Regulation of Diagnostics in Animal Health – Is It Needed?

PEER REVIEWED

At a time when evidence-based medicine is encouraged, and where evaluation and monitoring are key components of the cycle of health management, there is an increasing dependence on diagnostics. Renewed interest in the development of diagnostics for animal infections and diseases is motivated by the belief that suitable diagnostics will assist in the control of diseases.
This stimulates a number of questions about the regulation of, and standards for, diagnostic evaluation and monitoring tools, particularly in comparison to pharmaceuticals involved in the action to manage health problems that may be identified (Fig. 1).
This article will evidence the lack of regulation of, or standards for, veterinary diagnostics, in comparison with regulatory frameworks for veterinary pharmaceuticals (including biologicals). It will seek to address whether any differences matter, focussing on parasitic infections of farm and companion animals in particular.

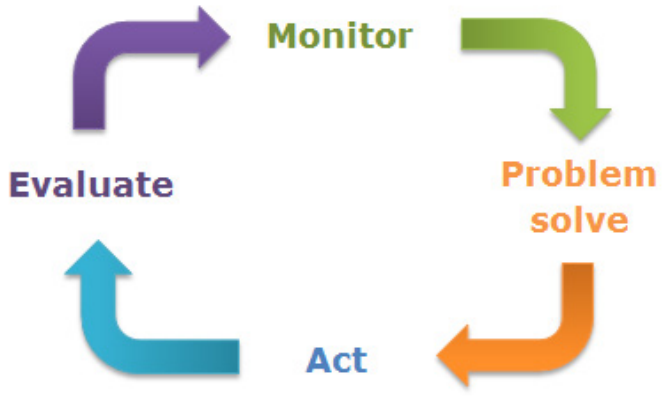

Figure 1: Cycle of health management (adapted from Tisdall et al. 2017)

Integrity and Utility of Diagnostics for Parasitic Infection and Disease

Confirmation of internal or external parasitism of farm or companion animals relies on accurate diagnosis, and is an implicit part of evidence-based medicine. Diagnosis underpins treatment and prevention programmes that may rely on parasiticide usage.

Diagnostics may be used most simply as a qualitative test, and for some parasites such as *Sarcoptes scabiei var. canis,* where there is zero tolerance for the parasite, this level of information may be sufficient. In other situations, for example to estimate the magnitude of helminth infections in calves, enumeration of parasites may be important. It is often necessary to rely on an available surrogate for the measurement of interest, which may, in the example of the calf, be faecal egg counts (FECs), pepsinogen levels or anti-helminth antibody titres. Interpretation may be confounded by the relationship between the parameter of interest and the chosen surrogate. Once calves have grazed for a period of approximately two months, and as immunity develops, the direct relationship between FECs and worm burden disappears, with worm burdens being under-represented by the FEC result (Shaw *et al.* 1998). Conversely, a reliance on antibody titres may over-estimate a parasite burden, because antibody titres often respond slowly to a reduction in the parasite burden, for example after parasiticide treatment.

There are ambitions to take diagnoses of parasite infections a stage further by linking them to production parameters (Charlier *et al.* 2014). These examples illustrate how the results of diagnostic tests may have profound implications for anything from the care of an individual animal to strategic management decisions for a herd or larger population.

Ideally, diagnostics should be sensitive and specific, with a lack of cross-reactivity (false positivity) in similar disease conditions. Tests should ideally be easy to run and produce unambiguous results. For devices, inter- and intra-batch consistency should have been convincingly demonstrated; a further consideration is their long-term stability during anticipated travel or storage conditions.

Diagnostics can be broadly categorised as techniques or devices. Techniques typically involve multiple steps and may include a degree of expertise to assess the test sample, such as that involved in examining a faecal sample under a microscope to speciate and enumerate the parasite eggs. Devices that permit *in vitro* tests to be conducted may be simplified one-step devices or may involve a complex series of steps before producing a result. Interpretation of the result in the context of the animal(s) involved and evaluation of the best course of action is a critical further step.

Whether developed as techniques or devices, there is an initial research phase, followed by method development and verification, validation stages and, where appropriate, manufacturing and/or user studies (Fig. 2). In the case of devices, validation includes, for example (where relevant):

- Robustness
- Limits of detection and quantitation determinations
- Sensitivity and specificity
- Positive and negative predictive values
- Cross-reactivity and/or interferences
- Intra-batch and inter-batch variation
- Accelerated and real-time stability studies.

Diagnostic Sample Life Cycle

The success or failure of a diagnostic test does not rely solely on the processing of the sample; rather it relies on a series of steps from sample collection to the utilisation of results, to inform the actions necessary to treat, prevent or control infections (Fig. 3). At any of these stages, integrity of the sample and its resulting data relies upon appropriate

handling throughout the process. There may be a restriction to qualified personnel, such as veterinary surgeons, when collecting some types of sample, such as blood or tissue, which are normally collected under veterinary surgery regulations.

	Pharmaceutical	Diagnostic
R&D	Research studies Conducted under GLP and GCP	Research maybe conducted within ISO or GLP environment
	Defines a dose-rate and quality, safety and efficacy data	
Approval process	Dossier submitted to regulatory authority	No submission process
	Formal review resulting in marketing authorisation when application successful	
Manufacturing environment	GMP	Not proscribed, maybe GMP or ISO or neither
Sales	Advertising and marketing legally proscribed	Normally available to any customer
Customer	Prescribed within authorisation – maybe Vet only, pharmacy, suitably qualified person, pet owner or farmer	Centralised government laboratory, private laboratory, veterinary practice, diagnostic for pet owner or farmer to use

Figure 2: Development of veterinary pharmaceuticals and diagnostics

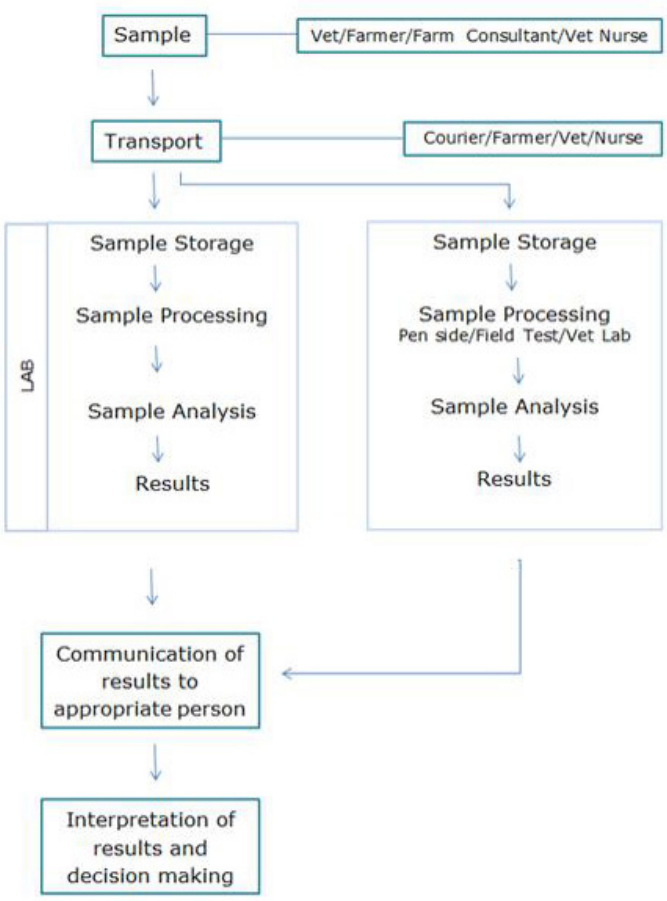

Figure 3: The diagnostic sample life cycle. It is worth noting that laboratory results may undergo QA or senior scrutiny before sign-off and release.

Tests may be run in the field ("pen side"), within the local veterinary practice or by remote, specialised laboratories. In companion animals, for example, tests typically used by veterinarians in practice include faecal flotation, on-site immunochromatographic and coproantigen tests, while serological techniques such as IFAT, ELISA and PCR are often outsourced to external laboratories (Kramer 2016). In either case, it is important to know how to obtain and preserve samples correctly as well as how to interpret results (Kramer 2016). Tests using canine blood samples for detection of dirofilariosis (heartworm) include DiroCHEK ELISA and for lungworm (*Angiostrongylus vasorum*) AngioDetect, while tests based on faecal samples include FLOTAC (Del Prete *et al.* 2015).

Regulations

Within the cycle of diagnosis and management (Fig. 1), highly regulated veterinary pharmaceuticals will often feature at the action stage; an overview of the regulation of veterinary pharmaceuticals and (human) clinical diagnostics are provided below to serve as a benchmark for comparison with regulation of veterinary diagnostics.

Veterinary Pharmaceuticals

Veterinary pharmaceuticals are highly regulated during development, with the process (Fig. 2) in Europe involving submission to the European Medicines Agency or the relevant national authority to demonstrate evidence of the quality, safety and efficacy of the treatment before it is approved for supply.

The formal application by the pharmaceutical sponsor company also contains specifications of the anticipated side-effects, claims and other characteristics that will ultimately appear in the summary of product characteristics, the package leaflet and the product labelling. The review process includes assessment of the instructions and advice to be provided to the end user of the pharmaceutical. In the event that the end user will be a farmer or companion animal owner, considerable efforts are made to ensure that the instructions are clear and unambiguous, with significant time spent on linguistic review to ensure that translation into different languages within Europe remain accurate. This strategy has been used successfully in safeguarding cats from the potential harm of pyrethroid products indicated for administration to dogs. Following a successful assessment, the sponsor company receives a marketing authorisation for the pharmaceutical. The marketing authorisation defines the route to market which may, in many cases, be limited to professional use only, including by pharmacists and veterinary surgeons who are trained to understand the instruction language and the methods of administration.

Post-authorisation monitoring is mandatory and consists of a well-developed pharmacovigilance system.

Whilst differing in detail, similar regulations exist for the development, validation, approval and marketing of veterinary biologicals and feed additives.

DIAGNOSTICS

Regulation of Laboratory Techniques

With a few exceptions, for example Good Laboratory Practice (GLP) accreditation, laboratory regulation of veterinary diagnostic laboratories is absent. Some laboratories, for example those that undertake analysis of samples for preclinical studies, where GLP compliance is mandatory and subject to inspection, will be subject to regulatory GLP inspection. In the UK this oversight is provided by the Medicines Healthcare Regulatory (MHRA) Agency of the Department of Health.

with the coverslip (Alcaino and Baker 1974; Egwang and Slocombe 1981). McCoy *et al.* (2005) demonstrated that the Fecpak system (using the McMaster method) was a reliable and accurate means of performing FECs in the hands of highly skilled and experienced operators, but was much less accurate when performed by flock owners, despite them having been trained in its use. They went on to state that it is necessary for anyone advocating the use of on-farm diagnostics to ensure that the skill of the operator is matched to the technique and equipment used and that a mechanism for QC be incorporated into the testing system to ensure accuracy and reproducibility. This point was highlighted again by Gates and Nolan (2009), who concluded that there is no substitute for good training, methodical approach and continuing education.

In various regions of the world, the absence of regulatory oversight of veterinary laboratory testing has been identified as a risk requiring addressing. In 2006, the Committee for Quality Assurance and Standards of the Society for Veterinary Clinical Pathology formulated a comprehensive document for QC standards applicable to all veterinary laboratories (www.asvcp.org/publications/qas-guidelinemenu.html). In Europe, the development and implementation of more complete QC programmes were recently identified as areas for improvement by a group of European veterinary medical laboratories seeking ISO 15189 certification (Freeman *et al.* 2006).

Lack of regulation in veterinary testing is one factor in QC monitoring not being well practised in veterinary hospital facilities, resulting in most users being left to follow the recommendations of suppliers of diagnostic instrumentation (Westgard QC 2015).

Quality assurance is defined in ISO 8402 as:
"all the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfil requirements for quality" (ISO1994).

Study results (Rishniw *et al.* 2012) suggested that the performance of "in clinic" biochemical analysers was significantly worse than that of reference laboratories, and commonly used in clinic analysers periodically and unpredictably failed quality assurance checks. 40-75%; 20% and 40% respectively of test errors occurred in the pre-analytic; analytic and post-analytic phase of diagnostic laboratory procedures respectively (Westgard QC 2015; Braun *et al.* 2015). In a 2007 survey of point of care instrumentation, analysis and quality assurance in veterinary practice, the majority of practices (316/374) utilised manufacturer-provided reference intervals without further adjustment or assessment, while one-third (126/374) used reference intervals from textbooks, which is discouraged by the American Society for Veterinary Clinical Pathology (Bell *et al.* 2014).

Current opinions on the regulation of medical devices, including *in vitro* tests in Europe, appear to reveal a concern that transparency and evidence-based data is lacking within the EU regulatory system as it is currently constructed (Eikermann *et al.* 2013). For example, the lack of a centralised registry means that post-marketing surveillance is fragmented. Eikermann *et al.* (2013) refer to the Poly Implant Prosthèse (PIP) case as an example of the problems associated with following up problems. Although unrelated to IVD, the similarity of the systems for implants

Regulation of Human and Veterinary Medical Devices
There is no regulation that applies to veterinary medical devices in Europe, however regulation of medical devices for human use exists. Within the EU, a **medical device** is defined as:

"Any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease
- diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap
- investigation, replacement or modification of the anatomy or of a physiological process
- control of conception, and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means."

This definition covers implanted devices such as hip replacements as well as *in vitro* devices. The boundaries are not clear cut and the document:
http://ec.europa.eu/consumers/sectors/medical-devices/files/meddev/2_1_3_rev_3-12_2009_en.pdf
has been developed to define the items within the overlapping areas between the Medical Devices Directive 93/42/EEC (MDD)3, the Active Implantable Medical Device Directive 90/385/EEC (AIMDD)4 and the Medicinal Products Directive 2001/83/EC5 (MPD). *In vitro* diagnostics are regulated by Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. OJ L 331 of 7 December 1998.

In order to be placed on the market, *in vitro* diagnostics (IVDs) in the EU must be Conformité Européene (CE) marked. There are a number of classes of IVDs, from simple, relatively harmless tests/methods to those covering potentially life-threatening and/or highly infectious disease diagnosis. The former may be CE marked by the producer, provided the company is accredited under ISO Standard EN13485 to do so. For the latter class of IVDs, the product sponsor nominates an organisation mandated to review applications for CE mark approval and submits a dossier, and in some cases product destined for sale.

In the USA, a similar raft of legislation and regulations is overseen by the Food and Drug Administration. IVDs of differing levels of risk must go through either a 510[k] review process or pre-market approval (PMA) before they are allowed to be sold.

In summary, there is no formal evaluation and approval process for veterinary diagnostics and no requirement for training of the end user or ensuring that the end user is suitably trained and retains competency.

Standards
A lack of mandatory regulation means that some veterinary laboratories and veterinary IVD diagnostics producers have identified relevant standards and have sought to implement them and have them recognised, where possible. Veterinary diagnostic laboratories may be GLP, Good Laboratory Clinical Practice (GLCP) or ISO Standard 17025 accredited, for example. While these standards differ in their detail, all work to ensure that procedures are standardised and appropriate and carried out by suitably informed/trained personnel. Similarly, manufacturers of veterinary IVD tests may be Good Manufacturing Practice (GMP) accredited or ISO 13485 accredited, and may elect to apply or obtain CE marks for individual tests, although there are no background legislation nor regulations to cover such situations. Adoption of agreed standards is consistent with the World Health Organisation recommendations (WHO 2003).

Evidence for the Integrity and Utility of Diagnostics
Correct diagnosis of parasite infection is crucial, with methods being either simple and carried out on farm or in veterinary practices, or complex and needing the use of specialised laboratories (Kramer 2016). Veterinary clinicians rely heavily on laboratory analyses for diagnosis and monitoring of treatment (Bell *et al.* 2014). Current guidance on best practice for parasite control in pets (ESCCAP 2010) recommends that all pets of all ages are screened at least four times per year for gastro-intestinal helminth infections. Similarly, best practice guidance on Sustainable Control of Parasites in Sheep (SCOPS) and Control of Worms Sustainably (COWS) recommend that the production species sheep and cattle, respectively, are monitored for parasite burdens and their requirements for parasite management and treatment based on this information. Diagnosis forms an integral part of evidence-based medicine (Fig. 1).

There are a large number of veterinary diagnostic tests and devices are currently used to identify and monitor parasite infections, and it is beyond the scope of this article to review every test. Instead, the widely used FEC technique will be used as an example of a commonly used technique where there have been initiatives to create standard kits to conduct the test and to make the technique more readily available to farmers and other animal keepers.

It is notable that there is no single FEC technique, with various different methodologies and a lack of between-laboratory standardisation. It has been recognised for some time (Stoll 1930) that the accuracy of FECs depends not only on the analytical sensitivity of the chosen flotation solution but also on the "personal factor" (Levine *et al.* 1960), an undefined source of variation that centres on technical proficiency, even when personnel conducting the test are following the same set of directions. Sample handling is another factor that can affect the apparent performance of all flotation techniques. It has been demonstrated (Foreyt 1986; Rinaldi *et al.* 2011) that many faecal preservation methods influence the detection of various parasite elements. Consensus as to centrifugation time when processing faecal samples for faecal flotation methods has not been established, in part, because the time will be influenced by the flotation media (Ballweber *et al.* 2014). Use of new methods without providing validation and quality control (QC) assessments should be discouraged (Ballweber *et al.* 2014).

The purpose of the exercise, cost-effectiveness, and technical capacity can also greatly influence the choice of method (Ballweber *et al.* 2014). Interpretation of the results requires an appreciation of the likely effects of age and grazing history, for example, on the FEC (Charlier et al. 2016).

Efforts to make a FEC in kit form include Fecpak and Fecpak mark 2 (G2). Some devices (e.g. Ovassay Plus (Zoetis Synbiotics) and Fecalyzer (Vetquinol USA)) incorporate a built-in specimen container and a flotation tube with a sieving basket and flat top for good contact

and IVDs suggests that similar situations could arise for IVDs.

Discussion

A review of the literature assessing the fitness for purpose of medical device regulation in Europe intimates that European regulation has not addressed all concerns. There is a sense that the USA regulation has some benefits over the European system. This may change with new European *in vitro* device regulations for medical devices due to be released in April or May 2017.

Regulation may have negative consequences; for example, it may increase the cost of bringing new products to market and, in doing so, inhibit innovation and decrease the number of new tests reaching the market.

The development of platforms, such as lateral flow devices, that can be used to develop pen side tests may mean that there are more tests available to the end user (the farmer, pet owner or local veterinarian), which means that such tests are out of the hands of professional laboratory techniques with their traditional laboratory management and report approval processes.

Conclusion

There are fundamental differences between the stringent regulation of veterinary medicines and the absence of regulation of veterinary diagnostics. Whilst there is a lack of clarity about the impact of the lack of regulation, or indeed the implications of introducing regulation, in an era of one health (OIE) for humans and animals, the differences between regulation of human and veterinary diagnostics merit further attention.

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