking in experimental design and statistics and may have difficulty accessing high quality training in this area (Howard *et al* 2009; Fry 2014). It is also notable that the deficiencies in experimental design in published work detailed in the studies above indicate that education to increase understanding in this area is needed for those already undertaking animal research as well as for those new to the field (Fry 2014). Therefore, the development and delivery of this unique industry accredited Training School in Experimental Design is an important and essential opportunity to address this gap in training provision and to facilitate further harmonisation of laboratory animal science standards and practice across Europe.

2.3. Learning outcomes

In order to meet the 3Rs objectives, the following skills and learning outcomes, which correlate with the requirements of Article 23 of Directive 2010/63/EU for personnel to be adequately educated and trained before carrying out procedures on animals (Function A) and before designing procedures and projects (Function B) are covered by the course material:

- 1. Describe the concepts of fidelity and discrimination (e.g. as discussed by Russell and Burch and others).
- 2. Explain the concept of variability, its causes and methods of reducing it (uses and limitations of isogenic strains, outbred stocks, genetically modified strains, sourcing, stress and the value of habituation, clinical or sub-clinical infections, and basic biology).
- 3. Describe possible causes of bias and ways of alleviating it (e.g. formal randomisation, blind trials and possible actions when randomisation and blinding are not possible).
- 4. Identify the experimental unit and recognise issues of non-independence (pseudo-replication).
- 5. Describe the variables affecting significance, including the meaning of statistical power and "p-values".
- 6. Identify formal ways of determining of sample size (power analysis or the resource equation method).
- 7. List the different types of formal experimental designs (e.g. completely randomised, randomised block, repeated measures [within subject], Latin square and factorial experimental designs).
- 8. Explain how to access expert help in the design of an experiment and the interpretation of experimental results.
- 9. Identifying suitable research objectives, controls and experimental units.
- 10. Identifying common basic faults in experimental design and data presentation.
- 11. Identifying the correct experimental design for a given objective.
- 12. Being aware of the ARRIVE Guidelines and designing an experimental protocol that would meet them.





3. Structure of the VetBioNet 3Rs Training School

The programme (see table 1) was taught by six expert tutors:

- Dr Derek Fry (University of Manchester)
- Dr Kate Millar (VetBioNet and University of Nottingham)
- Dr Michelle Hudson-Shore (VetBioNet, also acting as Training School Coordinator, University of Nottingham)
- Prof. Richard Preziosi (Manchester Metropolitan University)
- Dr David Lovell (St Georges, University of London)
- Dr Michael Festing (Consultant Statistician)

The format included lectures, group discussions, and individual exercises. The programme (table 1) is structured to lead the participants from simple experimental design and statistical ideas, through more complex methods and analysis to effective presentation of findings. Participants were also able to discuss their own research problems/experiences with the VetBioNet Training School tutors. This interactive approach strengthens and supplements the information given in the more traditional lectures. The School also fosters networking and dissemination of information between participants.

Table 1

VetBioNet Training School Programme, Nottingham 2019

Session	Time	Session Title and Content
Wednesday 9 January		Basic Principles
	08.50-09.15	Registration
1	09.15-09.45	Introduction to course: The 3Rs, legal and ethical aspects of Experimental Design.
2	09.45-10.15	Quiz 1
3	10.15-11.00	Principles of experimental design. Types of experiment (pilot, exploratory, confirmatory), objectives, controls, experimental units, replication, randomization, blinding.
	11.00-11.30	BREAK
4	11.30-12.15	Common failings: unclear objectives, bias, lack of power, failure to randomize/blind, pseudoreplication. Costs of poor design.
5	12.15-13.15	Group Exercise 1: Controls, experimental units.
	13.15-14.15	LUNCH
6	14.15-15.00	Revision of basic statistical inference. Null and alternative hypotheses, SD vs. SE, outliers, type I& type II errors, variables affecting significance, summary statistics.
7	15.00-15.45	Sources of variability in animal studies and how they may be controlled. Need for better design.
	15.45-16.15	BREAK





8	16.15-17.15	Simulating experiments and the importance of controlling variability. Randomisation, sampling, Type 1 and Type 2 errors.				
	19.00-21.00	DINNER				
Thursda	y 10 January	Experimental Designs and Statistical Analysis				
9	09.00-09.45	The analysis of variance, interactions, post-hoc tests, assumptions, data transformations.				
10	09.45-10.30	Group Exercise 2: Finding basic faults.				
	10.30-11.00	BREAK				
11	11.00-11.45	Completely randomised, randomised block and latin square designs. Power calculations, resource equation.				
12	11.45-12.30	Qualitative data, contingency tables, non-parametric tests.				
	12.30-13.30	LUNCH				
13	13.30-14.15	Factorial "designs".				
14	14.15-15.00	Group Exercise 3: Choosing the right design & over-night exercise.				
	15.00-15.30	BREAK				
15	15.30-16.15	Experiments to test relationship: correlation, regression.				
16	16.15-17.30	Power analysis, EDA and the pros and cons of software.				
	18.30-22.00	SOCIAL EVENT & DINNER				
Friday 1	1 January	Applied Experimental Design and Important Design Messages				
17	09.00-09.45	Discussion of overnight exercise. Planning an experimental programme.				
18	09.45-10.30	Group Exercise 4: Analysing and presenting results.				
	10.30-11.00	BREAK				
19	11.00-12.15	Parallel Session 1: Ask the Experts! Session A: Writing an experimental protocol, ethical review & 3Rs. Session B: Discussion of participants unresolved design problems. Session C: Searching for information on 3Rs and 3Rs resources.				
20	12.15-12.45	Quiz 2 and discussion.				
	12.45-13.30	LUNCH				
21	13.30-14.45	Parallel Session 2: Ask the Experts! FELASA accreditation exam [13.30-14.15] Session A: Writing an experimental protocol, ethical review & 3Rs (cont.). Session B: Discussion of participants unresolved design problems (cont.). Session C: Searching for information on 3Rs and 3Rs resources (cont.).				
22	14.45-15.00	Answers to Quiz 2 & take-home messages.				
		CLOSE				





4. Training School Participants

The Training School is targeted at those who are at postgraduate level or above. This course attracted participants from across Europe.

Table 2 lists the number of participants and their country of origin. As the host country the UK had the most representatives, but these were geographically spread from 17 different cities. The majority of participants were postgraduate or postdoctoral researchers, but the School also attracted senior members of staff, veterinarians and named persons among others (see table 3).

VetBioNet was represented well at the Training School with 16 participants (36% of all participants) coming from six VetBioNet partner institutions (Moredun Research Institution, Institute Zooprofilattico delle Venezie, The Roslin Institute / University of Edinburgh, University of Nottingham, INRA and APHA), covering three countries (UK, Italy and France). A number of EU participants had also be informed of the VetBioNet event by project partners.

Table 2	Origin of participants who attended the Training School, January 2019
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Country	Number of Participants
UK (17 Cities)	33
Croatia	3
Denmark	2
France	2
Italy	2
Finland	1
Norway	1
Portugal	1
Total	45

Table 3 Scientific roles represented among participants, January 2019

Scientific Position/Role

PhD Student

Post Doc Researcher

Scientist/Scientific Officer

Senior Scientist/Researcher

Researcher/Research Assistant Named Veterinary Surgeon

Head of Department

Deputy Study Director

DVM

Investigator

Biosciences Business Development Manager

Technical Developer

Scientific Manager

Veterinarian

Named Training and Competency Officer

Research Associate/Research Fellow

Director

Resident

Animal Welfare Officer





5. Outcomes

5.1. Key Outcomes

The main outcome is an increased awareness and understanding among participants of the need to reduce animal numbers in experiments and to refine procedures undertaken on them. This increase is evidenced by the raised understanding marks in the course quiz (see section 5.3) and the reported improvements in confidence in applying the 3Rs when planning an experiment in the course feedback (table 4). Of the 45 participants who took part, (100% of the distributed feedback forms were collected from the participants) only three reported no increase in confidence, but this may be attributed to their high confidence to begin with, as two of them rated their confidence as 10 and one rated it as 9 before attending the course. Of the remaining 42 participants over half (23) ranked their confidence in applying the 3Rs as improving by between 3 and 7 points, with eight giving an improvement of between 5 and 7 points. In addition, 93% (42) of participants indicated that the course had exposed them to new knowledge and practices. These improvements in awareness and understanding of the 3Rs are similar to the findings of Franco and Olsson 2014, and Fry 2014. Therefore, it is expected that this will translate more broadly into a reduction in the number of laboratory animals used and the suffering they may encounter.

Further, with a better understanding of how to properly design experimental programmes and effectively analyse results, participants will go on to produce higher quality science, which has made most efficient use of the resultant improved data.

5.2. Participant Feedback

The Training School was very well received with participant's providing very positive feedback. The majority of participants who provided feedback said that they would recommend the course to colleagues (98% with one participant recording "no opinion"), that the instructors provided helpful assistance and agreed that the course had exposed them to new knowledge and practices (see table 4 for a full summary of responses). Many participants made additional comments (see seven example comments below), such as:

'Course even better than expected. Course material was great with the books and website. Really great help from tutors. Best course I've been on for a long time. Learned a lot and will definitely conduct better experiments in the future!' (P2)

'This has been a FANTASTIC course in many ways. The topics covered 100% relevant to anyone doing experimental sciences, whether using animal models or not. It has been quite an eye-opening experience after nearly 20 years of scientific career due to the clarity of the concepts discussed here.' (P13)

'I would definitely recommend this course to colleagues and I would actually make it compulsory.' (P15)

'I think that this course should be undertaken by all research students. I wish I had done it during my PhD!' (P39)





'I was lucky enough to attend the 3Rs training workshop held in Nottingham this January. It was one of the most useful and enlightening training events I have ever attended. All staff attending from the [research institute] rated it very highly and we have given positive feedback to our senior management group at [research institute]. We have recommended the course as one all of our staff and postgrad student who are engaged in animal work and experimental design should be attending.' (Comments received via email following the course)

'The course was really useful; my only regret is that it was not available to me in this form and content early on in my professional career.' (Comments received via email following the course)

'I really enjoyed the course and I think it should be made more easily accessible to research students and staff through [research institute] short courses too. That would give a great boost to the quality of the research & publication outcomes and money savings! (Comments received via email following the course).

The VetBioNet team and the tutors were delighted to receive such positive feedback and this has encouraged the Nottingham VetBioNet team members to explore the possibility of hosting an additional Training School later in the project so that others can attend and benefit from this type of training. Opportunities to host a further 3Rs Training School at a VetBioNet partner institution will be explored.





Table 4

Training School Feedback Summary, Nottingham 2019

The Design of the Cou	rse						
The objectives of the course were clear to you			43	No opinion	1	Disagree	1
The course contents met with your expectations			45	No opinion	0	Disagree	0
The lecture sequence was	well planned	Agree	41	No opinion	2	Disagree	2
The contents were illustrate	ed with adequate examples	Too few	7	Enough	35	Too many	3
The academic level of the	course was appropriate	Too low	6	Correct	39	Too high	0
The course exposed you to	new knowledge and practices	Agree	42	No opinion	2	Disagree	1
You would recommend this course to your colleagues			44	No opinion	1	Disagree	0
The Conduct of the Co	ourse						
The lectures were clear and easy to understand			43	No opinion	2	Disagree	0
The course material provided was adequate			45	No opinion	0	Disagree	0
The group sessions were clear and easy to understand		Agree	40	No opinion	4	Disagree	1
The instructors provided helpful assistance		Agree	44	No opinion	1	Disagree	0
Background Informati	on						
Rate your confidence in applying the 3Rs when planning an experiment:	Decreased by	[1] [2] 0 0	[3] 0	[4] [5] [6] 0 0 0	[7] 0	[8] [9] 0 0	[10] 0
(1= no confidence, 10= very confident)	Increased by [0]	[1] [2] 9 10	[3] 11	[4] [5] [6] 4 5 1	[7] 2	[8] [9] 0 0	[10] 0
How did you hear about the School? *some gave >1 answer			14	Colleague	28	FRAME Website	1
Other, please specify 5		Flyer	0	Direct email	2	Facebook Twitter	1
Future Training							
I would be interested in furt the topics covered in this co	ther training to be able to teach ourse	Definitely	y 27	Maybe	14	Not at all	4

Responses: 45/45 (100%)





5.3. Knowledge Acquisition

To gain insight in to how successful training of this nature is, the Training School tutors designed a set of questions, which were given to the participants at the start and end of the course. The answers to these questions were collated and analysed to determine the participants' existing knowledge on the subject and then to establish if and what they had learned as a consequence of the training.

In line with previous events, there was an increase in understanding for all the areas tested (Figure 1) and the participants overall scores also increased after the training (Figure 2).

Figure 1 Comparison of the overall understanding by area of experimental design, before and after training by participants in the Nottingham Training School 2019

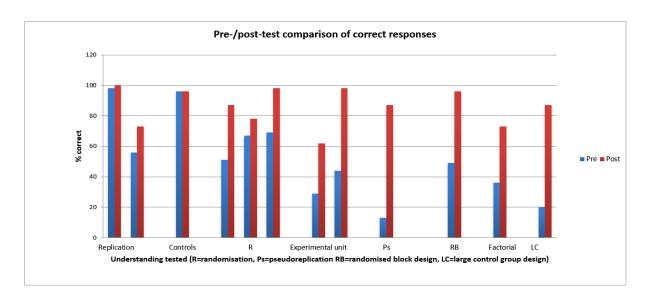
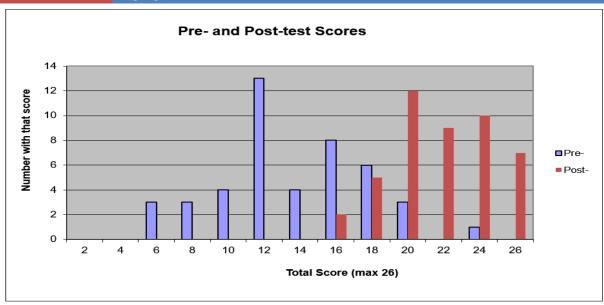






Figure 2 Comparison of participants' overall test scores before and after the Nottingham Training School, 2019.



5.4. FELASA Accreditation

Since 2017, the Training School has been accredited by Federation for Laboratory Animal Science Associations (FELASA) and is the first course of its kind to achieve this recognition. Therefore, it was possible to offer the participants the opportunity to take an examination to receive a FELASA certificate.

FELASA was established in 1978, it comprises of 21 independent European national and regional laboratory animal science associations that represent laboratory animal professionals in Europe. FELASA puts the 3Rs centre stage and advocates responsible scientific conduct with animals in the life sciences with particular emphasis on ensuring animal welfare. Historically, education and training have been a keystone of FELASA activities and an area in which it has had a great deal of influence in Europe (Guillen 2012). Indeed, in the absence of detailed standards for training in the European regulations the majority of European Union (EU) Member States follow the FELASA recommendations for courses aimed at researchers responsible for designing or conducting animal experiments (Franco and Olsson 2014).

The FELASA scheme is widely recognised within the European Laboratory Animal Science community and aims to fulfil two main purposes; 1) to promote quality education and training for all personnel based on both minimum requirements and competence and, 2) to recognise professional competence among European countries and facilitate the mobility of professionals across Europe (Guillen 2012).

Of the 45 participants taking part in the VetBioNet Training School, 41 opted to take the examination. All but one participant passed (this participant has accepted the opportunity to re-take the examination). As part of the accreditation process members of the FELASA Education and Training Board audit training courses. The 2019 event was subject to such an





audit. The VetBioNet team were very keen to continue the benefits of the FELASA accreditation as such were able to support the on-site audit process as part of VetBioNet Training School. The Training School received a positive audit result, with no formal recommendation being required.

6. Budget

The Training School was held in collaboration with VetBioNet, FRAME and the University of Nottingham. The Training School was coordinated by VetBioNet. The external tutors provided their time and expertise free of charge, so there is no associated remittance fee. As the host institution the University of Nottingham supplied the course facilities free of charge. VetBioNet provided facilitation and coordination of the course and covered the tutors' expenses. FRAME covered their administrative support cost. LASA (Laboratory Animals Science Association) and Laboratory Animals Ltd. kindly provided funding to supply all participants with two related text books.

Participants, through a registration fee, covered their own subsistence, accommodation, local transportation (between venue and accommodation), dinners and examination fees (for FELASA accreditation). Members of VetBioNet received funding from the project covering accommodation, subsistence and examination fee.

7. Conclusion

The aim of this deliverable (D5.4) was to organise a 3Rs training event, this report has outlined how this was very successfully achieved and provides impetus for further training of this nature. The FRAME/VetBioNet 3Rs Training School in Experimental Design, held at the University of Nottingham, 9-11 January 2019 met several of the VetBioNet Project aims and objectives including but not restricted to:

- providing graduate and post graduate training opportunities;
- defining and disseminating best practice, particularly in relation to the 3Rs and animal wellbeing;
- contributing to harmonisation of skills and best practice across the EU;
- facilitating researcher mobility and fostering new collaborations and knowledge exchange opportunities.

Exceptional organisation and highly skilled training provision resulted in the event receiving a very high level of positive feedback from participants and sector recognised auditors. The success of the course was also illustrated through the wide variety of participants and institutions motivated to attend it.





The examination and quiz results highlight the academic value of the course material and the continued financial support from additional funders as well as the industry recognised professional accreditation are indicative of the high regard in which this training is held.

In the process of delivering this training event some important limitations have become apparent. Even with an intense three-day programme it is not possible to adequately cover all aspects of experimental design and statistical analysis. It is clear that the development of extra courses reinforcing the subjects covered in this example and covering further topics within experimental design would greatly benefit researchers that use experimental animals (see Fry 2014 for suggested content).

A large number of participants (60%) said that they would definitely be interested in further training to be able to teach the topics covered in the Training School and 14 said that they may be interested, this is a total of 91% of participants showing interest in this further training. This indicates, as noted above, that there is a paucity in training of this nature available. It also suggests that again further training beyond the present course is needed, training where time is given to providing participants with examples and cases as well as background theory to enable them to feel confident in presenting and teaching the concepts of experimental design.

In order to preserve the interactive nature of the course and enable participants to maximise the opportunities to discuss the topic with fellow attendees and the tutors this Training School can only accommodate a maximum of 50 delegates. In the experience of the tutors and coordinators increasing the intake of the individual course would be detrimental to the learning experience and impact of the training. Therefore, there is an urgent need to embed this kind of course into large research programme as well as institutional training and competency programmes.

More broadly, while a few studies do exist (see above) there is a paucity of literature specifically examining the importance of education and training in the 3Rs and in particular in investigating the targeted and wider impacts this has on animal-based research and science more generally.

Given the success of this event and the factors discussed above the authors of this report would recommend continuing to deliver this Training School, for example working with VetBioNet partner institutions to develop a programme of inhouse education in this area. There is also great scope to design further training events with greater focus on Refinement and/or Replacement and to develop a 'Stage 2' Training School in Experimental Design. To support this rationale, it would be advantageous to conduct an empirical study on the availability of 3Rs training and the impacts that can result from it.





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