Batch Safety Testing of Veterinary Vaccines — Potential Welfare Implications of Injection Volumes

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Summary — This study examines the volumes administered by injection during the batch safety testing of veterinary vaccines according to the methods laid down in European Pharmacopoeia monographs. These are compared with maximum dose volumes recommended in good practice guidelines. The volumes administered during safety tests frequently exceeded recommended maximums, giving rise to concerns that these tests may seriously compromise animal welfare. This was particularly the case for live liquid vaccines and for vaccines recommended for intramuscular injection. The volumes used in testing vaccines for avian species exceeded the recommended maximums by the greatest amount. The administration of test doses should be performed in a way that minimises pain and discomfort. The refinement of tests by dividing large dose volumes and delivering them over multiple sites is discussed, but it is recognised that there are technical limitations, which in some cases may cause difficulties with fulfilling the purpose of the tests. Previously, the relevance of batch safety tests has been widely criticised, due to a lack of evidence that they contribute to the safety of veterinary medicines. By showing that the conduct of target animal safety tests can involve injection volumes that compromise the welfare of the animals involved, this analysis highlights the need for a more critical assessment of the necessity and justification for these tests.

Key words: *animal welfare, batch safety test, injection volumes, regulatory requirement, substance administration, veterinary vaccines.*

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Introduction

It is a regulatory requirement that safety tests are conducted on batches of veterinary vaccines. These tests require the use of a significant number of animals annually. In 2003, 6480 animals were used in batch safety tests for the release of immunological veterinary medicinal products in the UK alone, accounting for approximately one fifth of animals used in veterinary vaccine quality control tests (1). Animals are only believed to suffer during these safety tests if a vaccine displays residual virulence/toxicity, which is a rare event (2).

However, suffering may not be confined to infrequent adverse reactions, but may be intrinsic in the test methodology. A batch safety test typically requires animals of the target species to be administered an overdose of the vaccine. Overdoses are either ten times or double the recommended dose volume for live and inactivated vaccines, respectively. They are administered by a recommended route, to animals of the minimum age for which the vaccine is recommended. As vaccination is often performed in very young animals, the test can involve the injection of large volumes relative to the animal's size. The use of large volumes is a recognised welfare problem, because it can cause adverse physiological or pathological changes that compromise animal well-being. For example, volumes injected intramuscularly can physically distend the muscle, causing swelling at the injection site with associated tissue damage and pain (3).

Good practice guidelines have been developed with the goal of refining procedures for the administration of substances. Three of the most commonly used are those of Diehl *et al.* (4), Morton *et al.* (3) and Wolfensohn & Lloyd (5). These guidelines are established, authoritative texts, designed to provide practical and welfare-led perspectives, with input from veterinary, industry and animal welfare organisations. The Diehl *et al.* (4) guidance is the result of an initiative between the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM); that of Morton *et al.* (3) is the result of a collaboration between veterinary and animal welfare organisations, which was promoted by the UK Home Office (6); and that of Wolfensohn & Lloyd (5) was produced with veterinary and Home Office input and is used as a standard reference text in many establishments. Recommended maximum injection volumes vary between these guidelines, but tend to be of a similar order for a particular injection route and species.

This study compares the injection volumes required in batch safety tests of veterinary vac-

cines with maximum volumes recommended by the three guidelines described above. It highlights where recommended maximum volumes are exceeded, and therefore where batch safety testing has the greatest potential to compromise the welfare of the animals used in the testing.

Methods

The survey of commercially-available veterinary vaccine products and the calculation of volumes administered during safety tests

Datasheets for all the veterinary vaccines which are currently available commercially within the UK, were surveyed by using the NOAH Compendium of Data Sheets for Animal Medicines (7). All the injectable vaccine products containing a component corresponding to a specific monograph in the 6th edition of the European Pharmacopoeia (EP) were identified, and subsequent analysis was restricted to these products.

For each vaccine product included in the survey, the manufacturer's recommendations regarding target species, minimum age at vaccination, administration route and dose volume were recorded. The age of animal required by each vaccine monograph (generally the minimum age for which the vaccination is recommended) was used to estimate corresponding animal weights, based on standard growth curves for livestock and data provided by laboratory animal suppliers (e.g. the Charles River catalogue). This enabled the volumes administered in batch safety tests to be calculated on a ml/kg basis for each vaccine. Where a monograph specified an age range for animals to be used in the safety test, volumes were calculated for the youngest animals in this range, as these stood to receive the largest volume on a ml/kg basis.

Monographs typically require ten times or double the manufacturers' recommended doses to be used in the batch safety tests for live and inactivated vaccines, respectively. For freeze-dried live vaccines, the EP specifies that "the 10 doses are reconstituted in a suitable volume for the test". What is meant by "suitable volume" is not explained. Therefore, it is not possible to calculate the injection volumes that are implied. However, for liquid live vaccines, ten times the recommended dose volume is used in the test. Consequently, the analysis of live vaccines was restricted to those supplied in liquid form. The vaccine volume used in the test can vary between similar vaccines, depending on the recommended dose volume. Where there was more than one vaccine product available in the UK of a particular type (e.g. live canine adenovirus), the vaccine that would require the largest volume to be administered during the safety test (on a ml/kg basis) was selected for subsequent analysis. In instances where a vaccine product was recommended for administration by more than one route, all the recommended routes were considered.

Comparison with established good practice guidelines

The injection volumes required in batch safety tests for each vaccine included in the analysis were calculated (ten times the recommended dose for live vaccines; double the recommended dose for inactivated vaccines). These volumes were compared with maximum administration volumes recommended in the three good practice guidelines (3–5).

In one document, recommended maximum volumes were given for each species for an 'average' size animal of a specified weight (5). These values were adjusted to reflect the estimated weight of the animals used in the batch safety test, as per the authors' recommendations.

The guidelines used did not address injection volumes for cattle and horses. However, 20ml is the maximum dose volume recommended by manufacturers for a number of veterinary pharmaceutical products that are used in these animals (e.g. Depocillin, Intervet), which are delivered by intramuscular injection. Consequently, 20ml was selected as the maximum volume that should be given by intramuscular injection at a single site in these species.

Two guidelines used in the comparison (3, 5) give maximum injection volumes for administration at a single site. In addition, one of these guidelines indicates the maximum number of sites that should be used (5). Hence, for each vaccine considered, the number of injection sites that would be required to satisfy safety test requirements, without exceeding the maximum recommended injection volume at a single site, was calculated. This was compared with the maximum number of injection sites recommended.

Live and inactivated vaccines were analysed separately, due to their different test requirements. In the case of inactivated vaccines, the intra-muscular and subcutaneous routes were considered separately. Where a vaccine data sheet indicated that a vaccine could be administered by a choice of routes, all the possible routes were considered, as the wording of the monographs creates the potential for any of the recommended routes to be used during batch safety testing.

Results

Thirty-five types of injectable veterinary vaccine relating to a specific monograph of the EP, are mar-

keted in the UK, and therefore were eligible for inclusion in this study. These eligible vaccines comprised two live vaccines and 33 inactivated vaccines.

The analysis showed that the volume of vaccine administered during the batch safety test could exceed recommended maximums for both of the live vaccines considered and for 13 of the 33 inactivated vaccines. Only data relating to vaccines where volumes used in the safety tests potentially exceed the recommended maximums are shown in Tables 1–5.

Live vaccines

Table 1 summarises the manufacturers' recommendations for use, together with batch safety test requirements, for the two live vaccines where volumes used in tests potentially exceed recommended maximums. Table 2 shows how injection volumes required in batch safety tests for the vaccines in Table 1 compare with those recommended in the good practice guidelines.

The volumes required for testing live canine parvovirus can exceed the guideline recommendations, if they are injected at a single site. However, compliance with two guidelines (3, 5) is possible, if the volume used is split over multiple sites.

Marek's disease vaccines can be tested via the subcutaneous or intramuscular routes. In both cases, the guideline recommendations would be exceeded. For most species, Wolfensohn & Lloyd (5) indicate that large dose volumes administered subcutaneously can be split between up to four sites. However, this approach is not indicated for birds, suggesting that multiple injection sites are not considered appropriate for these species. If dose volumes were divided, the number of injection sites required to ensure that the volumes at a single site did not exceed recommendations, would be unacceptably high (between 9 and 56, depending on guideline).

If the intramuscular route is used for the batch safety testing of live Marek's vaccines, then the recommendations are exceeded to an even greater degree. Depending on the guideline used for comparison, between 444 and 889 injection sites would be required to administer the test volume without exceeding the maximum recommended volumes at a single site.

Inactivated vaccines

The vaccine volume required in batch safety tests could exceed the recommended maximum injection volumes for 13 of the 33 inactivated vaccines considered. Table 3 summarises the manufacturers' recommendations for the use of these 13 vaccines, together with the batch safety test requirements. Tables 4 and 5 show how injection volumes required in safety tests for vaccines in Table 3 compare with those recommended in the good practice guidelines for intramuscular and subcutaneous injections, respectively.

Intramuscular administration

There are 10 inactivated types of injectable vaccine, where volumes required in safety tests could exceed recommended maximums for intramuscular injection (Table 4). For six of these vaccines, there is at least one product marketed in the UK, where the manufacturer indicates that either the intramuscular or the subcutaneous route can be used.

Table 1: Live liquid veterinary vaccines marketed within the UK, for which volumes used in batch safety tests have the potential to exceed good practice guidelines

	Manufacturers' recommendations for use				Batch safety test requirements		
Vaccine	Target species	Minimum vaccination age	Dose volume (ml)	Administration route	Animal age	Estimated animal weight[a]	Dose volume (ml)
Canine parvovirus	Dog	6 weeks	1.0	Subcutaneous	6 weeks	2.5kg	10
Marek's disease	Chicken	1 day	0.2	Intramuscular or subcutaneous	1 day	45g	2

The Table shows a summary of manufacturers' recommendations for administration and corresponding batch safety test requirements. Where multiple vaccine products are available, values given are for the vaccine product that is recommended for use at the largest dose volume (ml/kg).

[a]*Where animals of a range of ages can be used, weights are based on the minimum age, as this involves the largest test dose volume on a ml/kg basis.*

Table 2: The batch safety testing of live veterinary vaccines

Vaccine	Species used	Dose volume required by test (ml)	Comparison with Morton *et al.* (3)		Comparison with Wolfensohn & Lloyd (5)[a]		Maximum volume in Diehl *et al.* (4) (ml)[b]
			Maximum recommended volume (ml/site)	No. of injection sites required to comply with guideline	Maximum recommended volume (ml/site)	No of injection sites required to comply with guideline	
Canine parvovirus (subcutaneous route)	Dog	10.0	5.0–12.5	1	**6.3**	2	**2.5 (5.0)**
Marek's disease (subcutaneous route)	Chicken	2.0	**0.09–0.225**	**9**	**0.036**	**56**	—
Marek's disease[c] (intramuscular route)	Chicken	2.0	**0.00225**	**889**	**0.0045**	**444**	—

The Table shows a comparison with good practice guidelines on maximum administration volumes. All vaccines where volumes used in the test can exceed maximum recommended volumes, are listed. Instances where recommendations are exceeded, are given in bold type.

[a]*Figures in Wolfensohn & Lloyd (5) have been calculated for the 'average' animal size for a particular species, but the authors recommended that the figures are adjusted, as necessary, for the actual weight of the individual. The figures in this table have been adjusted accordingly, based on the estimated weights in Table 1.*

[b]*According to Diehl* et al. *(4), figures to the left of the column are reflective of 'good practice', and the bracketed figures are possible maximal values where, "if exceeded, animal welfare or scientific implications may result."*

[c]*Vaccines have both subcutaneous and intra-muscular routes recommended as possible administration routes for at least one product marketed in the UK.*

If tested via the intramuscular route, the volumes used to test six avian and two canine vaccines exceed the maximum recommended for injection at a single site (3, 5). If divided over multiple sites, to ensure that volumes per site fall within the recommended limits, the number of injection sites required would range from 5 to 67, depending on the vaccine and guideline consulted (Table 4). The extent to which the recommended maximum volumes are exceeded is greatest for avian vaccines. The volumes required for the testing of canine vaccines exceeded those recommended by Diehl *et al.* (4).

The volumes required to test two pig vaccines exceed what is described as "good practice" by Diehl *et al.* (4), but fall within the limits of the possible highest volumes.

Subcutaneous administration

Dividing doses over multiple sites is not advocated for birds, yet the volumes required for the safety testing of inactivated avian vaccines by the subcutaneous route exceed the recommendations of Wolfensohn & Lloyd (5), if administered at a single site (Table 5). However, the degree to which the recommendations are exceeded is considerably less than for many live vaccines, or for inactivated vaccines administered intramuscularly. The volumes required for the testing of rabies and rabbit haemorrhagic disease vaccines exceed what is described as "good practice" by Diehl *et al.* (4).

Tetanus vaccines

The batch safety test for tetanus vaccines is the only one in which a target species is not used. Instead, the test is conducted in guinea-pigs of a given weight range. The monograph stipulates that the total dose volume should be split and administered as two equal volumes at separate injection sites. However, even when two injection sites are used, the volume administered at each site is still in excess of that recommended, and a minimum of three sites would be

Table 3: Inactivated veterinary vaccines marketed within the UK, for which volumes used in batch safety tests have the potential to exceed good practice guidelines

	Manufacturers' recommendations for use				Batch safety test requirements		
Vaccine	Target species	Minimum vaccination age	Dose volume (ml)	Administration route	Animal age	Estimated animal weight[a]	Dose volume (ml)
Avian Infectious Bronchitis	Chicken	14 weeks	0.5	Intramuscular or subcutaneous	14–28 days	300g	1.0
Avian Infectious Bursal Disease	Chicken	14 weeks	0.5	Intramuscular or subcutaneous	14–28 days	300g	1.0
Canine leptospirosis	Dog	6 weeks	1.0	Intramuscular or subcutaneous	6 weeks	2.5kg	2.0
Egg Drop Syndrome	Chicken	14 weeks	0.5	Intramuscular or subcutaneous	14–28 days	300g	1.0
Fowl cholera	Various avian	8 weeks	0.5	Subcutaneous	8 weeks	750g	1.0
Newcastle disease	Various avian	14 weeks	0.5	Intramuscular or subcutaneous	14–28 days	300g	1.0
Porcine parvovirus	Pig	5 months	2.0	Intramuscular	6 weeks–6 months	11kg	4.0
Rabbit haemorrhagic disease	Rabbit	10 weeks	1.0	Subcutaneous	10 weeks	2.0kg	2.0
Rabies[b]	Dog, cat	4 weeks	1.0	Intramuscular or subcutaneous	4 weeks	1.7kg	2.0
Salmonella enteritidis	Chicken	12 weeks	0.5	Intramuscular	12 weeks	1.1kg	1.0
Salmonella typhimurium	Chicken	12 weeks	0.5	Intramuscular	12 weeks	1.1kg	1.0
Swine erysipelas	Pig	6 weeks	2.0	Intramuscular or subcutaneous	6 weeks	11kg	4.0
Tetanus[c]	Various	Varies with species	Varies with species	Intramuscular or subcutaneous	Guinea-pig age not given	350–450g	5.0[d]

The Table shows a summary of manufacturers' recommendations for administration and corresponding batch safety test requirements. Where multiple vaccine products are available, values given are for the vaccine product which is recommended for use at the largest dose volume (ml/kg).

[a]*Where animals of a range of ages can be used, weights are based on the minimum age, as this involves the largest test dose volume on a ml/kg basis.*

[b]*Although the vaccine is used in both dogs and cats, only dogs are used in the batch safety test. The age of the animal used is not specified in the monograph, but here it is assumed to be the minimum age at which vaccination is recommended.*

[c]*The batch safety test for tetanus vaccine is performed in guinea-pigs, rather than in the target species.*

[d]*The monograph specifies that the dose is given subcutaneously, as two equally divided doses at separate sites.*

Table 4: Batch safety testing of inactivated veterinary vaccines (intramuscular administration)

Vaccine	Species used	Dose volume required by test (ml)	Comparison with Morton *et al.* (3)		Comparison with Wolfensohn & Lloyd (5)[a]		Maximum volume in Diehl *et al.* (4) (ml)[b]
			Maximum recommended volume (ml/site)	No. of injection sites required to comply with guideline	Maximum recommended volume (ml/site)	No of injection sites required to comply with guideline	
Avian Infectious Bronchitis[c]	Chicken	1.0	**0.015**	**67**	**0.03**	**33**	—
Avian Infectious Bursal Disease[c]	Chicken	1.0	**0.015**	**67**	**0.03**	**33**	—
Canine leptospirosis[c]	Dog	2.0	**0.125**	**16**	**0.42**	**5**	**0.625 (1.25)**
Egg Drop Syndrome[c]	Chicken	1.0	**0.015**	**67**	**0.03**	**33**	—
Newcastle disease[c]	Avian *spp.*	1.0	**0.025**	**67**	**0.05**	**33**	—
Porcine parvovirus	Pig	4.0	N/A	N/A	—	—	**2.75** (5.5)
Rabies[c]	Dog	2.0	**0.085**	**24**	**0.28**	**8**	**0.425 (0.85)**
Salmonella enteritidis	Chicken	1.0	**0.055**	**18**	**0.11**	**9**	—
Salmonella typhimurium	Chicken	1.0	**0.055**	**18**	**0.11**	**9**	—
Swine erysipelas	Pig	4.0	—	—	—	—	**2.75** (5.5)

The Table shows a comparison with good practice guidelines on maximum administration volumes. All vaccines where volumes used in the test can exceed maximum recommended volumes, are listed. Instances where recommendations are exceeded, are given in bold type.

[a]*Figures in Wolfensohn & Lloyd (5) have been calculated for the 'average' animal size for a particular species, but the authors recommended that the figures are adjusted, as necessary, for the actual weight of the individual. The figures in this table have been adjusted accordingly, based on the estimated weights in Table 3.*

[b]*According to Diehl* et al. *(4), figures to the left of the column are reflective of 'good practice', and the bracketed figures are possible maximal values where, "if exceeded, animal welfare or scientific implications may result."*

[c]*Vaccines have both subcutaneous and intra-muscular routes recommended as possible administration routes for at least one product marketed in the UK.*

required in order to comply with the published guidelines (3, 5; Table 5).

Intraperitoneal administration

The inactivated furunculosis vaccines used in fish, are administered by intraperitoneal injection. Of the vaccines currently available in the UK, the largest recommended dose is 0.2ml in fish of a minimum weight of 16g. The double dose of 0.4ml required for the safety test equates to 25ml/kg. This exceeds the recommended maximum volume of 10ml/kg (3).

Table 5: Batch safety testing of inactivated veterinary vaccines (subcutaneous administration)

Vaccine	Species used	Dose volume required by test (ml)	Comparison with Morton *et al.* (3)		Comparison with Wolfensohn & Lloyd (5)[a]		Maximum volume in Diehl *et al.* (4) (ml)[c]
			Maximum recommended volume (ml/site)	No. of injection sites required to comply with guideline[b]	Maximum recommended volume (ml/site)	No of injection sites required to comply with guideline[b]	
Avian Infectious Bronchitis	Chicken	1.0	**0.6**–1.5	1	**0.24**	**5**	—
Avian Infectious Bursal Disease	Chicken	1.0	**0.6**–1.5	1	**0.24**	**5**	—
Egg Drop Syndrome	Chicken	1.0	**0.6**–1.5	1	**0.24**	**5**	—
Fowl cholera	Avian *spp.*	1.0	1.5–3.75	1	**0.6**	2	—
Newcastle disease	Avian *spp.*	1.0	**0.6**–2.5	1	**0.24**	**5**	—
Rabbit haemorrhagic disease	Rabbit	1.0	1.5–3.75	1	1.125	1	**0.75** (1.5)
Rabies	Dog	2.0	3.4–8.5	1	4.25	1	**1.7** (3.4)
Tetanus	Guinea-pig	5ml (2 × 2.5ml at separate sites)	**0.7–2.25** (depending on size of animal)	**3** (monograph specifies 2)	**1.4–1.8**	**3–4** (depending on size of animal)	—

The Table shows a comparison with good practice guidelines on maximum administration volumes. All vaccines where volumes used in the test can exceed maximum recommended volumes, are listed. Instances where recommendations are exceeded, are given in bold type.

[a]*Figures in Wolfensohn & Lloyd (5) have been calculated for the 'average' animal size for a particular species, but the authors recommended that the figures are adjusted, as necessary, for the actual weight of the individual. The figures in this table have been adjusted accordingly, based on the estimated weights in Table 3.*

[b]*If dose volumes are divided, four is the maximum number of injection sites recommended in Wolfensohn & Lloyd (5) for subcutaneous injections for all species that are covered in the guideline, except birds. The guideline makes no recommendations about multiple sites in birds, suggesting that their use is not encouraged.*

[c]*According to Diehl* et al. *(4), figures to the left of the column are reflective of 'good practice', and the bracketed figures are possible maximal values where, "if exceeded, animal welfare or scientific implications may result."*

Discussion

The relevance of batch safety tests has been widely criticised, due to a lack of evidence that these tests contribute to the safety of veterinary vaccines. Consequently, there have been numerous calls for them to be omitted as a routine regulatory requirement (e.g. 8–13). The EP permits manufacturers to apply to competent authorities for permission to discontinue the test, if sufficient consecutive batches of a particular vaccine have previously passed it. Despite this, the batch safety test is still widely used.

The critics of batch safety tests have emphasised how the removal of the test would significantly reduce the numbers of animals used in vaccine quality control. The amount of suffering that can be involved, provides an additional incentive for removal of these tests.

The volumes of injectable veterinary vaccines used during routine safety tests frequently exceed the maximum volumes recommended in three established good practice guidelines. The guideline produced by Diehl *et al.* (4) contains two sets of figures. One of these is intended as a guide to good practice volumes, whilst the second set represents

the possible maximum values, where, "if exceeded, animal welfare or scientific implications may result". This study shows that both volumes are sometimes exceeded.

Although the maximum injection volumes recommended for a particular species and route can vary between guidelines, there are instances where the recommendations of all guidelines are exceeded. This is particularly the case for live liquid vaccines, where ten times the manufacturers' recommended dose volume is required in batch safety tests. However, even the administration of a double dose, required to test inactivated vaccines, can involve volumes that exceed the recommended limits.

Of greatest concern are tests that involve intramuscular injections, which cause more pain and have greater potential for nerve and tissue damage than do other administration methods (3). Little published research has examined the physiological and welfare consequences to animals of large injection volumes. However, the determination of serum creatine kinase (CK) has been used as an indirect and specific measure for the muscle damage resulting from intramuscular injection (14–16). Saline given at 0.25ml/kg has been found to increase serum CK activity after intra-muscular injection in pigs (16). This is a lower volume, on a ml/kg basis, than those that can be used in the batch safety testing of pig vaccines. Lower volumes of saline had less effect on CK activity (17). Increased levels of CK in pigs have also been demonstrated, following the intramuscular injection of saline at rates of 0.1ml/kg (14). In another study, the extent of tissue damage was proportional to the injection volumes, following intramuscular injections of antimicrobial compounds in both pigs and rabbits (15).

Clearly, intramuscular injection volumes should be kept as low as possible. The intramuscular route should only be used if a less painful alternative is impossible, and it should not be used for small animals, save in exceptional circumstances (3). The current analysis has highlighted the potential for this route to be used in day-old birds during batch safety tests. Such tests could involve volumes so large that up to 889 injection sites would be needed, if the volume administered at a single site was to comply with established good practice guidelines (i.e. for live Marek's disease vaccine). Fortunately, this vaccine can also be given subcutaneously. Vaccine monographs only require "a recommended route" to be used during the batch safety test. There is no requirement to demonstrate safety by using all the recommended routes. Thus, in cases where multiple routes are available, it is essential that batch safety testing is restricted to the subcutaneous route, in order to avoid additional suffering.

Testing vaccines by subcutaneous injection is preferable to intramuscular administration, but even when the subcutaneous route is used, the volumes required can still exceed good practice recommendations for some vaccines, particularly if the dose is administered at a single site. Unless there is sufficient loose skin at the injection site to allow the volume to be easily accommodated in the subcutaneous space, large volumes injected subcutaneously can cause discomfort. If doses are split over multiple sites, compliance with good practice guidelines can be achieved in many, but not all, cases. Despite this, only the monograph for tetanus vaccines specifically states that the test dose should be split over multiple sites. Even then, it is questionable whether the 'two sites' requirement by the monograph is sufficient to ensure that welfare is not compromised by the injection volume.

The use of multiple sites may have animal welfare consequences, because any pain or irritation at the injection site will be multiplied. If the sites for intramuscular injections are located in more than one limb, there is a danger of multiple limb lameness (3). Additionally, all injections carry a risk of bruising and infection, regardless of the route used. This risk increases with the number of sites used, and good technique is essential to the avoidance of adverse effects. Generally, the volumes used in safety tests should be split, if this will minimise pain and discomfort at the injection site, and this should be evaluated on a case-by-case basis.

Myxomatosis vaccines are administered by using a combination of the subcutaneous and intradermal routes, which creates the potential for the intradermal route to be used in safety testing. Injections via this route are painful (18), and, as there is no natural space to contain substances administered by this route, only small volumes should be injected at a single site (3). The volume required in the batch safety test exceeds that recommended for a single injection site, and so should be split over multiple sites, in order to reduce pain and discomfort at any individual site. A maximum of six sites is recommended for this route (3). However, 10 sites would be required for the volume at a single site to fall within recommended limits. Thus, either the recommended volume at a single site or the recommended number of injection sites must be exceeded, if the safety test requirements are to be met. This kind of dilemma will always exist, while batch safety testing continues.

Generally, the testing of avian vaccines is where the recommendations of good practice guidelines are exceeded to the greatest degree, regardless of the route of administration used. The impact on welfare is compounded by the requirement to use more animals for the batch safety testing of avian vaccines than for vaccines for mammalian species, despite the lack of scientific or statistical rationale for this difference (11).

In some cases, principally where very small animals are used, it would not be technically feasible to comply with the recommendations of both the EP and the welfare guidelines, e.g. the requirement of 889 maximum dose volumes of 0.0025ml/site for Marek's disease vaccine. It is not the intention of this study to provide solutions to this problem, but simply to point out that the use of volumes well in excess of the recommended maxima, is intrinsic to some tests.

Conclusions

The current study highlights how the batch safety test has the potential to cause considerable pain and discomfort in the animals that are used. This should provide an additional incentive for the vaccine manufacturers to apply to the competent authorities for permission to discontinue the routine use of the test. By seeking authorisation to discontinue routine safety testing, manufacturers can demonstrate commitment to the principles of the Three Rs. This would significantly reduce both the numbers and the suffering of animals used in quality control testing of veterinary vaccines. Priority should be given to the testing where there is the greatest potential for welfare to be compromised. These include the testing of avian vaccines, where the volumes used tend to be relatively larger than for vaccines for other target animal groups, and where splitting the dose over multiple injection sites is not recommended. Furthermore, the results of this study give additional animal welfare grounds for demanding that the need for the inclusion of the test as a monograph requirement should be reassessed, given that its scientific relevance has been questioned.

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References

1. Veterinary Medicines Directorate (2005). *Animal Usage in Quality Control Tests for the Release of Immunological Veterinary Medicinal Products in the United Kingdom in 2003.* Available at: http://www.vmd.gov.uk/Publications/Reports/Animal_Usage.pdf (Accessed 20.09.07).
2. Hendriksen, C.F.M. (2006). Towards eliminating the use of animals for regulatory required vaccine quality control. *ALTEX* **23**, 187–190.
3. Morton, D.B., Jennings, M., Bucknell, A., Ewbank, R., Godfrey, C., Holgate, B., Inglis, I., James, R., Page, C., Sharman, I., Verdchoyle, R., Westall, L. & Wilson, A.B. (2001). Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* **35**, 1–41.
4. Diehl, K.H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.M. & van de Vorstenbosch, C. (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology* **21**, 15–23.
5. Wolfensohn, S. & Lloyd, M. (2003). *Handbook of Laboratory Animal Management and Welfare*, 153pp. Oxford, UK: Blackwell Publishing Ltd.
6. Home Office (2003). *Animals (Scientific Procedures) Act 1986: PCD Circular 1/2003.* Available at: http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/publications/pcd-holders-circulars/pcdcircular1_2003.pdf (Accessed 20.09.07).
7. NOAH (2006). *Compendium of Data Sheets for Animal Medicines 2007.* Enfield, UK: National Office of Animal Health Ltd. Available at: http://www.noahcompendium.co.uk/Compendium/Overview/ (Accessed 20.09.07).
8. Roberts, B. & Lucken, R.N. (1996). Reducing the use of the target animal batch safety test for veterinary vaccines. *Developments in Biological Standardization* **86**, 97–102.
9. Pössnecker, A. & Cussler, K. (1998). Evaluation of the relevance of the target animal safety test for the quality control of veterinary immunological medicinal products. *ALTEX* **15**, 71–75.
10. Pössnecker, A. (1999). Investigation into the target animal safety test for the quality control of leptospira vaccines. *Pharmeuropa BIO* **99.2**, 17–24.
11. Bruckner, L., Bongers, J., Castle, P., Flore, P.H., Guittet, M., Halder, M., Jungbäck, C., Le Gross, F.X., Tollis, M., Nair, V.K., Wilhelm, M., Zeegers, J., Zigterman, G. (2000). Three Rs approaches in the production and quality control of avian vaccines. The report and recommendations of ECVAM Workshop 41. *ATLA* **28**, 241–258.
12. Halder, M. (2001). Three Rs potential in the development and quality control of immunobiologicals. *ALTEX* **18**, Suppl. 1, 13–46.
13. Advisory Group on Alternatives to Animal Testing in Immunologicals (2002). The target animal safety test — is it still relevant? *Biologicals* **30**, 277–287.
14. Steiness, E., Rasmussen, F., Svendsen, O. & Nielsen, P. (1978). A comparative study of serum creatine phosphokinase (CPK) activity in rabbits, pigs and humans after intramuscular injection of local damaging drugs. *Acta Pharmacologica et Toxicologica* **42**, 357–364.
15. Diness, V. (1985). Local tissue damage after intramuscular injections in rabbits and pigs: quantitation by determination of creatine kinase activity at injection sites. *Acta Pharmacologica et Toxicologica* **56**, 410–415.
16. Gueorguieva, T.M., Dimitrova, D.J., Dimitrov, D.S. & Gueorguiev, I.P. (1998). The effect of intramuscular injection of cloxacillin, dicloxacillin and flucloxacillin on serum creatine kinase activity in weanling pigs. *Revue de Médecine Vétérinaire* **149**, 1103–1106.
17. Gueorguieva, T.M., Kanelov, I.N., Gueorguiev, I.P.

& Eckersall, P.D. (1998). A comparative study of serum creatine kinase activity after intramuscular injections of two tylosin dosage forms in pigs. *Revue de Médecine Vétérinaire* **149**, 863–866.

18. Farley, J.S., Hustead, R.F. & Becker, K.E. (1994). Diluting lidocaine and mepivacaine in balanced salt solution reduces the pain of intradermal injection. *Regional Anesthesia* **19**, 48–51.