Veterinary
Medicines
Directorate

Animal usage in quality control tests for the batch release of Immunological Veterinary Medicinal Products (IVMPs) via the UK from 2007 to 2012.

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SUMMARY

This report reviews the status of animal use in the quality control (QC) of Immunological Veterinary Medicinal Products (IVMPs) released to the EU market via the UK between 2007 and 2012.

Key areas of improvement identified include:

- There was an 18% decrease in the total number of animals used over the six year period examined.
- 2011 and 2012 saw a substantial decrease in the numbers of animals used for the QC of IVMPs (down 37% from 2009), accompanied by a decrease in the average number of animals used per batch released (down 35% from 2010). There have been decreases in the use of larger animals such as cattle, sheep and dogs as well as hamsters.
- There has been a move away from challenge testing (71% in 2007 down to 58% in 2012), towards a less severe potency test based on serology (29% in 2007 up to 42% in 2012), in particular there has been a change from challenge potency test to serology test for all horse tetanus vaccines.
- A decrease of approximately 20% in the number of animals used for the Target Animal Batch Safety Test (TABST) of vaccines.
- A decrease of 40% in the number of animals involved in extraneous agents testing.
- 20% decrease in the number of animals used for the QC of leptospira vaccines from 2007 to 2012.

Key areas where improvements are ongoing

- With removal of the requirement for the TABST by the European Pharmacopoeia (Ph. Eur.) significant decreases will occur in the coming years. Based on 2012 figures this could result in an annual reduction of around 5,000 animals used in the QC of vaccines released via the UK from 2013 onwards.
- Further moves towards *in vitro* testing for potency of dog leptospira vaccines are expected in the future.
- Since April 2013 the monograph for Rabies vaccine (inactivated) for veterinary use includes a revision which gives further details on the serological potency assay to be used whenever possible as a less stressful alternative to the challenge potency assay using live animals. The serology assay will provide a significant improvement in terms of both the number of animals used and the severity of the test involved.
- Since April 2013 monographs for vaccines intended for fish (1521, 1580, 1581, 1950) no longer require the batch potency test to be performed on groups of not less than 30 fish. This allows flexibility in the design of these tests and the scope to justify the numbers of fish involved.

Key areas identified for improvement

- Multi-component clostridial vaccines represent only 1% of all UK authorised IVMPs however testing of these vaccines represents a disproportionately high animal usage (42% of all animals in 2010 for example), this consists predominantly of testing for toxicity and toxoid content in mice.
- Over 50% of UK authorised IVMPs base the release potency specification on a final product potency test performed in animals.
- *In vivo* extraneous agents testing is still required for some avian, bovine and porcine vaccines. Although vast improvements have been made in this area in recent years, developments in molecular techniques (for example Polymerase Chain Reaction) and increased measures to ensure consistency of production may further reduce the need for this test in the future.
- One way in which a reduction in animal testing could be achieved without any change to the authorised tests used would be for larger individual batches of products to be produced for release. This appears to be happening to some extent with fish vaccines and is something which could be an intermediate step in the reduction of animal numbers used in batch release testing.

INTRODUCTION

Background

Immunological Veterinary Medicinal Products (IVMPs), of which vaccines form the most common class of product, benefit human and animal health by preventing and controlling infectious agents that can cause disease and death. The routine use of quality control (QC) tests during and at the end of a manufacturing process is normal for all types of medicine. However, due to their biological origin, IVMPs have inherent variability and therefore a higher potential to vary from batch to batch. Consequently, coupled with a consistent manufacturing method, production requires the routine use of strict QC tests to ensure the consistent quality, safety and efficacy of each batch before it is released onto the market. Some of the tests employed can involve the use of animals and, depending on batch size and numbers of batches released to the market, relatively high numbers of animals can be used.

In Europe, the legal basis for the quality requirements for medicinal products is described in the European Pharmacopoeia (Ph. Eur.), which is supplemented by guidelines issued by the European Medicines Agency. IVMPs must comply with the Ph. Eur. monograph on veterinary vaccines and the accompanying texts, as well as specific monographs. Many of the requirements for QC testing are laid down in product specific Ph. Eur. monographs, many of which mention the use of animal tests.

In 1986 the EU adopted the concept to **R**educe, **R**efine or **R**eplace animals for experimental and other scientific purposes, known as the 3Rs concept (Russell and Burch, 1959), and consequently the Ph. Eur. continually reviews general texts and monographs to re-evaluate the relevance of the animal tests mentioned. As a result, many monographs now mention alternative *in vitro* methods to the traditional animal testing. For the remaining *in vivo* assays, strategies to promote the reduction or refinement of tests have been employed in recent years, for example the use of serological assays for control of potency as an alternative to challenge tests, and the introduction of humane endpoints. As a step further, from April 2013 the Ph. Eur. deleted the requirement for the target animal batch safety test (TABST) for all veterinary vaccines, which will result in a significant reduction in the number of animals used in the QC of veterinary vaccines from 2013 onwards. Changes to the Directive for the protection of animals used for scientific purposes (Directive 2010/63/EU), which came into full effect on 1st January 2013, strengthens the previous legislation and improves the welfare of those animals still needed to be used for scientific purposes. The new directive firmly anchors the principles of the 3Rs in legislation, and this can be expected to have a positive effect on the use of animals in the control tests of IVMPs in the future.

The tests for batch control do not necessarily have to be the same as those stated in the relevant Ph. Eur. monographs. A product must meet the specifications of the Ph. Eur., but the general monograph 'Vaccines for Veterinary Use' states "alternative test methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animal use or reduction of suffering". Any alternative test method must demonstrate that it can deliver equivalent results to those described in the Ph. Eur.

Scope

This report only examines the use of animals in the testing of batches of IVMPs released to the market, via the UK, for sale during 2007 to 2012. Products authorised as IVMPs include vaccines, immune sera, allergens, and *in vivo* diagnostics.

It should be noted that animals used during development of a product to demonstrate it is safe and efficacious do not fall within the scope of this report. Animals may also be used in the manufacture of products (although this is rare), as well as being a source of materials, such as serum, kidneys or chicken embryo fibroblasts, required for the conduct of certain tests. Such use of animals has not been included in this report as the VMD does not have access to such information. The term 'animal' throughout this report includes all vertebrates but not fertilised hens' eggs or primary cells derived from animals. The investigation reported on here did not include within its scope vaccines released for trial purposes under an animal test certificate (ATC), autogenous vaccines used to treat an outbreak of disease on a particular holding, or solvents and diluents.

Batch Release in the United Kingdom

The VMD is the competent authority for regulation of veterinary medicines in the UK. This means it is responsible for authorising the use of IVMPs in accordance with European (EU) and United Kingdom

(UK) legislation. A Marketing Authorisation (MA) is only granted after a detailed scientific assessment of the data relating to quality, safety and efficacy; this includes reaching an agreement on the release specifications and any required batch testing with the Marketing Authorisation Holder (MAH). QC tests for release of batches of IVMPs are performed by the manufacturer and must meet the specifications of the respective MA. Before a batch can be released onto the UK market the manufacturer submits the Batch Release Protocol (BRP) to the VMD for review. This contains details pertinent to the production of the product along with results from all in-process and finished product testing performed. If all the specifications are met, with no deviations to the authorised manufacturing procedure, the VMD issues a release certificate. This type of certificate (Article 81 of Directive 2001/82/EC) is a standardised EU certificate which is mutually recognised throughout the EU. As such the VMD will recognise EU Batch Release Certificates issued by other Member States under the same procedure. The VMD routinely operates this harmonised form of release which does not involve any re-testing, reliance being placed safely on the manufacturer's results. However, Member States are allowed to re-test batches of a number of specified product types considered to be higher risk (Article 82 of Directive 2001/82/EC) and some member states operate this routinely (Official Control Authority Batch Release (OCABR)). The VMD's approach helps avoid the unnecessary use of animals and we advocate this approach with the member states who continue to re-test certain products. This has meant that we have avoided the additional use of up to 5,000 animals for finished product control testing of IVMPs in 2012 alone (based on the current list of IVMPs for which OCABR has been agreed, https://www.edqm.eu/en/veterinary-biologicals-634.html).

Although this study examined all batches released to the EU market via the UK, it does not necessarily follow that all testing was performed in the UK. During authorisation of an IVMP the MAH must identify the GMP (Good Manufacturing Practice) compliant facilities responsible for all stages of production and QC testing. These may be located anywhere in the world, although all finished product testing must be performed at a site located within the EU. Therefore the numbers presented in this report cannot be correlated to figures released by the Home Office, which aim to estimate the numbers of animals used in all regulated procedures conducted in the UK (Home Office, 2012).

Purpose of Report

Competent Authorities and MAHs alike have a duty to ensure that animal usage is kept to a minimum and animal welfare legislation is upheld. The purpose of this study was to collate data the VMD holds on IVMPs authorised in the UK with records of all batches released via the UK between 2007 and 2012. This will enable analysis of how animal use fluctuates in the routine testing of IVMPs during production of batches for the market, and potentially highlight areas where efforts to advance alternative methods could be focused. It is also hoped that this report will promote the implementation of currently accepted alternative methods by the pharmaceutical industry as well as stimulating research in relevant areas.

Assumptions

It is important to note that all numbers presented in this report can only be considered an estimate. In collating the data held by the VMD the following assumptions were made:

- Retesting, in the case of failed outcomes or invalid tests, did not occur (under estimates numbers).
- The same control animals were not used for different batches (over estimates numbers).
- Unless stated, tests were not combined – which would result in the use of less animals (over-estimates numbers).
- In-Process Control Tests were performed on one antigen bulk per finished product (under-estimates numbers), and each bulk antigen only produced one finished batch (over-estimates numbers). In practice a number of antigen bulks are often blended to produce the finished product, with each antigen bulk often being split between a number of finished products.
- Finished product testing is generally performed on each batch of IVMP produced. However, in instances where a bulk IVMP is released in sub-batches, differing only in their vial size or filling session, some tests may be carried out only on the bulk IVMP or on one of the sub-batches. Sub-batches released during 2007 – 2012 have not been included in this study. It is assumed that QC tests involving animals would only have been performed on the finished bulk or first batch, and not each filling lot (without this assumption it is likely that the total numbers would be greatly over estimated, although the average per batch would remain unchanged).

ANALYSIS

Overview

Numbers of animals used for QC testing of all IVMPs from 2007 to 2012

At the beginning of this study (2007) a total of 283 IVMPS were authorised for use in the UK. During the period of the study an additional 107 MAs for new products were authorised, and the MAs for 89 products were expired. Throughout this period a total of 10,076 batches (not including sub-batches) were released, via the UK, which involved the use of approximately 300,000 animals in the respective quality control tests, these numbers are broken down by year in Table 1:

Table 1

	2007	2008	2009	2010	2011	2012
Total # animals used	56,039	54,423	72,477	72,386	60,673	45,776
Total # batches released	1,492	1,708	1,736	1,680	1,824	1,636
Average # animals per batch	38	32	42	43	33	28

From 2007 to 2010 there was an increase in the number of batches of IVMPs released onto the UK market, resulting in a concomitant increase of 32% in the number of animals used in the associated QC tests. There was also a 17% rise in the average number of animals used per batch of IVMP released. However, 2011 and 2012 saw a substantial decrease in the numbers of animals used (37% from the peak in 2009), accompanied by a decrease in the average number of animals used per batch released, down 35% from the peak in 2010, despite a similar number of batches being released.

Effect of Product Composition

It should be noted that the range in numbers of animals used in the QC of IVMPs to release a single batch is substantial: ranging from no QC test requiring the use of animals (of the 257 IVMPs released to the market during 2012, 30 required no QC testing in animals), to 592 animals involved in QC testing for one batch of finished product. The large range is attributable to the different types of products, for example inactivated vaccines generally require a greater use of animals than live vaccines, particularly for potency testing. Different active ingredients also require specific testing to ensure safety and efficacy of the product. This is discussed in greater detail throughout this report. The higher end of the range relates to the QC testing of three multi-component clostridial vaccines, each containing ten strains/serotypes.

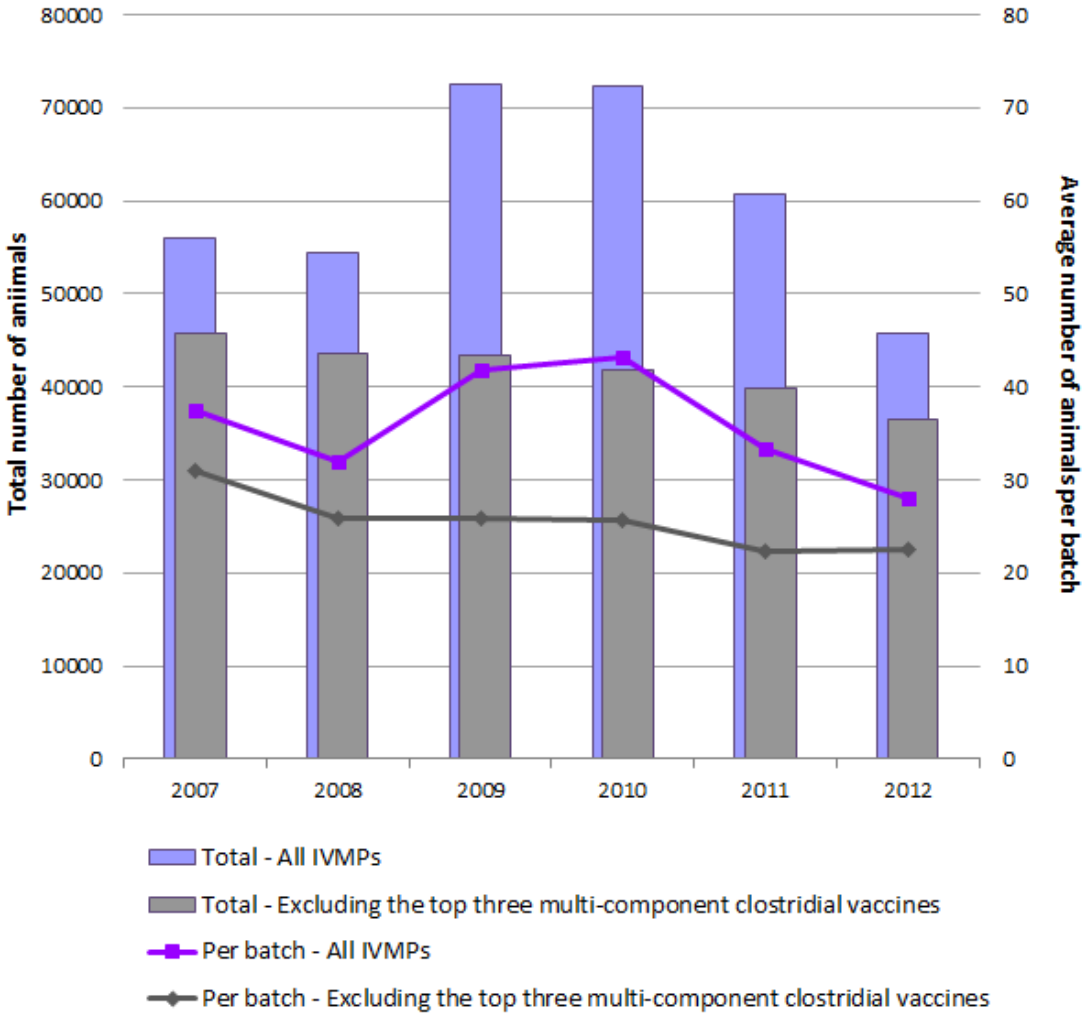
Peaks in numbers used

During 2009 and 2010 twice as many batches of the three multi-component clostridial vaccines were released onto the market than in the previous two years. This accounts for the large increases in the number of animals seen between 2009 and 2010 in the table above. Removing these three vaccines (which represent only 1% of all IVMPs authorised) from the analysis completely cancels out the increases seen in 2009/10 (see Figure 1 below). Indeed it is interesting to note that testing for these three vaccines alone represented 42% of all animals used in 2010 (mainly mice), but only 3% of all batches released (52 batches), this is discussed in greater detail later in the report.

Key Points

- A substantial decrease occurred in the numbers of animals used accompanied by a decrease in the average number of animals used per batch released in 2011 and 2012.
- The large increase in the number of animals seen between 2009 and 2010 can be accounted for by the release of multi-component clostridial vaccines which represented 42% of all animals used in 2010.

Figure 1. Animals used for QC testing of IVMPs for release to the market through the UK batch release system between 2007 and 2012



Species of animal used in QC testing of IVMPs

Figure 2 shows the total number of animals used in QC tests throughout the years 2007 to 2012 broken down by test species.

Mice are the most commonly used species for QC testing of IVMPs accounting for around half of the animals tested (this increased to 61% in 2009/2010). Chickens (17%), guinea-pigs (9%), hamsters (7%), and fish (6%) were the next most frequently used species. Animal testing on large mammals (dogs, cattle, cats, pigs, sheep and horses) accounted for only 3.5% of all animals tested over the six year period. A table indicating how these animals are used in the different types of QC tests can be found in the Supplementary Data section of this report.

Increases in total numbers used

There was an increase in the total numbers of certain species involved in QC tests over the six year period. However when analysed in terms of average numbers of animals used per batch released these represented decreases in some cases:

- Use of Chickens increased by 10%. However the number of batches of IVMPs released that involved QC tests on chickens also increased 32% over the same period and therefore this represents a decrease of 17% in the average number of chickens used per batch released.
- Use of pigs increased 18%. The number of batches of IVMPS released that involved QC tests on pigs also increased 44% over the same period and this represents a decrease of 18% in the average number of pigs used per batch released.
- Use of guinea-pigs increased 8%; this was with a concomitant rise in the average number of animals used per batch of 8%.

Decreases in total numbers used

There was a decrease in the numbers of some species involved in QC tests over the six year period:

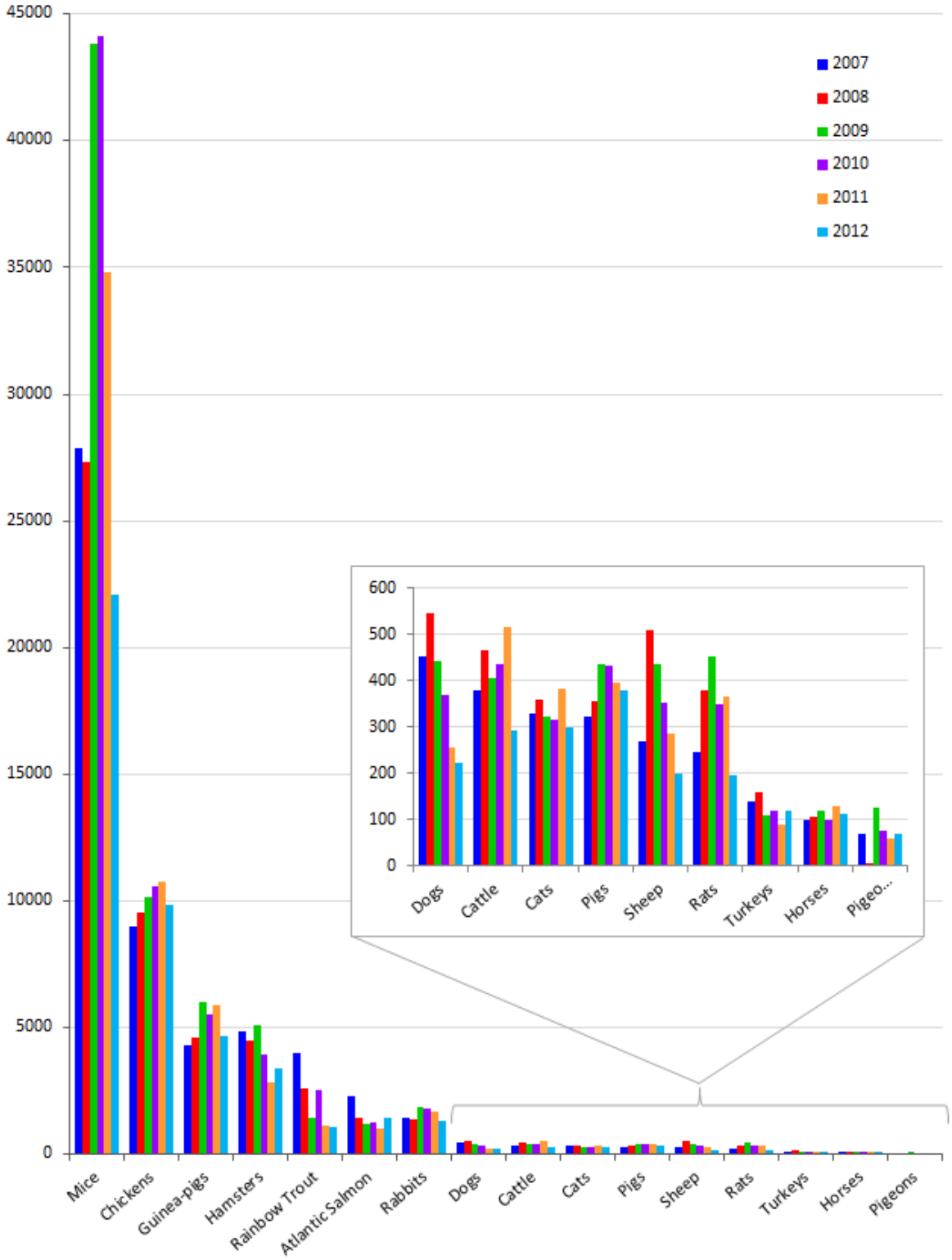
- Hamsters: total down 30%; per batch down 22%
- Dogs: total down 50%; per batch down 43%
- Cattle: total down 23%; per batch down 30%
- Sheep: total down 26%; per batch down 13%
- Rats: total down 20%; however there was in an increase in the average number of animals used per batch of 37%.

Numbers per batch are calculated based on the number of batches released for any IVMP which included an authorised QC test involving the stated animal at some point over the six year study period. Should a test have been removed during this time batches from later years are still included in the per batch figures to enable the analysis of any increasing or decreasing trend.

Key Points

- There have been decreases in the total and per batch use of larger animals such as cattle, sheep and dogs as well as hamsters.
- Over the six year period only one species, guinea pigs, has seen an increase in numbers used both in total and per batch.

Figure 2. Test animals used for quality control of vaccines from 2007 - 2012



Animals used in different types of QC tests

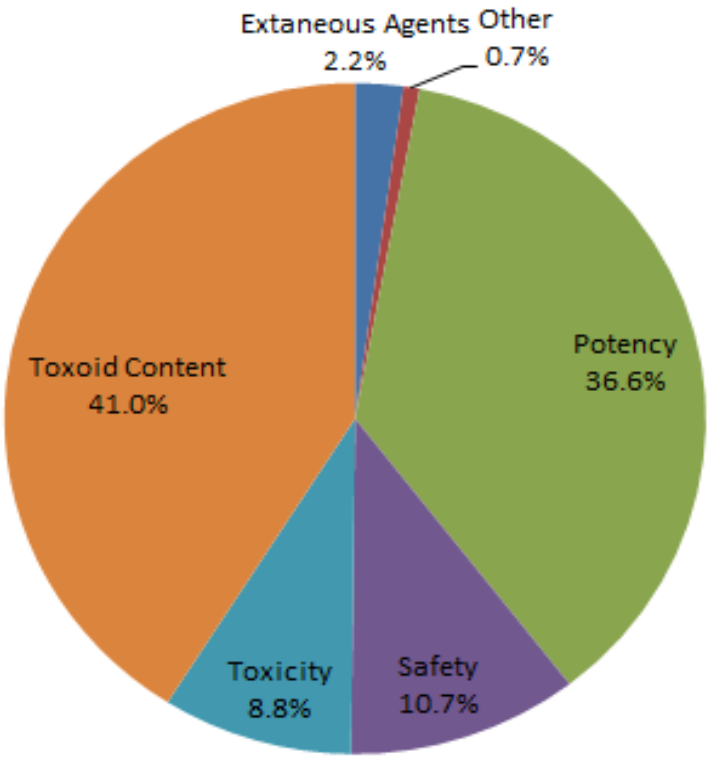
The majority of batch release tests do not require the use of animals, for example tests for bacterial and fungal sterility, absence from mycoplasma, tests for viral contamination using cell culture, virus titration in cell culture, live bacteria counts, and physico-chemical tests for adjuvant content, pH, etc.

Animal model systems are mainly involved in the testing of IVMPs for safety and potency. Safety tests can be either non-specific (extraneous agent testing, target animal batch safety test) or specific (freedom from specific toxicity, inactivation, minimal lethal dose test).

Averaged across the six year study period testing for toxicity and toxoid content, predominantly using mice (clostridial vaccines), accounted for 50% of all QC test procedures performed. Potency testing represented 37% of all tests performed, with safety testing making up 11%.

Figure 3 displays the percentage of animals used in different types of QC tests across the years 2007 - 2012:

Figure 3. Percentage of animals used in each QC test between 2007 - 2012



Animals used in QC Potency Testing

Potency of live vaccines is generally determined via a virus titration or bacteria count. However, tests for assessing potency of inactivated vaccines are frequently based on challenge models, in which vaccine-induced protection in the experimental animal is assessed by challenge with the virulent microorganism. As these tests involve disease modelling they are more severe than other options such as serology tests. Serology tests, based on measuring the serological response in the animal model after vaccination, are generally less severe and therefore are preferred to challenge tests.

Over 50% of authorised IVMPs base the release potency specification on a final product potency test performed in animals. The numbers of animals involved per year are displayed in Figure 4 and Table 2:

Figure 4. Animals used for potency testing of IVMPs

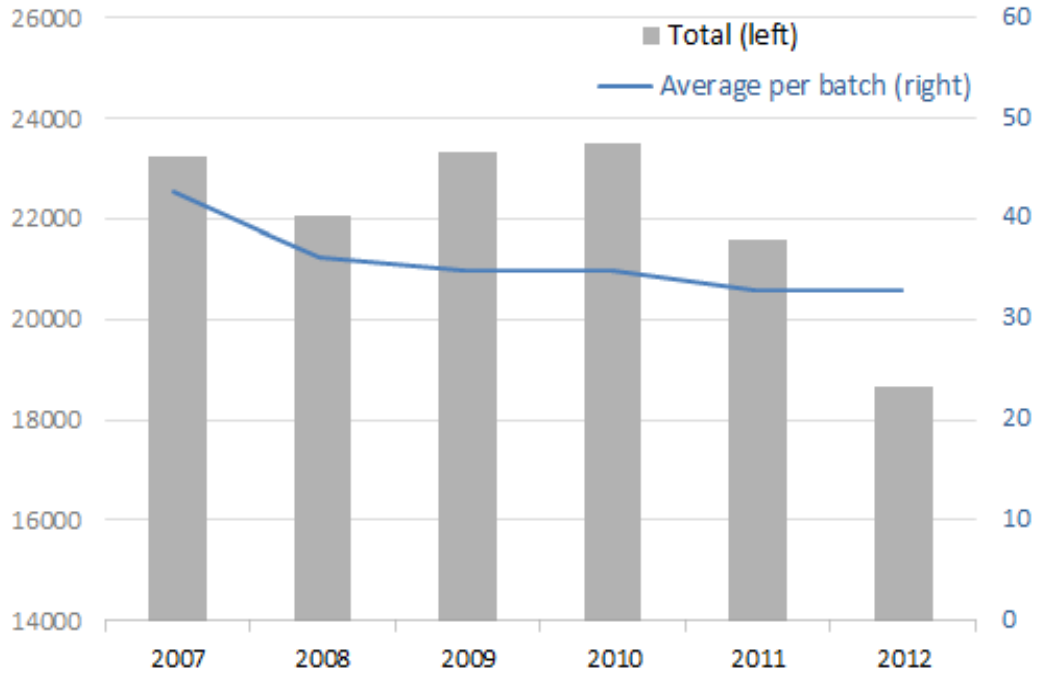
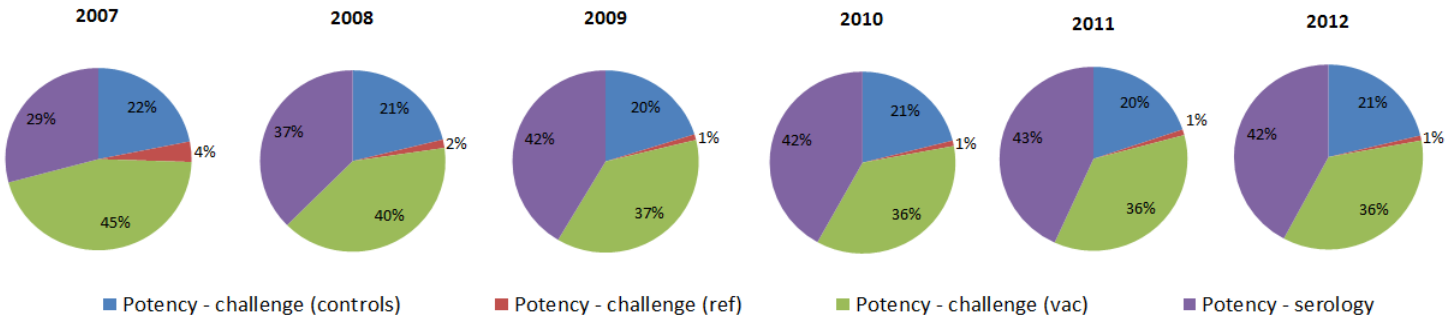


Table 2

	2007	2008	2009	2010	2011	2012
Total # animals used	23,237	22,045	23,308	23,476	21,575	18,671
Total # batches released	548	612	675	681	662	572
Average # animals per batch	42	36	35	35	33	33

As can be seen from Figure 4 the average numbers of animals used per batch released has decreased by approximately 23% over the six year period. Even more encouraging, as displayed in the pie charts in Figure 5, there has been a move away from challenge testing (71% in 2007 down to 58% in 2012), towards a less severe potency test based on serology (29% in 2007 up to 42% in 2012).

Figure 5. Distribution of animals used in the potency test of IVMPs from 2007 - 2012



The observed trend may in part be related to changes to certain Ph. Eur. monographs during the period of this study, for example:

- In 2007 a serology test for evaluation of potency of tetanus vaccines was introduced into the relevant monograph of the Ph. Eur.
- The Ph. Eur. allows for an alternative serological test or suitable validated *in vitro* test to assess the potency of canine leptospira vaccines. Although most products still employ the hamster challenge method for batch potency testing there are moves towards the less severe serological test by manufacturers with some products now employing the less severe alternative test.
- From April 2013 the monograph on Rabies vaccine (inactivated) for veterinary use was revised to include further details on the serological potency assay to be used whenever possible as a less stressful alternative to the challenge potency assay using live animals.

For some products the potency test is combined with tests for identity, safety, or extraneous agents, thereby reducing the number of animals required for release testing.

Key Points

- The average numbers of animals used per batch released has decreased by approximately 23% over the six year period examined.
- There has been a move away from challenge testing (71% in 2007 down to 58% in 2012), towards a less severe potency test based on serology (29% in 2007 up to 42% in 2012).

Animals used in the Target Animal Batch Safety Test (TABST)

Until 2013 it has been a requirement of the Ph. Eur. that a safety test should be performed on every batch of vaccine to ensure freedom from non-specific contamination. Since 2004 the general monograph on Vaccines for Veterinary Use has provided scope for the waiver of this test for established vaccines providing certain conditions are met. In 2012 the European Pharmacopoeia Commission adopted the deletion of the TABST from the Ph. Eur. for all veterinary vaccines (implementation date 1st April 2013).

This analysis looks at the use of the TABST over the period before removal of the requirements. The test is typically performed in the target species, generally involving two animals for mammals, at least 10 birds for avian vaccines, and at least 10 fish for fish vaccines.

The table below displays the number of animals involved in the finished product TABST of IVMPs from 2007 to 2012.

Table 3

	2007	**2008**	**2009**	**2010**	**2011**	**2012**
Total # animals used	7,344	6,697	6,312	6,539	5,774	5,884
Total # batches released	980	1,082	1,127	1,110	1,158	1,027
Average # animals per batch	8	6	6	6	5	6

The average number of animals used in the TABST per batch released has decreased 22% over the six year period from 2007 to 2012 (total number of animals used has reduced 18%). However, in 2012 there were still 5884 animals involved in the safety test for the release of IVMPs. The deletion of the TABST goes a step further than the previous option of waiving the test for established vaccines, and this is one area where we can expect to see a vast improvement in the numbers of animals involved in the QC testing of IVMPs in the coming years.

Key Points

- The average number of animals used in the TABST per batch released has decreased 22% over the six year period from 2007 to 2012 (total number of animals used has reduced by 18%).
- With removal of the requirement for the TABST by the Ph. Eur. significant decreases in the number of animals used will occur in the coming years. Based on 2012 figures this could result in an annual reduction of around 5,000 animals used in the QC of vaccines released in the UK from 2013 onwards.

Animals used in Extraneous Agents Testing

Despite the many precautions taken during IVMP development and production, the very nature of these biological materials means there is a potential for contamination of the IVMP with unknown extraneous agents. Detection of possible contaminants in the finished product is a requirement in a number of Ph. Eur. monographs, mainly for chicken vaccines, but also the Ph. Eur. includes the requirement in some product specific monographs for other species including cattle, pigs and rabbits.

All monographs for inactivated avian viral vaccines require extraneous agent testing to be performed in 10 chicks. For live avian viral vaccines, prior to the period examined, the Ph. Eur. introduced a new monograph '2.6.25. Avian live viral vaccines: tests for extraneous agents in batches of finished product'(5th Edition 2005) which replaced the previous requirement for extraneous agent testing to be carried out in chicks, and introduced tests in specific pathogen free (SPF) eggs and cell culture for this purpose instead. However, there is still a provision within the monograph to allow testing in chicks should the manufacturer justify the need.

During the period of this report 45 products authorised for use in the UK included a requirement for extraneous agent testing prior to release; the majority of these are indicated for use in avian species (chickens, turkeys, pigeons) and perform the extraneous agents test in SPF chicks, with two products indicated for use in rabbits, one for use in cows and one in pigs. Extraneous agent testing made up only 2.2% of the total animals used for the QC of IVMPs from 2007 to 2012. The numbers of animals involved per year are displayed in Figure 7 and Table 4:

Figure 7. Animals used for extraneous agent testing of IVMPs

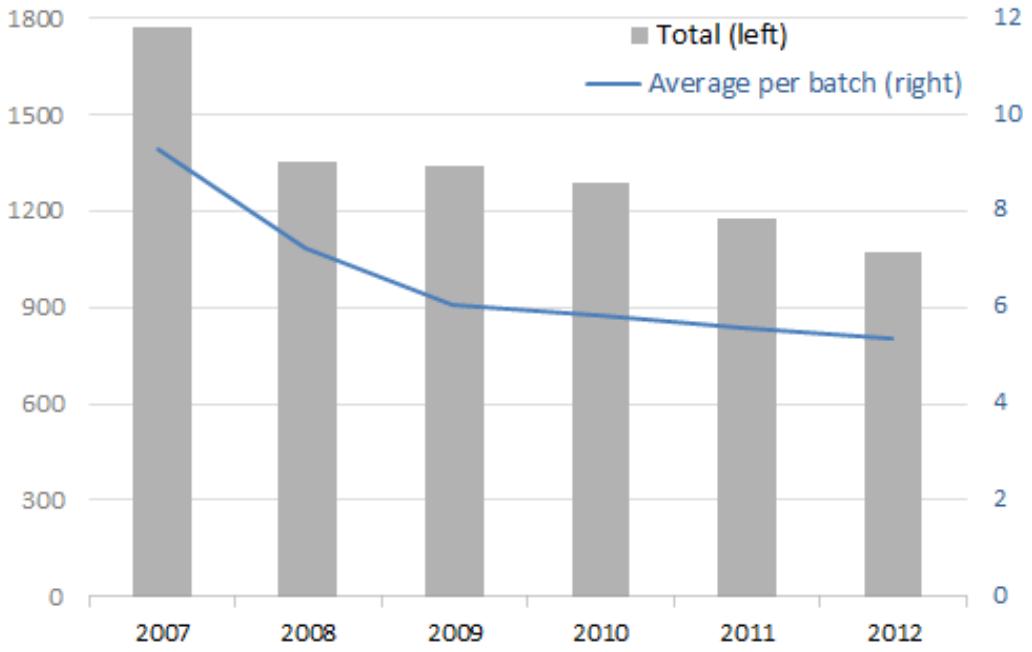


Table 4

	2007	2008	2009	2010	2011	2012
Total # animals used	1,772	1,350	1,336	1,290	1,178	1,074
Total # batches released	192	188	223	223	213	203
Average # animals per batch	9	7	6	6	6	5

For twenty-one of the products requiring an extraneous agent test in animals the test is performed concurrently with the target animal batch safety test, thereby helping to minimise the total number of animals used for the batch release of these products.

Since the implementation of the 5th Edition of the Ph. Eur. manufacturers have submitted variations for a number of vaccines to replace the extraneous agent testing with the *in vitro* tests described in the new monograph. As such there has been a 40% drop in the number of chicks used for extraneous agent testing of IVMPs since 2007. In 2012 only 28 of the 81 authorised poultry vaccines still had a requirement for extraneous agent testing in chicks remaining on their authorisation.

Key Points

- There has been a 40% drop in the number of chicks used for extraneous agents testing.
- Many products requiring an extraneous agent test in live animals perform the test concurrently with the target animal batch safety test, thereby helping to minimise the total number of animals used for the batch release of these products.

Animals used in Toxicity Testing

The only toxicity tests detailed in the Ph. Eur. are the toxicity testing of tuberculin in guinea-pigs, absence of toxin and irreversibility of toxoid of *Clostridium tetani* in guinea-pigs, and residual toxicity of *Clostridium botulinum*, *Clostridium novyi* (type B), *Clostridium perfringens*, and *Clostridium septicum* in mice. However, there is also a requirement in the General Monograph 'Vaccines for Veterinary Use' for tests for toxoiding to be carried out immediately after toxoiding and on the bulk blend of toxoids before adding the adjuvant. As a result a relatively large number of toxicity tests are performed during the manufacture of clostridial vaccines. Production of clostridial vaccines involves growing the bacteria to produce large amounts of toxins, then inactivating the culture so that it is no longer toxic. The manufacturer must demonstrate that the inactivated bulk antigen is potent, non-toxic and non-viable. A test for estimation of toxoid content performs the first task, an absence of toxin test the second and toxicity testing performs the final task. Most of these toxins rely on measurement in an animal model (a mouse) to determine their presence.

From 2007 to 2012 the number of animals involved in the tests for toxicity and toxoid content combined are displayed in Table 5 and Figure 8:

Table 5

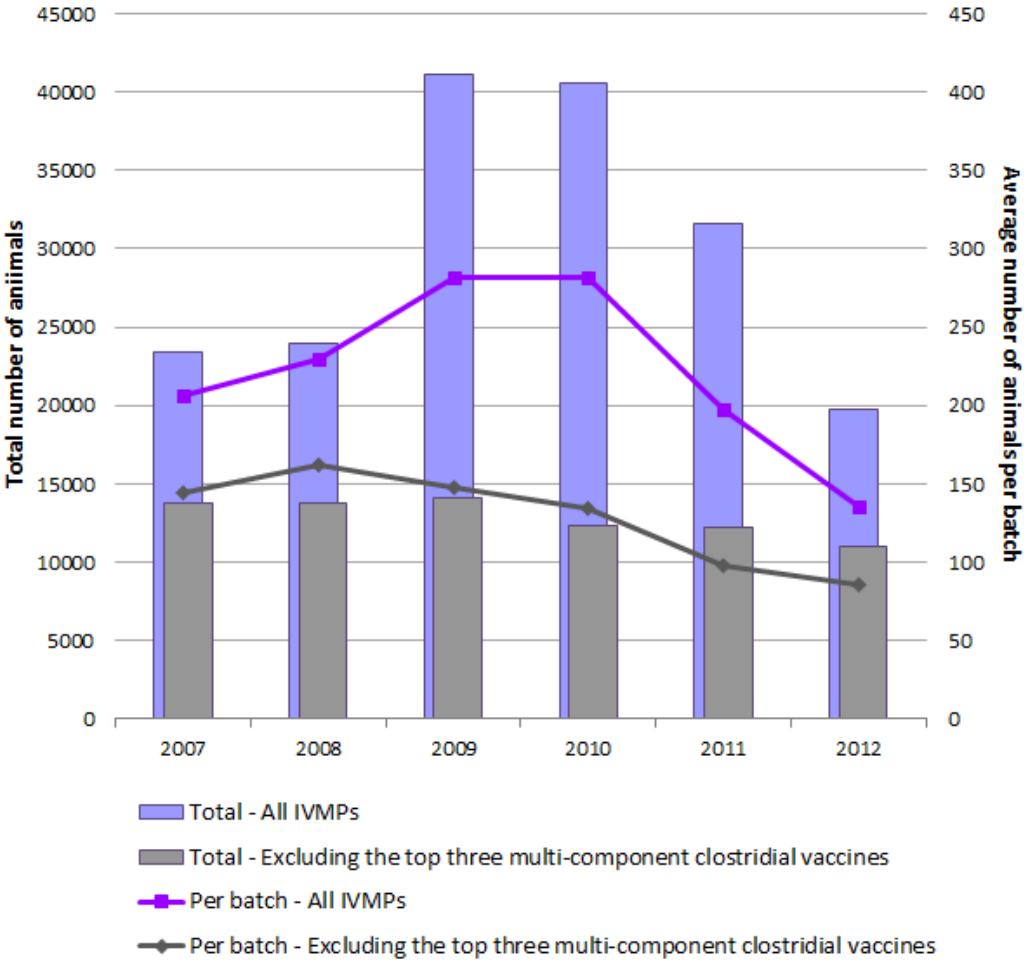
	2007	**2008**	**2009**	**2010**	**2011**	**2012**
Total # animals used	23,373	23,910	41,112	40,572	31,565	19,704
Total # batches released	113	104	146	144	160	145
Average # animals per batch	207	230	282	282	197	136

As previously observed in the overview of animal usage in all IVMPs, for the three clostridial vaccines which require the most animal testing, twice as many batches were released in 2009/10 than in the two previous years – as the predominant use of animals for QC of these vaccines is in the toxicity and toxoid content tests Figure 8 also displays the numbers of animals used for toxicity testing excluding these three vaccines for comparison. This clearly demonstrates the relatively large difference they make to the numbers of animals used.

Key Points

- Toxicity and toxoid testing combined requires the use of the most animals for the release of IVMPs.
- Toxicity and toxoid testing is mainly required to ensure the safety of clostridial vaccines, and is predominantly performed in mice.

Figure 8. Animals used for toxicity testing of IVMPs



Vaccine Type

As mentioned previously in this report the range of animals used in the QC of IVMPs to release a single batch is substantial; ranging from no QC test requiring the use of animals, to 592 animals involved in QC testing for one batch of finished product alone.

The higher end of this range relates to the QC testing of clostridial vaccines. Vaccines indicated for use in fish, chickens, and those indicated to provide protection against leptospira or rabies also tend to use large numbers of animals for batch release testing. For this reason, in the following section of this report, all products authorised for use in the UK have been divided in to the following categories:

- Clostridial vaccines
- Rabies vaccines
- Leptospira vaccines
- Vaccines for use in chicken
- Vaccines for use in fish
- All other IVMPs

This enables further analysis of the trends and fluctuations in animal usage in the production of IVMPs.

Animals used for QC testing of clostridial vaccines from 2007 to 2012

Clostridial vaccines are the category of IVMP which requires the largest number of animals to ensure the quality, safety and efficacy of each batch of product released as seen from analyses earlier in this report.

Clostridial bacteria are widely distributed in the environment, in addition to being present in the gut of animals. The bacteria are responsible for a large proportion of the unexplained sudden livestock deaths that most farms experience. Tetanus is the most well-known of the clostridial diseases, and is especially problematic for horses. Infected horses usually die or require euthanasia. Additional clostridial bacteria include *C. chauvoei* (cause of blackleg), *C. novyi* (causes black disease), and *C. haemolyticum* (causes bacterial redwater). Other clostridial bacteria causing sudden death in cattle and sheep include *C. sordelli* and *C. perfringens*. Treatment of clostridial diseases with antitoxins and large doses of antibiotics is expensive and generally not successful, nor does it follow the principles of responsible use of antibiotics. The use of vaccines is required to give animals protection against these diseases, thereby avoiding the use of antibiotics.

The total numbers of animals involved in the testing of all clostridial vaccines from 2007 to 2012 are displayed in Figure 9 and Table 6:

Figure 9. Animals used for QC testing of clostridial vaccines

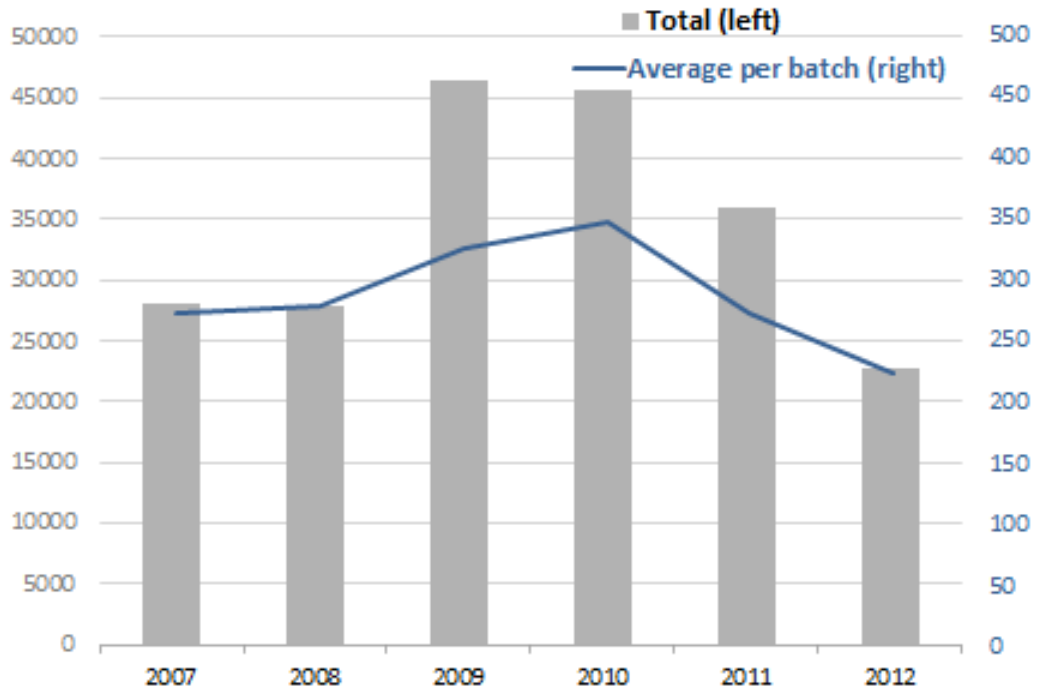


Table 6

	2007	2008	2009	2010	2011	2012
Total # animals used	27,994	27,894	46,416	45,703	36,022	22,703
Total # batches released	103	100	143	132	132	101
Average # animals per batch	272	279	325	344	273	225
Range of animals per batch	32 - 592	22 - 592	22 - 592	22 - 592	22 - 592	22 - 592

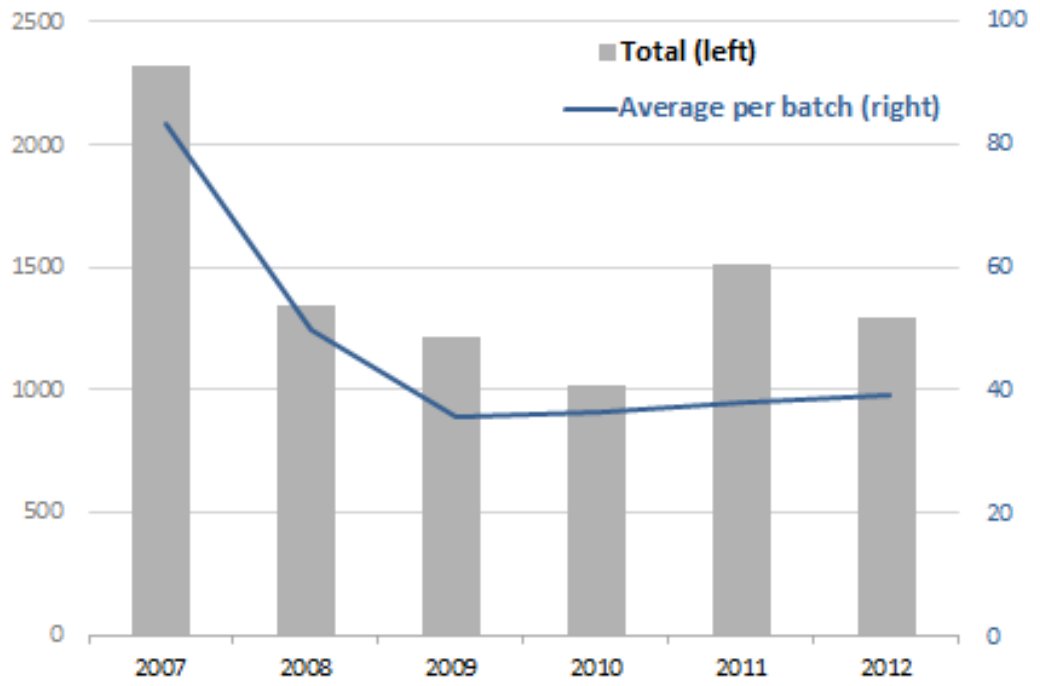
There were 23 authorised clostridial vaccines available in the UK over the period of this report, with the testing of clostridial vaccines accounting for 50% up to 64% (in 2009) of all animals used in the QC of IVMPs.

Clostridial vaccines are usually multi component products containing up to 11 different clostridial strains/serotypes. They are also often combination vaccines against additional disease pathogens for example *E. coli*, Equine influenza virus, *Mannheimia haemolytica*, and *Pasteurella trehalosi*.

Consequently the vaccines contained in this category may contain from one up to 20 different inactivated active ingredients, each of which requires testing to demonstrate potency, and for some components freedom from toxicity, in animal models which means that the number of animals involved in the QC of clostridial vaccines to release one individual batch can range from 22 up to 592. If these numbers are further sub-divided between the number of active ingredients each vaccine contains, this range reduces to approximately 4 to 59 animals per active ingredient.

For almost every product the animals used in both 'in process' and 'finished product' testing have remained constant across the six years. This is with the exception of *C. tetani* vaccines indicated for use in horses where there has been a decrease of over 50% in the numbers of animals used per batch as displayed in Figure 10. A serology test for evaluation of potency of tetanus vaccines was introduced into the relevant monograph of the Ph. Eur. in 2007 and for all vaccines with UK MAs the manufacturers have since implemented this test.

Figure 10. Animals used for QC testing clostridial vaccines indicated for use in horses



Key Points

- Clostridial vaccines contribute a disproportionately high number of animals in testing to the overall figures because of the complexity of product combinations and the need for toxicity and toxoid tests.
- The number of animals used per batch have stayed stable over six years with the exception of *C. tetani* vaccines indicated for use in horses where there has been a decrease of over 50% in the numbers of animals used per batch released.
- A 2013 meeting between the European Commission, Regulatory Authorities and Industry focused on the need to replace animal testing in clostridial vaccines. The background to the meeting was the development of proof of principle *in vitro* cell culture methods as alternatives to the animal models for toxicity and antigenicity (Redhead et al., 2012). In the longer term it is hoped that replacement of the mice models for toxicity and potency testing of clostridial vaccines may be possible.
- It is acknowledged that implementation of any new test for existing products would be a lengthy and complicated process, requiring extensive validation.

Animals used for QC testing of rabies vaccines from 2007 to 2012

The UK is considered free of rabies in terrestrial animals. However, in order to maintain the country's rabies free status pet owners must get their dog or cat vaccinated against the disease before they can enter the UK. During the period of this report a total of six veterinary vaccines against rabies were authorised for use in the UK (one for use in foxes which wasn't marketed in the UK), 13 to 21 batches are released each year requiring the following number of animals for batch release:

Figure 11. Animals used for QC testing of rabies vaccines

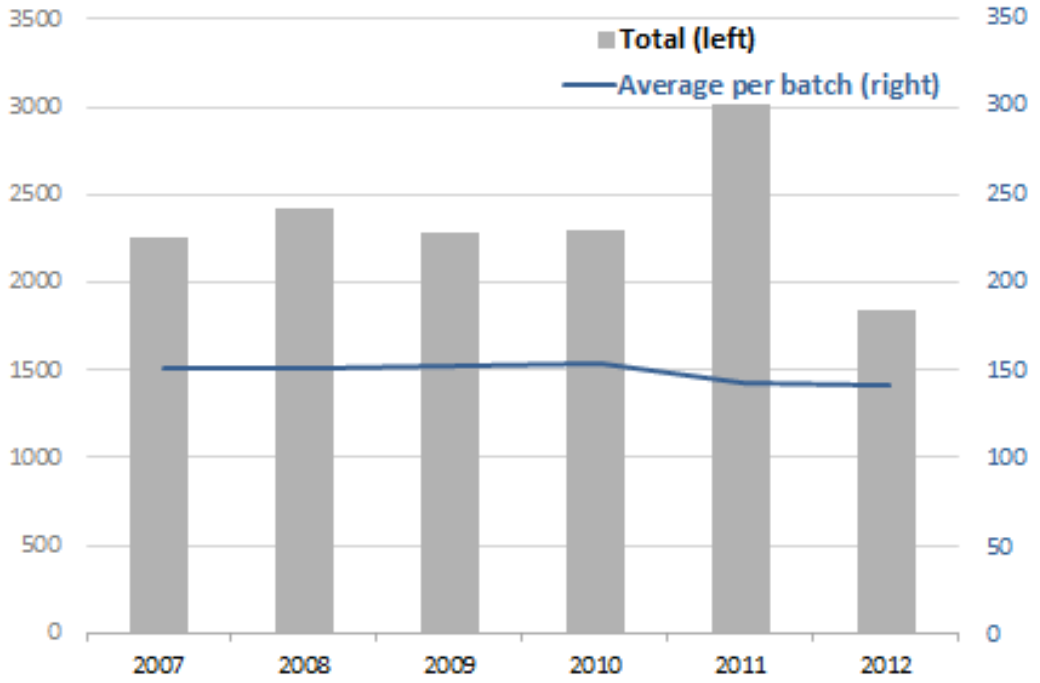


Table 7

	2007	2008	2009	2010	2011	2012
Total # animals used	2,260	2,422	2,290	2,300	3,012	1,846
Total # batches released	15	16	15	15	21	13
Average # animals per batch	151	151	153	153	143	142
Range of animals per batch	142-162	142-162	142-162	142-162	2 - 162	2 - 162

All inactivated rabies vaccines for veterinary use authorised in the UK are tested for potency before release using the challenge method in mice, as required by the Ph. Eur. (monograph 0451). This is the most widely used and internationally recommended potency assay for batch testing of inactivated rabies vaccines and requires a relatively large number of animals, 140 mice for each test.

However, from April 2013 the monograph on Rabies vaccine (inactivated) for veterinary use was revised to include further details on the serological potency assay to be used whenever possible as a less severe alternative to the challenge potency assay using live animals. The serology assay will provide a significant improvement in terms of both the number of animals used and the severity of the tests involved (Krämer et al., 2010).

To date no manufacturers with UK MAs have replaced the challenge test with the serological test. It is acknowledged that this would be a lengthy process requiring both time and money to validate the new methodology for use with specific vaccines. However, at least one manufacturer has been in communication with the VMD regarding the implementation of a new serology test for rabies vaccines. Other manufacturers will be strongly encouraged to follow suit.

In February 2011 a rabies recombinant canarypox vaccine for use in cats was authorised. Since the vaccine is a recombinant product, potency can be measured in such a way that means there is no need for the challenge test in mice, and hence vastly reducing the number of animals required for batch release.

Key Points

- The number of animals used per batch for the QC testing of vaccines indicated for use against rabies have stayed stable over six years.
- Discussions the VMD has held with manufacturers of rabies vaccines suggests future implementation of a new serology test for rabies vaccines, replacing the more severe challenge test, may be possible.
- Regulatory authorities acknowledge that implementation of the serology test for existing products would be a lengthy and complicated process.

Animals used for QC testing of leptospira vaccines from 2007 to 2012

Leptospirosis is a potentially fatal disease of animals and humans caused by infection with the spirochete *Leptospira,* which is spread via the urine of infected animals. The bacteria are divided into strains (or serovars), based on the characteristic of their surface proteins. Leptospirosis is a common infection in dairy and beef herds, resulting in infertility, abortion, and poor milk yield. It is also a common disease in dogs, and can result in liver and kidney damage if left untreated. Vaccination is used to control the spread of the disease, with each batch of vaccine being tested for potency in a laboratory animal model prior to release.

During the period of this study a total of twenty vaccines against leptospira were authorised in the UK, two of which were indicated for use in cattle, with the remaining being used for the vaccination of dogs, often in combination with antigens against other dog pathogens. The numbers of animals involved in the release testing for these vaccines are displayed in Table 8 and Figure 12.

Figure 12. Animals used for QC testing of leptospira vaccines

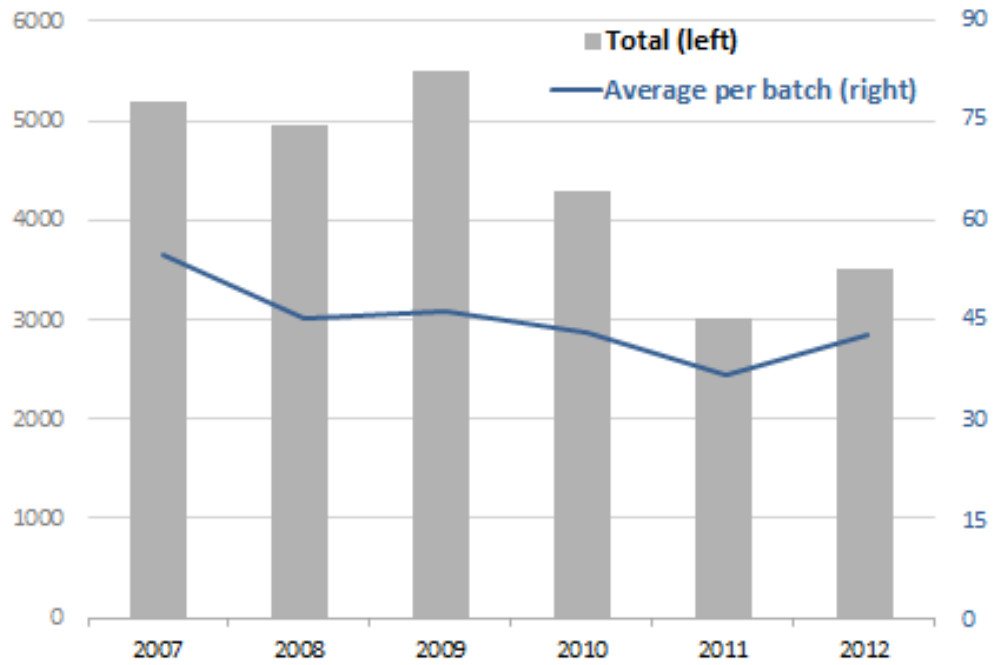


Table 8

	2007	2008	2009	2010	2011	2012
Total # animals used	5,187	4,967	5,511	4,292	3,022	3,512
Total # batches released	95	110	119	100	82	82
Average # animals per batch	55	45	46	43	37	43
Range of animals per batch	22 - 144	22 - 84	22 – 84	20 - 84	2 - 84	2 - 84

The Ph. Eur. includes monographs on canine leptospirosis and bovine leptospirosis vaccines (inactivated).

The hamster challenge test is applied for batch potency testing of canine vaccines. However the Ph. Eur. also explicitly allows for an alternative serological test or suitable validated *in vitro* test, to determine the content of one or more antigenic components indicative of protection, to be performed.

The monograph for bovine leptospirosis vaccines describes the use of a serological test in guinea pigs, or alternative validated method.

The decrease in number of animals used per batch (approximately 20%) seen in Figure 12 relates mainly from a reduction in the total number of animals used in the hamster challenge test over this

period. Additionally, one bovine leptospira vaccine manufacturer moved entirely to *in vitro* testing for potency during the period of this report.

Encouragingly, at a January 2012 Ph. Eur. workshop, the suitability of the hamster potency test was questioned and unanimous agreement was reached that moving toward complete *in vitro* testing is possible and should be promoted (Bruckner, 2013). During the period of this report no UK MAH for dog leptospira vaccines had submitted a variation to implement the more humane alternative tests to the hamster challenge test. It is acknowledged that this would be a lengthy process requiring both time and money to fully validate the new methodology. Nevertheless in the future it is likely that manufacturers will start to generate the data in order to implement *in vitro* testing for leptospira vaccines and indeed the VMD is aware of moves in this direction.

Key Points

- 20% decrease in the number of animals used for the QC of leptospira vaccines from 2007 to 2012.
- Move to *in vitro* potency testing for one bovine leptospira vaccine.
- Further moves towards *in vitro* testing for potency of dog leptospira vaccines expected in the future.

Animals used for QC testing of chicken vaccines from 2007 to 2012

Vaccines indicated for use in chickens are the largest group of vaccines consisting of 74 UK authorised products during the years 2007 to 2012, many of which are multi-valent vaccines. As a result a relatively large number of animals, almost exclusively chicks, are involved in the QC testing of this category of IVMP, as displayed in Figure 13 and Table 9.

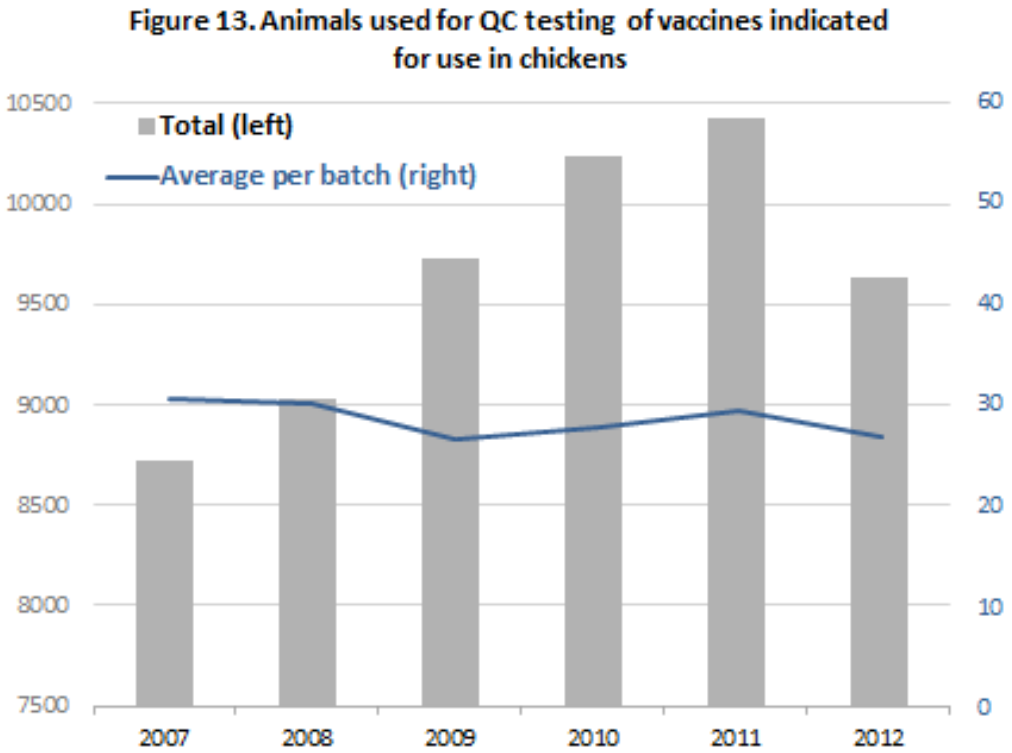


Table 9

	2007	2008	2009	2010	2011	2012
Total # animals used	8,725	9,025	9,735	10,235	10,432	9,635
Total # batches released	285	299	366	367	356	358
Average # animals per batch	31	30	27	28	29	27
Range of animals per batch	0 - 85	0 - 130	0 - 130	0-130	0 - 220	0 - 85

Currently the Ph. Eur. has 20 product specific monographs in relation to vaccines against chicken pathogens, many of which mention the use of animals for QC testing of the finished product, for example:

- All monographs for inactivated viral vaccines require extraneous agent testing to be performed in 10 chicks; they also include scope for potency to be assessed via serology or challenge in chicks.
- All monographs for inactivated bacterial vaccines allow for potency to be assessed in chicks via challenge or serology.
- All live viral avian vaccines now require extraneous agent testing to be performed in SPF eggs and cell culture as a replacement to testing in chicks. However, as mentioned previously there is a provision within the Ph. Eur. to allow testing in chicks should the manufacturer justify the need and this is often the case for Newcastle Disease vaccines where it is technically necessary due to difficulties neutralising the vaccine virus.
- Vaccines against coccidiosis (Eimeria vaccines) which are multi component (containing 5 to 8 Eimeria species) require a relatively large number of chicks for determination of potency; of the four products authorised for use in the UK this ranges from 60 animals per batch up to 220.

Key Points

- Across the entire range of chicken vaccines the total number of animals has increased, in line with an increase in the number of batches released, however the average number of animals used for QC purposes per batch released has remained consistent, averaging 26 – 30 animals per batch.
- Twelve products have replaced extraneous agent testing in chicks with testing in SPF eggs and cell culture during the six year study period, and two products have reduced the number of animals used for batch safety testing.
- Safety testing makes up approximately a third of all testing of chicken vaccines. From April 2013 no UK authorised vaccine for use in chickens will have a requirement for a finished product safety test in chicks remaining on their licence.
- No change to the batch potency test for any UK authorised chicken vaccine was implemented during the study period. Currently, potency testing accounts for just over half of all animal testing for chicken vaccines, and challenge tests make up two thirds of this number.

Animals used for QC testing of fish vaccines from 2007 to 2012

At present the Ph. Eur. contains four monographs related to vaccines indicated for use in salmonids (salmon and trout):

- Furunculosis vaccine (1521)
- Vibriosis (cold-water) vaccine (1580)
- Vibriosis vaccine (1581)
- Yersiniosis vaccine (1950)

Between 2007 and 2012 fourteen UK authorised vaccines for use in trout and salmon were released via the UK, requiring between 80 and 260 fish to be tested for safety and potency per batch released, total numbers are displayed in Figure 14 and Table 10.

Figure 14. Animals used for QC testing of vaccines indicated for use in fish

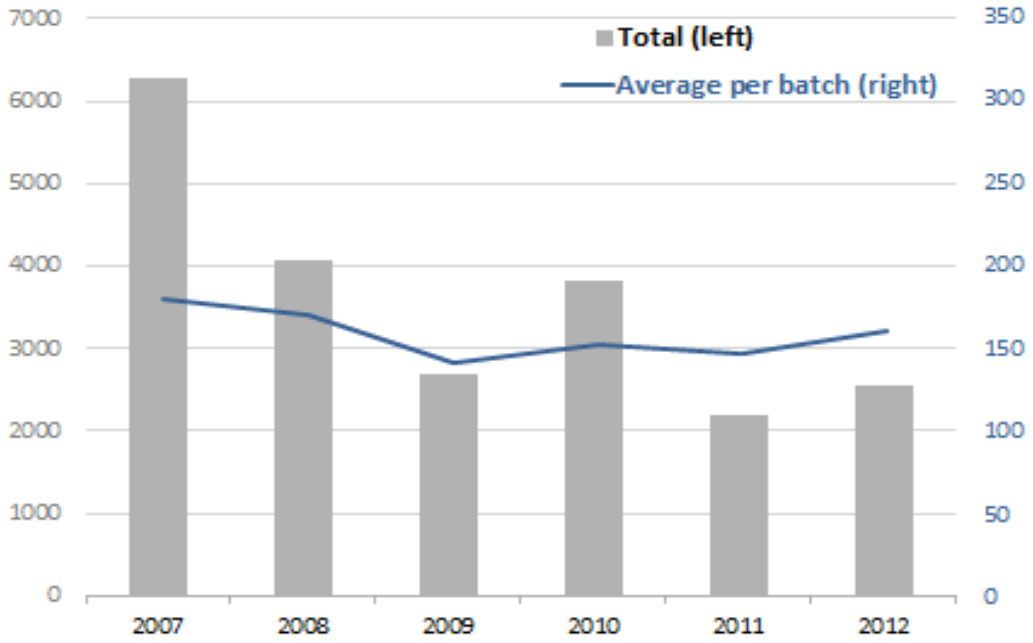


Table 10

	2007	2008	2009	2010	2011	2012
Total # animals used	6,280	4,080	2,680	3,820	2,200	2,560
Total # batches released	35	24	19	25	15	16
Average # animals per batch	179.4	170.0	141.1	152.8	146.7	160.0
Range of animals per batch	80 - 260	80 - 260	80 - 260	80 - 260	80 - 260	80 - 260

All authorised products are inactivated vaccines, many are multi-valent, and as such require the use of relatively large numbers of animals to assess potency. Over the period of study the EU guidance and the Ph. Eur. Monographs have had different recommendations regarding numbers of fish to be used in testing. Consequently most vaccines indicated for use in fish and tested over the period examined in this report perform the batch potency test in groups of 30 or more fish. However, revisions of guidance and monographs are on-going, allowing scope for the number of animals involved in these tests to be justified by the MAH. The current monographs also indicate that, alternatively, a potency test based on antibody response may be acceptable.

Although the total number of fish involved in the QC testing of vaccines has decreased over the six year period this is in fact due to a decrease in the number of batches released each year during this time. There has been no change in the number of animals per batch of product released across the years 2007 to 2012. However, there is some suggestion that larger batch sizes for some products were being manufactured and released in the second half of the period examined, allowing the same

numbers of fish to be treated, whilst at the same time reducing the numbers of fish required to release the products.

Batch safety testing of fish vaccines is responsible for around 40% of all fish used, and with the removal of this test from April 2013 we can expect to see a dramatic decrease in the number of fish required to batch release this category of vaccine in the coming years.

Key Points

- Total numbers of fish involved in the QC testing of vaccines has decreased although the average number required to release one batch has remained constant.
- Removal of the requirement for the TABST from the Ph. Eur. should result in a 40% decrease in the number of fish involved in the control testing of fish vaccines.
- There is some evidence to suggest larger batches of product are being manufactured, resulting in a decrease in the total number of fish required for QC each year.

Animals used for QC testing of all other IVMPs from 2007 to 2012

This category includes all other IVMPs authorised for use in the UK which do not fall in to any of the preceding classifications. This is the largest category of IVMP with approximately 1,000 batches released via the UK each year however it is also the category which uses the least number of animals per batch released, with an average of less than 6 animals per batch, and a slight downward trend observed over the period of the study, see figure 15 and Table 11.

Figure 15. Animals used for QC testing of all other IVMPs

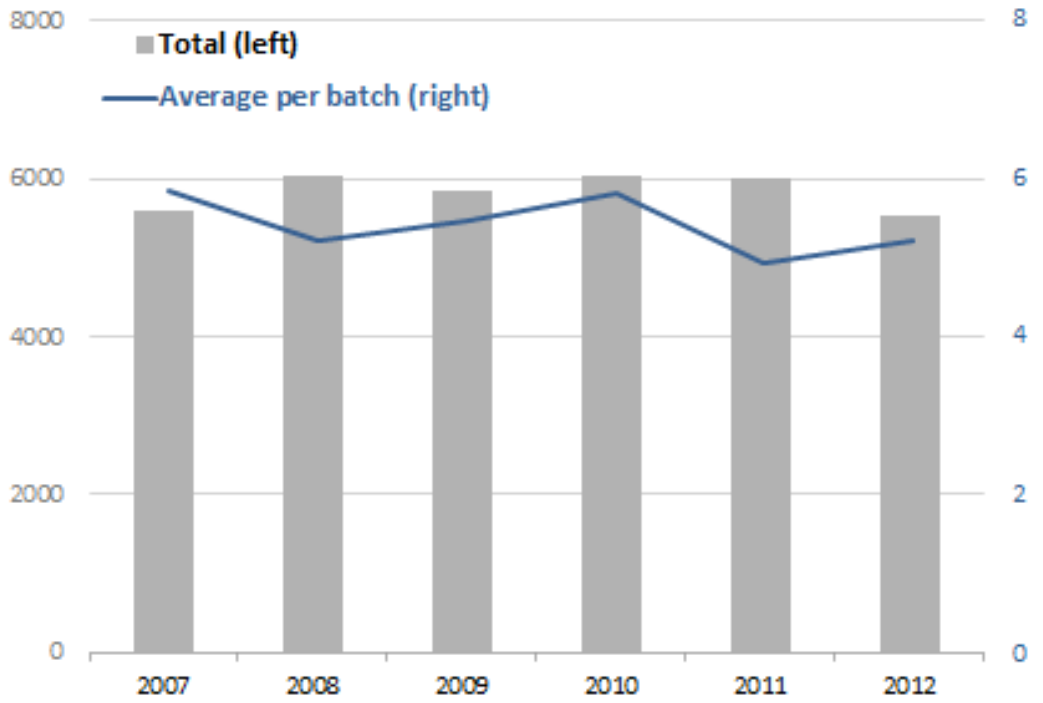


Table 11

	2007	2008	2009	2010	2011	2012
Total # animals used	5,593	6,035	5,845	6,036	6,021	5,547
Total # batches released	959	1,159	1,074	1,041	1,219	1,066
Average # animals per batch	6	5	5	6	5	5
Range of animals per batch	0 - 277	0 - 224	0 - 224	0 - 137	0 - 137	0 - 137

The main use of animals in the QC of IVMPs which fall into this category is for the determination of potency via serology. For products which determine potency using an *in vivo* test the predominant test is serology ranging from 81% in 2007 to 99% in 2012. Although a challenge test was not replaced with a serology test for any product during the time period examined two products where potency was assessed via a challenge test, for use against *E. coli* and Erysipelothrix, using relatively large numbers of animals per batch, were discontinued in 2009 and 2010 respectively. Alternative products for these diseases use a less severe serology test to determine potency and the use of considerably fewer animals (7 – 45 for *E. coli* products; 20 – 41 for Erysipelothrix products).

The TABST was removed from the MA for 24 of the products which fall into this category over the period studied.

Conclusions

This report reviewed the status of animal use in the quality control of authorised IVMPs for use in the UK, and released to the EU market via the UK, between 2007 and 2012.

It is evident from the analysis that a degree of improvement has been made in applying the principles of the 3Rs. Efforts have been made by both the Regulatory Authorities and MAHs to modify the tests performed for the release of IVMPs in order to either reduce the number of animals required, the severity of the testing or to replace the animal tests completely by *in vitro* methods.

Although the replacement of *in vivo* testing has to be the ultimate aim, additional considerations in developing appropriate *in vitro* assays needs to be acknowledged, especially in regards to potency testing. These include the correlation of the *in vitro* test to target animal efficacy, interference by adjuvants (in particular for inactivated vaccines), cross-reactivity of other antigens and the specificity and sensitivity of the assay. Replacement of an *in vivo* test by an *in vitro* test may need careful and pragmatic decisions to be taken in terms of the degree of validation necessary to demonstrate equivalence. As a result it can take many years, and considerable expense, for alternative methods to be implemented. Nevertheless, new assay development is being encouraged, with particular interest in products currently implementing challenge models, and future improvements in this area are likely.

One way in which a reduction in animal testing could be achieved without any change to the authorised tests used would be for larger individual batches of products to be produced for release. This appears to be happening to some extent with fish vaccines and is something which could be an intermediate step in the reduction of animal numbers used in batch release testing.

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Supplementary Data

How animals are used in the different types of QC tests required for the release of IVMPs

	Extraneous Agents	Identity	Inactivation	Potency - challenge	Potency - serology	Safety	Sensitising effect	Toxicity	Toxoid content
Atlantic Salmon				+		+			
Cats						+			
Cattle	+				+	+			
Chickens	+	+		+	+	+			
Dogs					+	+			
Guines-pigs		+	+	+	+		+	+	
Hamsters				+	+				
Horses						+			
Mice		+	+	+	+	+		+	+
Pigeons					+	+			
Pigs	+				+	+		+	
Rabbits	+		+	+	+	+			
Rainbow Trout				+		+			
Rats					+				
Sheep				+	+	+			
Turkeys						+			

Species of animals used for QC testing of IVMPs from 2007 to 2012

Test Animal		2007	2008	2009	2010	2011	2012	Trend and % change
Atlantic Salmon	Animals	2300	1460	1220	1280	1040	1460	-36.5
	Batches	15	11	11	12	9	10	-33.3
	Average	153.3	132.7	110.9	106.7	115.6	146.0	-4.8
Cats	Animals	330	360	322	316	382	298	-9.7
	Batches	147	164	144	145	171	130	-11.6
	Average	2.2	2.2	2.2	2.2	2.2	2.3	2.1
Cattle	Animals	380	466	407	435	515	292	-23.2
	Batches	123	152	138	150	181	134	8.9
	Average	3.1	3.1	2.9	2.9	2.8	2.2	-29.5
Chickens	Animals	8995	9560	10150	10600	10755	9870	9.7
	Batches	290	318	382	390	381	382	31.7
	Average	31.0	30.1	26.6	27.2	28.2	25.8	-16.7
Dogs	Animals	452	546	444	368	256	224	-50.4
	Batches	191	234	203	197	184	166	-13.1
	Average	2.4	2.3	2.2	1.9	1.4	1.3	-43.0
Guinea-pigs	Animals	4328	4647	6008	5534	5908	4663	7.7
	Batches	182	192	226	216	212	181	-0.5
	Average	23.8	24.2	26.6	25.6	27.9	25.8	8.3
Hamsters	Animals	4850	4515	5080	3945	2835	3395	-30.0
	Batches	101	115	127	109	89	91	-9.9
	Average	48.0198	39.2609	40	36.1927	31.8539	37.3	-22.3
Horses	Animals	100	106	120	98	128	112	12.0
	Batches	50	53	60	49	64	59	18.0
	Average	2.0	2.0	2.0	2.0	2.0	1.9	-5.1
Mice	Animals	27844	27327	43854	44126	34809	22091	-20.7
	Batches	167	169	211	207	209	161	-3.6
	Average	166.731	161.7	207.8	213.2	166.6	137.2	-17.7
Pigeons	Animals	70	5	125	75	60	70	0.0
	Batches	8	1	13	7	7	7	-12.5
	Average	8.8	5.0	9.6	10.7	8.6	10.0	14.3
Pigs	Animals	322	357	435	433	397	380	18.0
	Batches	126	130	156	167	179	182	44.4
	Average	2.6	2.7	2.8	2.6	2.2	2.1	-18.3
Rabbits	Animals	1434	1406	1854	1815	1686	1307	-8.9
	Batches	124	114	155	150	141	111	-10.5
	Average	11.6	12.3	12.0	12.1	12.0	11.8	1.8
Rainbow trout	Animals	3980	2620	1460	2540	1160	1100	-72.4
	Batches	20	13	8	13	6	6	-70.0
	Average	199.0	201.5	182.5	195.4	193.3	183.3	-7.9
Rats	Animals	246	378	452	348	366	196	-20.3
	Batches	12	18	20	15	15	7	-41.7
	Average	20.5	21.0	22.6	23.2	24.4	28.0	36.6
Sheep	Animals	268	510	436	353	286	198	-26.1
	Batches	105	119	147	123	123	89	-15.2
	Average	2.6	4.3	3.0	2.9	2.3	2.2	-12.8
Turkeys	Animals	140	160	110	120	90	120	-14.3
	Batches	14	16	11	12	9	12	-14.3
	Average	10.0	10.0	10.0	10.0	10.0	10.0	0.0

Animals: Total number of animals used.
Batches: Number of batches released – this includes all batches released for products that included an authorised QC test in the test species at some point during the six year study period. Should a test have been removed during this time batches from later years are still included in the batch figures.
Average: Average number of animals used per batch released.

Numbers of animals used in the different types of QC test from 2007 to 2012

Test Type		2007	2008	2009	2010	2011	2012	Trend and % change
Extraneous Agents	Animals	1772	1350	1336	1290	1178	1074	-39.4
	Batches	192	188	223	223	213	203	5.7
	Average	9.2	7.2	6.0	5.8	5.5	5.3	-42.7
Identity	Animals	97	141	115	142	320	186	91.8
	Batches	16	20	17	27	43	46	187.5
	Average	6.1	7.1	6.8	5.3	7.4	4.0	-33.3
Inactivation	Animals	204	274	294	343	225	185	-9.3
	Batches	15	22	26	31	26	23	53.3
	Average	13.6	12.5	11.3	11.1	8.7	8.0	-40.9
Potency challenge (controls)	Animals	5088	4673	4705	4951	4276	3969	-22.0
	Batches	242	262	291	278	233	204	-15.7
	Average	21.0	17.8	16.2	17.8	18.4	19.5	-7.5
Potency challenge (ref)	Animals	837	330	236	228	220	162	-80.6
	Batches	35	30	33	38	39	30	-14.3
	Average	23.9	11.0	7.2	6.0	5.6	5.4	-77.4
Potency challenge (vacc)	Animals	10542	8782	8701	8455	7757	6668	-36.7
	Batches	273	294	324	309	270	231	-15.4
	Average	38.6	29.9	26.9	27.4	28.7	28.9	-25.2
Potency serology	Animals	6770	8260	9666	9842	9322	7872	16.3
	Batches	381	438	502	507	495	423	11.0
	Average	17.8	18.9	19.3	19.4	18.8	18.6	4.7
Safety	Animals	7344	6697	6312	6539	5774	5884	-19.9
	Batches	980	1082	1127	1110	1158	1027	4.8
	Average	7.5	6.2	5.6	5.9	5.0	5.7	-23.5
Sensitising effect	Animals	12	6	0	24	36	72	500.0
	Batches	2	1	0	7	22	35	1650.0
	Average	6.0	6.0	0.0	3.4	1.6	2.1	-65.7
Toxicity	Animals	4709	4526	6568	6424	5485	4118	-12.6
	Batches	112	103	145	140	152	136	21.4
	Average	42.0	43.9	45.3	45.9	36.1	30.3	-28.0
Toxoid content	Animals	18664	19384	34544	34148	26080	15586	-16.5
	Batches	79	76	111	108	98	75	-5.1
	Average	236.3	255.1	311.2	316.2	266.1	207.8	-12.0

Animals: Total number of animals used in QC test.
Batches: Number of batches released – this includes all batches released for products that had a requirement for the QC test on their MA at some point during the six year study period. Should a test have been removed during this time batches from later years are still included in the batch figures.
Average: Average number of animals used per batch released.