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The Impact of Infection and Inflammation on Drug Metabolism, Active Transport, and Systemic Drug Concentrations in Veterinary Species[S]

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ABSTRACT

Within human medicine, it is recognized that the pharmacokinetics (PK) of many compounds can be altered by the presence of inflammation or infection. Research into the reason for these changes has identified pathways that can influence drug absorption, clearance, and tissue distribution. In contrast, far less is known about these relationships within the framework of veterinary medicine. Rather, most of the PK data generated in veterinary species employs healthy subjects, raising the question of whether these studies are founded on an assumption that healthy animal PK reflect that of the diseased animal population. Accordingly, there is a need to explore the PK changes that might be overlooked in studies that recruit only healthy animals to assesses drug PK. To meet this objective, we surveyed the published literature for studies focusing on the impact of disease on the dose-exposure relationships in food-producing and companion animal species. We found that, consistent with humans and laboratory species, both up- and downregulation of the various cytochrome isoenzymes and/or transporters have occurred in response to an increase in inflammatory mediators. These findings suggest that, as observed in human medicine, the potential for differences in the drug PK in healthy versus animal patients points to a need for acquiring a greater understanding of these changes and how they may influence the dose-exposure-response relationships of veterinary pharmaceuticals.

SIGNIFICANCE STATEMENT

This review delivers a much-needed summary of published information that provides insights into how disease and inflammation can influence the appropriateness of extrapolating laboratory-based dose-exposure-response relationships to what will occur in the actual veterinary patient. As part of this review, we also examine some of the method-associated issues to be considered when assessing the reported nature and magnitude of these changes.

Introduction

A characterization of drug pharmacokinetics (PK) in healthy human subjects often fails to adequately describe dose-exposure-response relationships occurring in the targeted patient population (Renton, 2005; Morgan, 2009). A landmark example is the severe theophylline toxicity precipitated by an unexpected elevation in theophylline serum concentrations in children during the influenza epidemic of 1982 (Kraemer et al., 1982). This toxicity has been attributed to a disease-associated downregulation of CYP1A2 (Christmas, 2015).

Typically, the PK of veterinary therapeutics is assessed in normal, healthy animals, with no additional assessments conducted in the intended patient population (representatives of the target animal species with or without the indicated disease condition). Thus, there is an underlying assumption of comparability between the dose-exposure (blood and tissue) relationships in healthy versus diseased animals. There is ample evidence that such an assumption can be incorrect.

On the other hand, although disease can alter plasma protein binding, such modifications rarely lead to a need for dose adjustments. For example, in humans, disease and inflammation may decrease the levels of serum albumin (Don and Kaysen, 2004) and increase levels of α1-acid glycoproteins (Huang and Ung, 2013). Either of these changes could potentially affect the relationship between total versus free drug concentrations in the plasma. If assessed solely on the basis of total plasma drug concentrations, changes in protein binding would be interpreted as changes in the drug PK. Nevertheless, unless changes in protein binding are differently expressed in plasma versus tissues, there are very few situations in which such changes will affect free (unbound) drug

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ABBREVIATIONS: ABCB1 (or Abcb1), ATP-binding cassette (ABC) transporter protein B1 (P-glycoprotein); BCRP (or Bcrp), breast cancer resistance protein; CL, clearance; CL/F, clearance divided by fraction of an administered dose that is systemically available; dpi, days postinfection; FBZ, fenbendazole GI gastrointestinal; GI, gastrointestinal; IBR, infectious bovine rhinotracheitis; IL, interleukin; LPS, lipopolysaccharide; Mdr, multidrug resistance protein; MRP, multidrug resistance–associated protein; MRT, mean residence time; NSAID, nonsteroidal anti-inflammatory drug; OXF, oxfendazole; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PK, pharmacokinetics; PRRSV, porcine reproductive and/or respiratory syndrome virus; $t_{1/2}$, half-life; T_{max}, time to peak drug concentrations; TNFα, tumor necrosis factor-α; Vd, volume of distribution; Vd/F, volume of distribution divided by fraction of an administered dose that is systemically available.

exposure. Generally, it is the free drug concentrations that are clinically relevant (Benet and Hoener, 2002; Schmidt et al., 2010; Gonzalez et al., 2013; Heuberger et al., 2013; Stern et al., 2016). Thus, dose adjustments would not be needed. Unfortunately, this disparity between disease-induced changes in free versus total drug concentrations complicates the interpretation of most in vivo disease model studies conducted in veterinary species in which only total drug concentrations are measured. This gap renders it more difficult to identify disease-induced changes in drug metabolism or transporter activity.

Based upon what is known from laboratory animal studies and human medicine, we considered it important to ask the following questions:

- Is there published evidence that a change in immune state (due to infection, stress, or inflammation) can alter drug PK in veterinary species?
- What data are available to inform us about the relationship between specific metabolizing enzymes and/or transporters, the different inflammatory mediators, and the corresponding magnitude of impact it may have on drug PK?

To explore these questions, we surveyed the literature for published veterinary clinical pharmacology studies describing disease-related changes in PK and considered potential pathways responsible for these effects as suggested by published in vitro data generated in veterinary-derived tissues. It is important to note that all studies cited in this manuscript were reviewed solely from the perspective of PK changes associated with infection and inflammation. They were not considered from the perspective of addressing any regulatory requirements (e.g., target animal safety and human food safety). Furthermore, when evaluating potential PK changes in edible tissues, it is vital to recognize that meat and milk from diseased animals should not enter the human food supply.

This review is divided into sections, first to provide a high-level overview of the general aspects of the relationship between disease, inflammation, and infection and secondly to consider the relationships as described for the various drug classes. Accordingly, several of the published investigations are cited in multiple sections of this manuscript, allowing us to emphasize different aspects of the study results.

Evidence of Disease-Induced Changes in Drug PK

Based upon published animal and in vitro study data, it is important to consider the potential for microbial, parasitic, and inflammatory diseases to influence drug PK in veterinary species. As depicted in Fig. 1, inflammation and infection are inter-related, leading to numerous changes in host physiology. Details on these pathways and their relationships can be obtained from Cavaillon and Adib-Conquy (2002), who describe the cytokine cascade, and Morgan (2017), who discusses the relationship between inflammation and acute inflammatory responses.

For any given cytochrome P450, there is specificity in the relationship between the particular cytokine released, the nature of the infection, and altered enzyme activity. Consequently, changes in drug metabolism tend to be infection-specific (Renton, 2005). The complexity of this relationship is exemplified by the downregulation of murine hepatic Cyp2a5 (ortholog of human CYP2A6/13) when administered low doses of lipopolysaccharide (LPS), the component of the cell wall of Gram negative bacteria that acts as endotoxin, but not by higher doses of LPS. In contrast, other cytochromes (Cyp1a1/2 and Cyp2b9/10) were downregulated, but only in the presence of high doses of LPS (De-Oliveira et al., 2015). Similarly, the change in cytochrome P450 activity is influenced by pathogen, body site, and time postinfection. For example, murine schistosomiasis resulted in a significant upregulation of hepatic Cyp1a2, 2c29, 2e1, 2j5, 3a11, 4f13, and 4f18 at 30 days postinfection (dpi) but a 30%–96% downregulation of most of these same cytochrome P450s at 45 dpi (exceptions included cyp4a12, 4f16, and 4f18) (Mimche et al., 2014). In broiler chickens, induced colibacillosis (an infection caused by *Escherichia coli*) statistically significantly decreased the Cyp3a37 mRNA in liver and kidney but not in the duodenum, jejunum, and ileum. Yet there was a simultaneous statistically significant upregulation in the Abcb1-mRNA expression levels of the kidney, jejunum, and ileum. Statistically significant differences in Abcb1-mRNA expression levels were not observed in the liver and duodenum (Guo et al., 2014).

These observations lead to questions pertaining to the underlying mechanisms responsible for these complexities.

Studying Mechanisms Underlying Disease-Induced PK Changes

It is believed that the cytokines released in response to inflammation and infection can act through pre- and post-transcriptional changes (Morgan *et al.*, 2002; Renton, 2005; Ho and Piquette-Miller, 2006). However, this may not always be the case: there can be a disconnect between altered transcription activity versus protein expression. For example, looking again at the study by Mimche et al. (2014), their mouse schistosomiasis model was associated with a 3.8-fold upregulation of microsomal Cyp2a levels at 30 dpi, even though there was no corresponding change in its hepatic mRNA. For those mRNAs upregulated on day 30, there was no matching increase in the microsomal levels of the other corresponding cytochrome P450s. Conversely, results seen on 45 dpi showed similar changes in the levels of specific cytochrome P450s and their mRNA.

Théron et al. (2003) examined the influence of tumor necrosis factor-α (TNFα) on the multidrug resistance protein 1a,b (Mdr-1a,b), mRNA, and P-glycoprotein (P-gp) expression of immortalized rat brain endothelial cells. They concluded that disease-induced changes may be influenced by the species studied, the cell model type, the culture procedures used, and the treatment protocol. Of importance was their observation that changes in transporter activity may not correspond to changes in protein expression or to changes in the level of mRNA. Moreover, even though the changes in mRNA may have suggested either an increase or no change in the levels of P-gp, there was an apparent TNFα concentration-dependent and time-dependent inhibition of P-gp activity. These conclusions were reiterated by Poller et al. (2010), thus reinforcing the concern that an interpretation of study results involving changes in the levels of mRNA should proceed with great caution.

The molecular mechanism by which an assault to the immune system impacts the PK of drugs in animals has been studied in pigs (Monshouwer et al., 1995b, 1996a,b; Li et al., 2016), Frisian calves (Maffei Facino et al., 1984), broiler chickens (Bártíková et al., 2010; Guo et al., 2014), sheep (Klingenberg, 1958; Saitoh et al., 1999; Zhang et al., 2014), dogs (Lambert et al., 1991), rabbits (Garfinkel, 1958; Haritova et al., 2008), and fish (Reynaud et al., 2008). A variety of in vitro methods have been used to probe drug disposition–related changes after infection (Maffei Facino et al., 1984; Monshouwer et al., 1995b, 1996a; Saitoh et al., 2000; Haritova et al., 2008; Bártíková et al., 2010; Guo et al., 2014; Li et al., 2016), exposure to a toxin (Zhang et al., 2014), and disease (Lambert et al., 1991). These include the measurement of total cytochrome P450 content, drug metabolism activity (by the cytochrome P450s as well as by other enzymes), mRNA expression, transporter expression, cytokine levels, protein expression, quantitative polymerase chain reaction (PCR), and immunohistochemistry. Table 1 summarizes the in vitro methods used and assay results.

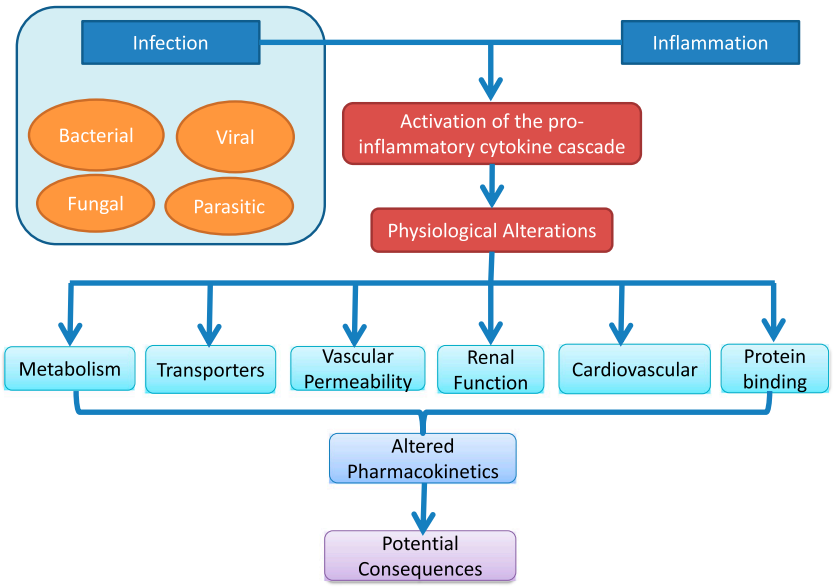

Fig. 1. Inter-relationships associated with the impact of inflammation and infection on drug pharmacokinetics. Refer to Cavaillon and Adib-Conquy (2002), Renton (2005), and Morgan et al. (2002) for additional details regarding activation of the cytokine cascade and its potential physiologic/PK consequences.

When interpreting published data from in vitro studies, it is important to identify the species specificity of probes used to assess cytochrome P450 or transporter activities. If probe selection is based upon human-derived DNA or RNA, there could be bias introduced into the data interpretation. Interspecies mismatch in DNA sequences limits the quantitative information that these assays can provide. Therefore, these studies should be evaluated on the basis of qualitative rather than quantitative trends. Nevertheless, because of the paucity of data for veterinary species, these findings are included in our summary. Methodologies are summarized in Supplemental Material, Part 2.

Impact on Drug Metabolism. The nature of the proinflammatory cytokines released depends upon the stimulus (Medzhitov and Horng, 2009; Contreras and Rao, 2012; Muralidharan and Mandrekar, 2013), with the most potent cytokines being interleukin (IL)-6, TNFα, IL-1b, and interferon-c (Harvey and Morgan, 2014). In this regard, it was recently noted that changes in human drug metabolism can be related not only to transcriptional suppression but also to potential post-translational protein modification by proinflammatory cytokines (Storelli et al., 2018). An additional point of caution is that several enzymes can be involved in drug metabolism, rendering it difficult to ascribe an observed change in CL to a specific metabolic pathway. For example, in humans, CYP3A4, CYP2C9, CYP2C18, CYP1A2, and CYP2B6 all appear to be involved in the formation of antipyrine metabolites (Engel et al., 1996). Similarly, it is challenging to ascribe a specific cytochrome P450 as being responsible for the observed *Actinobacillus pleuropneumonia*–associated decrease in the clearance of antipyrine and caffeine in swine (72% and 68%, respectively) (Monshouwer et al., 1995a).

As with that associated with bacterial diseases, parasitic infections can lead to substantial increases in total drug exposure in human patients. For example, the area under the concentration versus time curve (AUC) values of praziquantel (metabolized primarily by CYP2D6 and 3A4) and propranolol (metabolized largely by CYP2D6 and 1A2) were increased 2- to 5-fold in humans infected with schistosomiasis (Watt et al., 1988; Mandour et al., 1990). A similar outcome was obtained in a chronic murine schistosomiasis infection model (Mimche et al., 2014), with the majority of cytochrome P450s downregulated (protein levels and mRNA) 45 dpi, reflecting chronic infection. In contrast, the mRNAs at 30 dpi were either slightly upregulated or remained unchanged, with cytochrome P450 protein levels typically remaining unchanged by the infection (except for Cyp2a, as previously noted). The observed time-associated variation in disease-induced PK changes may be particularly important for those drugs whose systemic concentrations remain elevated for weeks or months, due either to repeated administrations or to the use of extended-release formulations.

Selectivity of hepatic cytochrome P450 effects was also observed in dogs with congestive heart failure, with significant decreases observed in total cytochrome P450s and Cyp2b, but not in Cyp3a. Interestingly, congestive heart failure in humans has been linked with an increase in TNFα, IL-6, and IL-1β (Gullestad et al., 2012), which is consistent with the cytokines associated with inflammation-associated changes (down-regulation) in cytochrome P450s. In fact, in humans, congestive heart failure is associated with an increase in the gene expression (measured as mRNA) of several cardiac cytochrome P450s and the simultaneous downregulation in the expression of several hepatic cytochrome P450s (Zordoky and El-Kadi, 2008). Thus, some physiologic diseases may also alter drug PK, at least in part, via activation of inflammatory pathways.

Transporters. Both influx and efflux transporter activity can be influenced by the proinflammatory cytokines (Le Vee et al., 2009).

Proinflammatory cytokines released in response to inflammation and infection can affect the expression of multidrug resistance–associated proteins (MRPs), breast cancer resistance protein (BCRP), and P-GP (Ho and Piquette-Miller, 2006). In a rat model of rheumatoid arthritis, there was an 85% increase in the duodenal permeability of a Chinese herbal medication, which was apparently due to a downregulation of membrane P-gp activity (Duan et al., 2017). In contrast, P-gp upregulation was observed during the assessment of an in vitro murine model of bovine mastitis caused by *E. coli* (reflected in the administration of LPS) or *Staphylococcus aureus* (Yagdiran et al., 2016). Murine mammary epithelial HC11 cells differentiated into a secreting phenotype exhibited a statistically significant increase in P-gp expression when incubated with LPS and *S. aureus* as compared with that associated with the control cell cultures. This outcome was interpreted as reflecting potential increases in drug and toxin secretion into the milk of lactating dairy cattle (Yagdiran et al., 2016).

In that regard, it is interesting to note that the PK of moxidectin in sheep was significantly altered by the presence of *Haemonchus contortus* infection, including an increase in CL/F after subcutaneous injection (Lespine et al., 2004). This increase was (at least in part)

TABLE 1

Highlights of publications exploring the mechanism by which changes in the immune system impact drug pharmacokinetics

The methods referenced in the "Marker for Evaluation" and "Nature of Change" columns are described in detail in the Supplemental Material, Part 2 of this report, such that each letter (i.e., A, B1, B2, C, etc.) corresponds to a method description. In general, the impact of a change in immune function is to decrease cytochrome P450 expression and activity. This may lead to increased residue concentrations. Abbreviations are defined at the end of the table.

Animal	Source of Inflammation	Metabolism or Transporter	Marker for Evaluation	Nature of Change	Reference
Pigs	Endobronchial *A. pleuropneumoniae* N = 9 pigs inoculated in the bronchia N = 6 control pigs	Cyp3a, 1a1, 2b, 2e1	**A:** Total cytochrome P450 **B1:** Cytochrome P450 activity •Testosterone (3a) •7-ethoxyresorufin (1a1) •Pentoxyresorufin (2b) •Aniline (2e1) **B2:** UDP activity •1-napthol •Morphine •Chloramphenicol •Paracetamol **C:** RNA hybridization •3A4 cDNA[a]	24 h postinfection: **A:** Cytochrome P450 content ↓40% **B1:** Cytochrome P450 activity •Testosterone ↓50% •7-Ethoxyresorufin ↓50% •Pentoxyresorufin ↓60% •Aniline ↓33% **B2:** UDP activity No change **C:** RNA hybridization: Cyp3a mRNA ↓	Monshouwer et al., 1995b
Pigs	*E. coli* LPS-induced acute phase response model N = 6 pigs injected with 17 μg/kg LPS every hour for five doses N = 6 saline-injected pigs	Cyp3a, 1a1, 1a2, 2e1	**A:** Total cytochrome P450 **B1:** Cytochrome P450 activity •Testosterone (3a4) •7-ethoxyresorufin (1a1) •Aniline (2e1) •Caffeine (1a2) **B2:** UDP activity •1-napthol **D:** Cytokine assays •IL-6[b] •TNFα [c] **E:** Western blot •Cyp1a •Cyp3a **F:** Plasma concentration •Antipyrine	Postinfection: **A:** Total cytochrome P450 ↓25% **B1:** Cytochrome P450 activity •Testosterone ↓45%–80% •7-ethoxyresorufin ↓45% •Aniline ↓35% •Caffeine ↓60% **B2:** UDP activity No significant change **D:** Cytokine assays •↑IL-6: T_{max} 3 h •↑TNFα: T_{max} 1 h **E:** Western blot •Cyp1a ↓ •Cyp3a ↓ **F:** Plasma concentration CL ↓75% $t_{1/2}$ ↑3.6× AUC ↑4.2×	Monshouwer et al., 1996a
Cows	*Fasciola hepatica* (parasite) 30 Frisian calves Group 1: adult parasites Group 2: flukes Group 3: no infection	Cytochrome P450	**B1:** Cytochrome P450 activity •*p*-Nitroanisole •Aminopyrine •Aniline **G:** Liver tissue activity •Nitroxynil metabolism	**A:** Cytochrome P450 content ↓60% **B1:** Cytochrome P450 activity •*p*-Nitroanisole ↓60% •Aminopyrine ↓60% •Aniline ↓60% **G:** Liver tissue activity •Nitroxynil metabolism: ↓80% in infected cows inhibited by mild disease had milder impact	Maffei Facino et al., 1984
Chickens (broilers)	*E. coli* from infection with colibacillosis injected into pectoral muscle N = 5 infected N = 5 healthy	Cyp3a, P-gp: Abcb1 gene	**F:** Plasma concentration in infected and healthy: •Enrofloxacin 10 mg/kg •Enrofloxacin 10 mg/kg + verapamil 15 mg/kg **H:** qPCR [d] •Primers specific for Abcb1, Cyp3a, and β-actin **I:** Immunohistochemistry for P-glycoprotein •Liver •Small intestine	**F:** Plasma concentration PK enrofloxacin infected: •C_{max} ↓66% •$AUC_{0–12}$ ↓50% •T_{max} ↑120%× PK enrofloxacin with P-gp inhibitor verapamil: •C_{max} ↓30% •$AUC_{0–12}$ ↓12% •T_{max} ↓9% (Personal comparison of means provided in the publication: see comments in text) **H:** qPCR: disease resulted in significantly higher Abcb1 mRNA levels in kidney, jejunum, ileum. No change in liver. •Cyp3a37 mRNA significantly decreased in liver and kidney **I:** Immunohistochemistry for P-gp •Healthy birds: P-gp visualized on bile canicular membrane	Guo et al., 2014

(*continued*)

TABLE 1—*Continued*

Animal	Source of Inflammation	Metabolism or Transporter	Marker for Evaluation	Nature of Change	Reference
				Kidney: P-gp visualized on apical plasma membranes of proximal tubule cells •Infected birds: internalized in cytoplasm away from bile canicular membrane. Kidney: distributed in cytoplasm.	
Sheep	Mycotoxin *N* = 27 sheep	Cytochrome P450	**J:** RNA-Seq analysis **H:** qPCR	**J:** RNA-Seq analysis Multiple cytochrome P450s were identified in the RNA-Seq analysis. **H:** qPCR Only Cyp2c8 and Cyp1a2 were confirmed by qPCR.	Zhang et al., 2014
Dogs	Congestive heart failure *N* = 14 mongrel dogs	Cyp2c8, 1a2, 2e1, 3a, 2b	**A:** Total cytochrome P450 **B1:** Cytochrome P450 activity •Aminopyrine (2c8) •7-ethoxycoumarin (1a2, 2e1) •Aniline (2e1) **E:** Western blot •Cyp3a •Cyp2b	**A:** Total cytochrome P450 ↓40% **B1:** Cytochrome P450 activity •Aminopyrine ↓ •7-ethoxycoumarin: no Δ •Aniline: no Δ **E:** Western blot •Cyp3a: no change •Cyp2b: ↓40%	Lambert et al., 1991
Pigs	Incubate hepatocytes with cytokines: IL-1β, TNFα, IL-6 for 12 or 24 h Livers from three pigs	Cytochrome P450 and UDP GT	**B1:** Cytochrome P450 activity •Testosterone (3a4) •Ethylmorphine (2d6 and 3a4) **B2:** UDP activity •1-napthol •Paracetamol •Morphine	**B1:** IL-6 caused significant inhibition of metabolism of all substrates tested: 30%–50% decrease. **B2:** IL-1α [e] and TNFα significantly reduced metabolism of 1-napthol, paracetamol, and morphine: 20%–30%.	Monshouwer et al., 1996b
Rabbits	*E. coli* LPS *N* = 20 rabbits	Cytochrome P450	**A:** Total cytochrome P450 **B1:** Cytochrome P450 activity •Aminopyrine •Aniline •Caffeine **B2:** UDP activity •*p*-Nitrophenol **E:** Western blot •Anti-Cyp1a1/a2 •Anti-Cyp2e1 **F:** Plasma concentration •Antipyrine	**A:** Total cytochrome P450 ↓25% **B1:** Cytochrome P450 activity For all: no change in Km V_{max} ↓45% •Aminopyrine •Aniline •Caffeine **B2:** UDP activity •*p*-Nitrophenol: no Δ **E:** Western blot •Anti-Cyp1a1/a2 ↓ •Anti-Cyp2e1 ↓ **F:** Plasma concentration •Antipyrine AUC ↑1.5×	Saitoh et al., 1999
Chickens (broilers)	Experimentally induced colibacillosis *N* = 36 chickens	Mdr1 [f], Mrp2 [b]	**H:** qPCR •Mdr1 •Mrp2	**H:** qPCR •Mdr1 ↓Mdr1 mRNA levels in the duodenum, jejunum, caeca, and liver •Mrp2 ↓ Mrp2 in liver	Haritova et al., 2008
Sheep	*H. contortus* *N* = 12 lambs	Cyp3a	**B1:** Cytochrome P450 activity •7-ethoxyresorufin (1a) •7-methoxyresorufin (1a) •7-pentoxyresorufin (2b) •7-benzyloxyresorufin (3a) •7-methoxy-4-coumarin demethylase (2c9) •Clorzoxazone (2e1) **B2:** UDP activity *p*-Nitrophenol •Flavine monoxygenase Thiobenzamide	**B1:** Cytochrome P450 activity •7-ethoxyresorufin (1a) ↓12% •7-methoxyresorufin (1a) ↓20% •7-pentoxyresorufin (2b) ↓10% •7-benzyloxyresorufin (3A) ↓40% •7-methoxy-4-coumarin demethylase (2c9) ↓20% •Clorzoxazone (2e1) ↓40% •Flavine monoxygenase Thiobenzamide ↓50%	Bártíková et al., 2010

↓, decrease; ↑, increase; qPCR, quantitative PCR.

[a]cDNA is synthesized from a single-stranded RNA template in a reaction catalyzed by a reverse transcriptase.

[b]IL-6 is a cytokine that can express both pro- and anti-inflammatory activities (Scheller et al., 2011).

[c]TNFα is a proinflammatory cytokine that has a key role in the pathogenesis of chronic immune-mediated diseases (Chu, 2013). It significantly reduced metabolism of 1-napthol, paracetamol, and morphine 20%–30%.

[d]qPCR is used for its ability to determine the relative or absolute amounts of amplified DNA in samples.

[e]IL-1α is released from the cell upon death and is a potent inflammatory cytokine (van de Veerdonk and Netea, 2013; Di Paolo and Shayakhmetov, 2016);

[f]Mdr1 is the multidrug resistance gene that encodes for the efflux transporter P-glycoprotein.

[g]Mrp2 is a unidirectional efflux transporter that primarily transports organic anions. It is most highly expressed in the liver, where it typically transports compounds into the bile.

attributed to increased intestinal and biliary secretion, which they noted may be modified by loperamide. Because this relates to the moxidectin transport via P-gp (Lespine et al., 2011), it would have been informative if data were available to determine whether changes in intestinal P-gp levels occurred in the presence of this parasitic infection.

Interestingly, a given inflammatory mediator may differentially impact gene expression in a tissue-specific manner. For example, in LPS- or turpentine-treated mice, P-gp was downregulated in the liver, a response attributed to increased levels of IL-6 (Hartmann et al., 2001), but was upregulated in the kidney (Hartmann et al., 2005). This apparent discrepancy underscores the interpretation bias that can be introduced when trying to extrapolate the impact of infection and inflammation across tissues, even with the same animal species or experimental model.

The other interesting observation is the apparent contradiction between the findings of Guo et al., 2014 versus Haritova et al., 2008. Haritova and colleagues report a downregulation of P-gp (Abcb1 mRNA expression) after *E. coli* infection in broiler chickens. These changes were observed in the duodenum, jejunum, cecum, and liver within 24 hours postinfection. In contrast, Guo et al. reported an upregulation in the expression of Abcb1 mRNA in the kidney, jejunum, and ileum (no significant changes observed in the liver or duodenum) after an induced *E. coli* infection in broiler chickens. When examining these two studies, the principle difference appears to be the manner of infection. For Haritova's group, inoculation was via tracheal administration (0.2 ml containing 2.6×10^8 colony forming units). In the Guo investigation, inoculation was via pectoral injection (0.5 ml containing 1.5×10^8 colony forming units). Given the high specificity seen in terms of variables impacting the expression of transporters and drug metabolizing enzymes in response to infection and inflammation, it would not be surprising if this difference in site of inoculation influenced the nature of the response. Such an effect would be consistent with the observed relationship between the site of pathogen vaccination versus the magnitude and nature of the host immune response (Belyakov and Ahlers, 2009).

The study by Guo et al. (2014) has several interesting aspects. They observed that plasma enrofloxacin concentrations after oral administration were lower in diseased broilers within 12 hours after inoculation with *E. coli* versus that of healthy broilers (Guo et al., 2014). Similar changes in plasma enrofloxacin concentrations were reported by Soliman (2000) for intravenous enrofloxacin within 48 hours after *E. coli* administration. Although plasma levels were lower rather than higher in the diseased birds, hepatic Cyp3a37 mRNA was downregulated in the infected broilers (Guo et al., 2014). Simultaneously, there was an upregulation of Abcb1 mRNA levels in kidney, jejunum, and ileum (but not in the liver). Guo et al. suggested that the decrease in plasma concentrations was a function of increased activity of the intestinal efflux transporter. Using oral verapamil to block the P-gp, they observed that groups of birds receiving verapamil exhibited higher enrofloxacin plasma concentrations as compared with the healthy and diseased birds that were not coadministered verapamil. In fact, the verapamil-treated infected birds exhibited AUC values similar to those of verapamil-treated healthy birds. Although it can be argued that the effects of verapamil likely reflect inhibition of both P-gp and Cyp3a enzymes (Wang et al., 2004), the much larger magnitude of increase in blood levels in the infected birds suggests an important role of disease effects on the drug transporter system.

In terms of Bcrp, Su et al. (2014) observed that both Abcg2 (the ATP-binding cassette super-family G member 2, Bcrp) mRNA expression and Bcrp protein levels were statistically significantly different (lower rather than higher) in the liver, jejunum, and ileum of broilers infected by *E. coli* or sporulated oocyst suspension of *Eimeria necatrix* and *E. tenella* (injected into the pectoral muscles) as compared with that of healthy birds. Thus, when comparing the results of Su et al. versus Guo et al. studies, it would appear that *E. coli* infection can exert opposing effects on the levels of P-gp versus Bcrp of broilers.

Impact of Disease as a Function of Drug Class

The information below provides a summary of published studies on the influence of disease on the PK of drugs in farm and companion animal species. Of the studies identified, 17 report an increase in systemic drug exposure, whereas 25 report either no change or a decrease in exposure. Table 2 highlights the published studies on cephalosporins, fluoroquinolones, nonsteroidal anti-inflammatory drugs (NSAIDs), macrolides, or antiparasitic agents. A more detailed version of this table is provided in the Supplemental Material, Part 1.

For completeness, we provide both a synopsis of the investigator conclusions and our comments based upon the mechanisms of disease effects as discussed above.

Cephalosporins. In lactating dairy cattle, total ceftiofur plasma concentrations after repeated intramuscular injections tended to be lower in mastitic versus healthy animals (Gorden et al., 2016). This decrease in exposure appeared to be associated with an increase in CL/F. Lower concentrations were also attributed to an increase in the total systemic volume of distribution (Vd)/F. It is interesting to note that although these disease-associated changes were seen after multiple administrations, similar PK differences were not evident after dose 1. The authors acknowledged that their study design introduced a possible bias by using a blood sampling schedule that failed to capture steady-state peak drug concentrations (both in healthy and diseased cattle), leading to a potential exaggeration of healthy versus diseased differences in total drug exposure (AUC), CL/F, and Vd/F. Furthermore, because the study was complicated by concomitant treatment with flunixin and fluids to the diseased cattle, it was not possible to ascertain the extent to which disease versus the administration of fluids and flunixin was responsible for the observed PK differences. Drug concentrations in milk were not reported.

Cephalosporin drug concentrations in milk were measured in two studies: intravenous ceftriaxone administered to healthy and diseased (endometritis) cows (Kumar et al., 2010) and intramammary infusion of ceftiofur hydrochloride to healthy and mastitic cattle (Han et al., 2017). Han's group did not observe any significant differences in either the milk or serum total ceftiofur concentrations as a function of infection. Rather, the productivity of the quarter was far more important than was the presence of a disease state. In that regard, drug concentrations in milk derived from high-production quarters were significantly lower and depletion was more rapid than those from low-production quarters. Conversely, Kumar et al. (2010) reported that ceftriaxone levels were lower and that CL was higher in diseased versus healthy cattle. A statistical analysis was not conducted to support this conclusion. The mean milk ceftriaxone concentrations were greater in healthy cows as compared with diseased cows at the first milking 12 hours postdose. Again, no statistical comparison of these values was provided. At all other sampling points, the biologic relevance of any apparent numerical differences in mean milk ceftriaxone was difficult to assess because of the large S.D. seen, particularly in the milk of endometritic cows.

In swine, after intramuscular injection of ceftiofur hydrochloride, AUC was lower, CL/F was greater, C_{max} was lower, and Vd/F was greater in nonpregnant, nonlactating swine artificially infected with porcine reproductive and/or respiratory syndrome virus (PRRSV) as compared with their healthy counterparts (Tantituvanont et al., 2009; Day et al., 2015). Furthermore, although Tantituvanont et al. (2009) suggested that the change in CL/F could be attributed to disease-associated changes in plasma protein binding, we consider this to be

TABLE 2

Impact of Infection on PK

A more detailed summary of these study reports is provided in the Supplemental Material).

Title	Author Year	Pathogen	Drug	Method of Drug Administration	Species	Consequence
Influence of induced disease states on the disposition kinetics of imidocarb in goats	Salam Abdullah and Baggott, 1986	LPS, *Trypanosoma evansi*, IBR virus	Imidocarb	Intravenous	Goats	LPS and IBR reduced Vd and CL. Infection with *T. evansi* resulted in an increase in Vd and CL.
Pharmacokinetics of difloxacin in healthy and *E. coli*-infected broiler chickens	Abo El-Ela et al., 2014	*E. coli*	Difloxacin	Intravenous and oral	Chicken	After intravenous administration, disease resulted in an increase in CL and Vd and a decrease in AUC.
Oxytetracycline concentrations in healthy and diseased calves	Ames et al., 1983	Pneumonia caused by BVDV plus *P. haemolytica*	Oxytetracycline	Intravenous	Calves	Pneumonia resulted in an increase in Vd, $t_{1/2}$, and oxytetracycline lung concentrations.
Effects of trypanosomal infection on the pharmacokinetics of diminazene aceturate in dogs	Anika and Onyeyili, 1989	*Trypanosoma brucei*	Diminazene	Intravenous	Dogs	Infection with *T. brucei* resulted in a decrease in Vd and CL.
Pharmacokinetics of tulathromycin in edible tissues of healthy and experimentally infected pigs with *Actinobacillus pleuropneumoniae*	Bladek et al., 2015	*A. pleuropneumoniae*	Tulathromycin	Intramuscular	Swine	Infection resulted in a change in tulathromycin tissue concentration-time profile, characterized by an increase in elimination $t_{1/2}$ and AUC in liver, kidney, muscle, skin, and injection site.
Impact of an experimental PRRSV and *Streptococcus suis* co-infection on the pharmacokinetics of ceftiofur hydrochloride after intramuscular injection in pigs	Day et al., 2015	PRRSV and *S. suis*	Ceftiofur	Intramuscular	Swine	Coinfected pigs had lower AUC and C_{max} values but greater Vd and CL values than that of healthy pigs.
Pharmacokinetics of tilmicosin in healthy and experimentally *Pasteurella multocida* infected lactating goats	El-Komy et al., 2016	*P. multocida*	Tilmicosin	Subcutaneous	Goats (lactating)	Plasma tilmicosin concentrations were substantially lower in *P. multocida*–infected goats.
Pharmacokinetics of flunixin after intravenous administration in healthy and endotoxaemic rabbits	Elmas et al., 2006	LPS	Flunixin	Intravenous	Rabbit	LPS resulted in a decrease in CL and an increase in AUC and $t_{1/2}$.
The influence of *Actinobacillus pleuropneumoniae* infection on tulathromycin pharmacokinetics and lung tissue disposition in pigs	Gajda et al., 2016	*A. pleuropneumoniae*	Tulathromycin	Intramuscular	Swine	Greater tissue AUCs were observed in pneumonic pigs as compared with healthy pigs, but significance was not detected.
Altered plasma pharmacokinetics of ceftiofur hydrochloride in cows affected with severe clinical mastitis	Gorden et al., 2016	*E. coli* or *Klebsiella* spp.	Ceftiofur	Intramuscular	Cattle (lactating dairy)	Mastitic cows had significantly higher plasma Vd and CL and lower AUC and C_{max} as compared with healthy cows.
E. coli infection modulates the pharmacokinetics of oral enrofloxacin by targeting P-glycoprotein in small intestine and Cyp450 3a in liver and kidney of broilers.	Guo et al., 2014	*E. coli*	Enrofloxacin with or without oral verapamil	Oral	Chicken	By 12 h postinfection, there was a significant upregulation of Abcb1 mRNA in kidney, jejunum, and ileum. Expression of Cyp3a37 mRNA significantly decreased in liver and kidney. Significant decrease in enrofloxacin C_{max} and AUC but later T_{max}. Disease-induced changes in systemic exposure were reduced by verapamil.
Elimination kinetics of ceftiofur hydrochloride in milk after an 8-day extended intramammary administration in healthy and infected cows	Han et al., 2017	*S. aureus*	Ceftiofur	Intramammary	Cattle (lactating dairy)	No differences in milk or serum PK. Quarter production efficiency but not disease influences drug conc. in milk.
Pharmacokinetic-pharmacodynamic indices of enrofloxacin in *E. coli* O78/H12 infected chickens	Haritova et al., 2011	*E. coli*	Enrofloxacin	Oral	Chicken	Mdr1 mRNA expression was significantly lower in infected animals but was partially restored with 5 days of oral danofloxacin or enrofloxacin treatment. No blood PK samples were collected.
Comparative kinetic disposition of oxfendazole in sheep and goats before and during infection with *Haemonchus contortus* and *Trichostrongulus colubriformis*	Hennessy et al., 1993	*H. contortus*, *T. colubriformis*	[^{14}C]OFZ	Intraruminal	Goats and Sheep	No change in the PK of FBZ or FBZ-SO_2, but significant decrease in OFZ C_{max} and AUC in both goats and sheep
	Ismail and El-Kattan 2007	*M. haemolytica*	Marbofloxacin	Intramuscular and intravenous	Calves	Infection resulted in a decrease in CL (intravenous) and an increase in $t_{1/2}$ (intramuscular and intravenous),

(*continued*)

TABLE 2—*Continued*

Title	Author Year	Pathogen	Drug	Method of Drug Administration	Species	Consequence
Comparative pharmacokinetics of marbofloxacin in healthy and *Mannheimia haemolytica* infected calves						AUC (intramuscular and intravenous), and C_{max} (intramuscular). There were no changes to protein binding.
Effect of *Haemonchus contortus* infection on the clearance of antipyrine, sulfobromophthalein, chloramphenicol, and sulfathiazole in lambs	Kawalek and Fetterer 1990	*H. contortus*	Antipyrine, sulfobromophthalein, chloramphenicol, sulfathiazole	Intravenous	Lambs	During infection, significant decreases were observed in the AUC of sulfathiazole, antipurine, and chloramphenicol. However, only antipyrine was associated with a significant increase in CL. Therefore, the reliability of the conclusions is unclear.
Comparison of pharmacokinetics and milk elimination of flunixin in healthy cows and cows with mastitis	Kissell et al., 2015	Mastitis (*E. coli* or *Klebsiella* spp.)	Flunixin	Intravenous	Bovine	Mastitis resulted in a substantial decrease in CL and increase in milk flunixin concentrations.
Plasma pharmacokinetics and milk levels of ceftriaxone following single intravenous administration in healthy and endometritic cows	Kumar et al., 2010	Endometritis (unknown)	Ceftriaxone	Intravenous	Bovine	Only mean parameters provided (no statistics). Data suggest increase in CL, Vd, and $t_{1/2}$ but decrease in AUC. Ceftriaxone milk excretion was initially greater in healthy cows, but some differences in mean values were observed at hour 36 postdose (drug levels in milk of healthy cows = 7.5 μg/ml; that of cows with endometritis = 22.9 μg/ml). However, the variability (CV) observed in milk levels of diseased cows was substantially greater at hours 24 and 36 (46% and 64% CV, respectively) as compared with that of healthy cows (10.9% CV and 2.9% CV at hours 24 and 36, respectively) (n = 8 per group).
The influence of a heavy infection with sensitive and resistant strains of *Ostertagia circumcincta* and with *Trichostrongylus colubriformis* on the pharmacokinetics of febantel on lambs.	Landuyt et al., 1995	*O. circumcincta*, *T. colubriformis*	Febantel	Oral	Lambs	Authors suggest that PK changes (monitored for febantel metabolites) were dependent on the infecting parasitic species. Although there was a consistent decrease in mean AUC (compared with the animals prior to infection), the change in rate of metabolite appearance (C_{max} and T_{max}) differed as a function of the nature of the infection. In general, differences in mean values were small.
The influence of parasitism on the pharmacokinetics of moxidectin in lambs	Lespine et al., 2004	*H. contortus* and *T. colubriformis* mix (natural infections)	Moxidectin	Oral and subcutaneous	Sheep	Increase in CL/F (oral), decrease in mean residence time (oral and subcutaneous), and decrease in AUC (oral). C_{max} values were difficult to interpret because of the very large intersubject variability.
Pharmacodynamics and pharmacokinetics of carprofen, a non-steroidal anti-inflammatory drug, in healthy cows and cows with *E. coli* endotoxin-induced mastitis	Lohuis et al., 1991	LPS	Carprofen	Intravenous	Bovine	Mastitis resulted in a reduction in carprofen CL, increase in AUC, an increase in $t_{1/2}$, and greater excretion of carprofen into milk.
Effect of parasitism with *Ostertagia circumcincta* on pharmacokinetics of fenbendazole in sheep	Marriner et al., 1985	*O. circumcincta*	FBZ	Oral	Sheep	Consistently lower blood levels of fenbendazole and its metabolites when animals were infected. This was accompanied by lower drug and metabolite exposures in the abomasum.
Comparative pharmacokinetics of diminazene in noninfected Boran (Bos indicus) cattle and Boran cattle infected with *Trypanosoma congolense*	Mamman et al., 1993	*Trypanosoma congolense*	Diminazene	Intramuscular	Cattle	Drug PK of each animal was determined before and during acute and chronic phases of infection. Acute infection increased absorption rate and decreased Vdss but did not affect CL/F.
Effect of parasitism with *Nematodirus battus* on the pharmacokinetics of levamisole, ivermectin and netobimin	McKellar et al., 1991	*N. battus*	Levamisole, ivermectin, netobimin	Oral and subcutaneous	Lambs	No differences in PK were reported.
Effect of parasitism on the pharmacokinetic disposition of ivermectin in lambs	Pérez et al., 2006	*Ostertagis, Trichostrongylus, Cooperia* mix	Ivermectin	Subcutaneous	Lambs	Parasite infection resulted in a decrease in AUC. Although C_{max} tended to be lower in infected animals, the difference was not significant. CL/F and Vd/F were not reported

(*continued*)

TABLE 2—*Continued*

Title	Author Year	Pathogen	Drug	Method of Drug Administration	Species	Consequence
Pharmacokinetics of florfenicol after intravenous administration in *E. coli* lipopolysaccharide-induced endotoxaemic sheep	Pérez et al., 2015	LPS	Florfenicol	Intravenous	Sheep	Endotoxemia resulted in higher florfenicol plasma concentrations because of a decrease in CL.
The pharmacokinetics of oxytetracycline following intravenous administration in healthy and diseased pigs	Pijpers et al., 1990	*A. pleuropneumoniae*	Oxytetracycline	Intravenous	Swine	Significantly lower CL, Vd, and $t_{1/2}$ were in diseased vs. healthy pigs when dosed at 10 mg/kg but were not different when dosed at 50 mg/kg.
The influence of disease on feed and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs	Pijpers et al., 1991	*A. pleuropneumoniae*	Oxytetracycline	Oral	Swine	CL/F was significantly lower in diseased pigs, resulting in an increase in AUC and $t_{1/2}$.
Influence of porcine *A. pleuropneumoniae* infection and dexamethasone on the pharmacokinetic parameters of enrofloxacin	Post et al., 2002	*A. pleuropneumoniae*	Enrofloxacin	Intravenous	Swine	Disease resulted in a decrease in Vd and $t_{1/2}$, but CL was unaffected. APP did not affect the metabolism of enrofloxacin to ciprofloxacin.
The effect of endotoxin and dexamethasone on enrofloxacin pharmacokinetic parameters in swine	Post et al., 2003	LPS	Enrofloxacin	Intravenous	Swine	Administration of LPS was associated with a decrease in enrofloxacin CL, leading to an increase in AUC and $t_{1/2}$.
Effects of endotoxin-induced fever and probenecid on disposition of enrofloxacin and its metabolite ciprofloxacin after intravascular administration of enrofloxacin in goats	Rao et al., 2000	*E. coli*	Enrofloxacin	Intravenous	Goat	Disease reduced the CL of enrofloxacin, resulting in an increase in AUC and $t_{1/2}$. Ciprofloxacin plasma concentrations decreased, and $t_{1/2}$ was increased.
The impact of acute phase response on the plasma clearance of antipyrine, theophylline, phenytoin and nifedipine in rabbits	Saitoh et al., 2000	LPS	Antipyrine, theophylline, phenytoin, nifedipine	Intravenous	Rabbits	Lower CL and a longer $t_{1/2}$ were observed for antipyrine, theophylline, phenytoin, and nifedipine in infected animals. An increase in Vd was observed for phenytoin and nifedipine.
Tissue distribution and disposition kinetics of enrofloxacin in healthy and *E. coli*–infected broilers	Soliman, 2000	*E. coli*	Enrofloxacin	Intravenous and oral	Chicken	Following intravenous administration, the CL significantly increased, and AUC and $t_{1/2}$ significantly decreased, but the increase in Vd was not statistically significant when comparing healthy vs. diseased chickens. Nine days postdose with enrofloxacin, breast muscle concentrations were significantly greater in infected birds. There were no other differences in the other tissues assayed or at other time points.
Pharmacokinetics and efficacy of tilmicosin in the treatment of *Pasteurella haemolytica* bronchopneumonia in calves	Soliman and Ramadan Ali Ayad, 2014	*P. haemolytica*	Tilmicosin	Intravenous and subcutaneous	Calves	Following intravenous administration, CL and Vd were significantly lower in diseased vs. healthy calves.
Pharmacokinetics of ceftiofur hydrochloride in pigs infected with porcine reproductive and respiratory syndrome virus	Tantituvanont et al., 2009	PRRSV	Ceftiofur	Intramuscular	Swine	PRRSV-infected pigs had higher CL and Vd and lower AUC, C_{max}, and $t_{1/2}$ compared with their healthy counterparts.
Effect of tick-borne fever and trypanosomiasis on the pharmacokinetics of sulfadimidine and its metabolites in goats	Van Gogh et al., 1989	*E. phagocytophila* and *T. brucei* 1066	Sulfadimidine	Intravenous	Goats	Both parasitic infections resulted in lower CL and Vd and larger AUC and $t_{1/2}$ values.
Influence of *Escherichia coli* endotoxin-induced fever on the pharmacokinetic behavior of marbofloxacin after intravenous administration in goats	Waxman et al., 2003	*E. coli*	Marbofloxacin	Intravenous	Goat	Disease resulted in a decrease in CL and Vd and an increase in AUC.
Pharmacokinetics of tilmicosin in healthy pigs and in pigs experimentally infected with *Haemophilus parasuis*	Zhang et al., 2017	*H. parasuis*	Tilmicosin	Oral	Swine	No significant differences in tilmicosin PKs were observed in healthy vs. infected pigs.

BVDV, bovine viral diarrhea virus. App

highly unlikely. In that regard, ceftiofur protein binding in swine is only about 70% (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000079/WC500065945.pdf).

Therefore, we believe that factors other than disease-associated changes in protein binding were responsible for the observed lowering of total drug exposure (as estimated by AUC). Several other possibilities that may have contributed to this outcome in swine include the following:

1. Altered tissue pH: In this regard, a recent investigation demonstrated that ceftiofur contains several pK_a values (Ribeiro and Schmidt 2017). Such a change could affect the relative amounts of ionized drug in the system. Therefore, we need to consider the possibility that a decrease in tissue pH (due to infection-induced physiologic changes) may have led to a trapping of drugs within the tissues.
2. A decrease in drug absorption from the site of injection: Potentially, an infection-induced decrease in blood flow to peripheral tissues (injection site) could compromise drug absorption when administered via intramuscular injection (Prescott and Drusano, 1999). Because noncompartmental methods of data analysis were used, a change in fraction absorbed could have impacted estimates of CL/F or Vd/F. Although the time to peak concentrations (T_{max}) was not affected by disease, either an increase in the ionization and/or a decrease in local blood flow could have contributed to a decrease in the fraction of drug absorbed.
3. An increase in renal drug elimination: The analytical method employed in both studies employed a derivatization process rendering the parent and metabolite indistinguishable. Because the metabolites are primarily eliminated by the kidney, an increase in the amount trapped in the urine, and therefore renally eliminated, could have been responsible for the observed decrease in estimated plasma drug concentrations. Because there does not appear to be an active transport mechanism influencing the elimination of the drugs studied in these reports, any change in renal clearance that may have occurred would likely be attributable to other disease-associated factors, such as an increase in urine acidification. This possibility is worth considering in light of potential changes in urine pH that can occur in the presence of the decreased respiratory efficiency associated with pneumonia (Seifter and Chang, 2017).

Fluoroquinolones. The impact of disease on fluoroquinolone PK was studied in bovine pneumonia (Ismail and El-Kattan, 2007), *A. pleuropneumoniae* infection in swine (Post et al., 2002), endotoxin-induced systemic shock in swine (Post et al., 2003) and chickens (Soliman, 2000; Guo et al., 2014), and endotoxin-induced fever in goats (Rao et al., 2000; Waxman et al., 2003).

In naturally occurring bovine pneumonia, Ismail and El-Kattan (2007) observed an increase in terminal elimination half-life ($t_{1/2}$), a decreased CL, and an increase in the AUC values estimated in diseased versus healthy cattle after intravenous marbofloxacin administration. Vd did not differ. In the same study, marbofloxacin was administered intramuscularly to cattle, resulting in a disease-associated increase in $t_{1/2}$, C_{max}, and AUC.

After intravenous enrofloxacin administration to healthy pigs or to pigs experimentally infected with *A. pleuropneumoniae*, Post et al. (2002) observed that Vd and $t_{1/2}$ were lower in the infected pigs, whereas AUC and CL did not differ. In a similar study, intravenous injection of enrofloxacin to pigs challenged with LPS resulted in an increase in AUC and $t_{1/2}$ and a lower CL but no change in the Vd of the challenged versus normal pigs (Post et al., 2003).

Soliman (2000) reported that, as compared with healthy broilers, diseased birds exhibited higher CL after intravenous administration, resulting in lower systemic drug concentrations after both single intravenous and oral enrofloxacin administrations. They also noted a statistically significant longer absorption $t_{1/2}$ (but not a different mean absorption time) and smaller AUC but no difference in estimates of F (based upon between-bird AUC ratios of mean oral/intravenous AUC values) after oral administration to diseased versus healthy birds. After repeated oral administration, organ enrofloxacin concentrations did not differ in the liver, kidney, spleen, lung, heart, brain, thigh muscle, fat, and skin of diseased and healthy chickens. In breast muscle, disease did not significantly increase enrofloxacin concentrations at 1, 3, and 6 days after the last administration, but statistically significant differences were observed at 9 days postadministration.

Using a model similar to that of Soliman (2000), Guo et al. (2014) also observed a decrease in broiler plasma enrofloxacin concentrations after oral drug administration to chickens with an induced *E. coli* infection (injected into the pectoral muscle) versus that of healthy birds. They report a decrease in Cyp3a37 mRNA expression in the liver and kidney and an increase in Abcb1 mRNA levels and in the P-gp localization in the kidney, jejunum, and ileum. With a decrease in cytochrome P450 activity, one would have expected an increase rather than the observed decrease in plasma drug concentrations. Based upon data generated after the administration of verapamil (correcting the drop in systemic drug exposure), they suggested that the paradoxical relationship between expected and observed results was a function of a simultaneous disease-associated increase in intestinal P-gp activity (thereby constraining the fraction of the oral dose absorbed) and decrease in drug elimination (see section on *Transporters* for additional discussion of this work). Although Soliman and Guo et al. both report a decrease in enrofloxacin AUC, the defined mechanisms responsible for this drop are not the same. Soliman observed an increase in CL after intravenous injection but no difference in F. Conversely, Guo et al. observed a decrease in Cyp3a37 mRNA expression and an increase in P-gp activity (suggesting that the lower enrofloxacin AUC after oral administration to diseased vs. healthy broilers was a consequence of decreased drug absorption). The reason for these disparities is not evident from the publications.

In terms of goats, after intravenous administration, enrofloxacin $t_{1/2}$ and AUC were greater and CL was lower in endotoxin-challenged goats as compared with their healthy counterparts (Rao et al., 2000). Vd did not differ. After intravenous marbofloxacin administration, Vd and CL were lower and AUC was greater in LPS-challenged goats compared with healthy goats following. The $t_{1/2}$ did not differ (Waxman et al., 2003). Thus, similar PK changes were reported for these two fluoroquinolones as a function of disease.

Macrolides. This was the most extensively studied drug class on this topic in veterinary species.

The results from Bladek et al. (2015) followed a general trend of statistically significantly higher concentrations of tulathromycin in kidney, liver, muscle, skin with fat, and injection site of pigs experimentally infected with *A. pleuropneumoniae* as compared with those of healthy pigs after a single intramuscular injection. In contrast, Gajda et al., (2016) did not observe higher tulathromycin concentrations in the plasma of *A. pleuropneumoniae*–infected pigs. Rather, plasma concentrations of tulathromycin were greater in healthy pigs than in the infected pigs at 0.5 and 2 hours after administration. No differences were observed at time points beyond 2 hours posttreatment, likely due to the high variability observed in the plasma concentrations of both groups. Plasma tulathromycin C_{max} values were greater in healthy versus infected pigs, but no statistically significant differences were observed for any of the other plasma PK parameters. In lung tissue, concentrations of tulathromycin did not differ between healthy pigs and pigs

experimentally infected with *A. pleuropneumoniae* until 360 and 792 hours after administration, at which time lung concentrations of tulathromycin were greater in lung tissue from infected pigs. Furthermore, lung tissue T_{max} and AUC values were greater in pigs experimentally infected with *A. pleuropneumoniae* as compared with the healthy pigs (Gajda et al., 2016). Considering the results of these two investigations, the potential for infection to induce a greater partitioning of tulathromycin from plasma to tissues should be considered.

For a different target animal species (goats) and pathogen (*Pasteurella multocida*), Smith et al. (2019) reported that although there was a trend toward higher plasma tulathromycin concentrations in healthy versus diseased goats, no statistically significant differences were detected in the tulathromycin plasma C_{max}, T_{max}, $t_{1/2}$, AUC, and mean residence time (MRT) values after subcutaneous injection. However, they did observe that the Vd/F was higher in infected versus healthy goats. In muscle, liver, and fat tissues collected at 13 days postdose, concentrations of CP-60,300 (marker residue for tulathromycin) did not differ between healthy goats and infected goats. However, the CP-60,300 kidney concentrations were greater in the healthy goats. Although not specifically addressed by these authors, it is important to note the trend toward a greater magnitude of variability in plasma and tissue tulathromycin concentrations observed in the presence of disease.

In terms of the drug tilmicosin, reported findings were inconsistent, with examples of blood levels not changing, increasing, or decreasing in response to disease. Whether these discrepancies were due to study-specific differences in the route of drug administration (oral vs. subcutaneous injection vs. intramuscular injection), animal species' response to disease (pigs vs. goats vs. calves), pathogen used, or species-specific differences in tilmicosin PK is unclear. Plasma concentrations of tilmicosin after oral gavage did not differ between healthy pigs or in pigs inoculated intranasally with *Haemophilus parasuis* (Zhang et al., 2017). Conversely, lower C_{max}, AUC, and MRT values were observed in lactating goats experimentally infected with *P. multocida* as compared with healthy lactating goats after a single subcutaneous injection of tilmicosin (El-Komy et al., 2016).

Tilmicosin concentrations in serum were measured after intravenous and subcutaneous injections in experimentally *P. haemolytica*–infected versus clinically healthy calves (Soliman and Ramadan Ali Ayad, 2014). After intravenous administration, $t_{1/2}$ did not differ between infected and healthy calves, but Vd and CL were lower in infected calves, and the initial plasma concentrations and AUC values were higher in infected calves. No disease-associated differences in serum drug concentrations were observed after subcutaneous administration. In terms of bronchial secretions after intravenous administration, tilmicosin concentrations were higher in diseased versus healthy lungs (statistically significantly higher AUC values). It is important to note that the Soliman and Ramadan Ali Ayad (2014) study relied upon a microbiological assay. Although this approach allows for the unbound drug concentrations to be measured, thereby eliminating misinterpretations that could have resulted from assessing total rather than free drug concentrations, it necessitates an assumption of negligible activity associated with any tilmicosin metabolite.

Tetracyclines. In pneumonic calves (*P. haemolytica*), intravenous oxytetracycline Vd, $t_{1/2}$, and lung residues were higher than those observed in healthy animals (Ames et al., 1983). However, serum CL and the mean oxytetracycline concentrations in liver, kidney, and serum did not differ.

Pijpers et al. (1990) used a sequential study design in which pigs were administered oxytetracycline before and then after experimental infection with *A. pleuropneumoniae*. In their 1990 study, disease increased plasma AUC and decreased plasma CL, Vd, and $t_{1/2}$ after intravenous administration of 10 mg of oxytetracycline per kilogram. When the dose was increased to 50 mg of oxytetracycline per kilogram, these differences were not observed. The investigators followed a similar approach to examining the impact of disease on orally administered oxytetracycline (50 mg of oxytetracycline per kilogram via oral gavage), reporting that infection with *A. pleuropneumoniae* increased plasma $t_{1/2}$ and AUC and decreased plasma CL/F and C_{max} (Pijpers et al., 1991). Taken together, these two investigations are indicative of the importance of both dose and route of administration in determining the effect of disease on the PK parameters.

NSAIDs. Two intravenous injection studies were published on the effect of bovine mastitis on NSAID PK: one involving carprofen (Lohuis et al., 1991) and the other involving flunixin (Kissell et al., 2015). Both investigations observed a substantial lowering of CL and increase in total drug exposure in diseased as compared with healthy animals.

Lohuis et al. (1991) reported that after intravenous injection, mastitic cows exhibited higher plasma carprofen $t_{1/2}$ and AUC, with a corresponding decrease in plasma CL, as compared with that of healthy cows. In terms of drug concentrations in milk, although carprofen concentrations were nondetectable in the milk of healthy cows, it remained above the limit of detection in the milk from diseased cows.

Kissell et al. (2015) reported that after intravenous administration of flunixin meglumine, mastitic cows exhibited greater plasma AUC and reduced CL as compared with healthy cows. Furthermore, the comparative concentrations of 5-hydroxy flunixin (marker residue) in milk of mastitic versus health cattle varied as a function of time. They were greater in milk from healthy compared with mastitic cows at 2 and 12 hours after flunixin administration and were no different at 24 hours postdose, but by 36 hours postdose, concentrations of 5-hydroxy flunixin were above the limit of quantification in the milk of eight of the 10 mastitic cows but not in the milk of any of the healthy cows. At hour 48 postdose, concentrations of 5-hydroxy flunixin were below the analytical method limit of quantification in both groups. Parent flunixin concentrations were greater in the milk from mastitic cows at all time points. It should be noted that both healthy and mastitic cows were simultaneously treated with intramuscular injections of ceftiofur and group-matched intramammary infusions of cephapirin, and the effects of these drugs on disease-associated changes in milk residues were not determined. There was also large variability in the milk concentrations of the diseased animals, with some animals presenting with milk concentrations similar to those of the healthy controls, whereas others had levels that were substantially higher.

Antiparasitic Agents. Febantel (the administered compound) is metabolized to fenbendazole (FBZ) and oxfendazole (OXF), both of which are active substances that undergo reversible metabolism (Debackere et al., 1993). The inactive metabolite, fenbendazole sulphone, is the final step in the metabolism of this drug. Thus, it is difficult to determine the extent to which changes in the PK of febantel are associated with altered drug absorption versus drug metabolism. This is an important consideration because studies of this compound involved oral drug administration and because the targeted parasites reside in the GI tract.

Landuyt et al. (1995) evaluated plasma concentrations of febantel, its two active metabolites and the inactive metabolite, after an oral dose of 7.5 mg/kg febantel in lambs before and 28 days after parasite infection. The pathogens were *Ostertagia circumcincta* [G1 = susceptible parasites (n = 5), G3 = drug resistant parasites (n = 3)] or a susceptible strain of *Trichostrongulus colubriformis* (G2, n = 5). Although no statistically significant differences were observed as a consequence of infection (paired Student's t test), the authors noted a trend toward a decrease in mean drug exposure as a function of disease. All groups were associated with high intersubject variability (only interanimal variability reported). Nevertheless, a disease-associated decrease in drug exposure appeared

to be a repeatable observation. In a different study, statistically significantly lower FBZ AUC (orally administered as the parent compound) and its metabolite, OXF, was reported in sheep heavily infected with *O. circumcincta* (Marriner et al., 1985).

Comparable results were observed by Debackere et al. (1993). Because this was published by the same research team as Landuyt et al. (1995), and because the study outcomes were similar, the results of the Debackere investigation are not included in Table 2. One of the fundamental differences between the two investigations was the parasite load used to generate the artificial infections. As discussed by Landuyt et al., intestinal parasitic infections cause many changes within the GI tract, any of which could have contributed to altered drug concentration–time profiles. This includes parasite-induced changes in abomasal pH, intestinal permeability, and intestinal transit time (occasionally presenting as diarrhea). Although the Debackere et al. study did include an intravenous arm, it was administered as a third period (4 weeks after the oral treatment of infected animals). Therefore, it was not possible to determine whether disease-associated effects similar to those observed after oral drug administration would have occurred after intravenous injection (solvent used to solubilize the febantel was dimethyl sulfoxide).

An infection-associated decrease in drug exposure was also reported for the avermectins. Pérez et al. (2006) observed a significant decrease in ivermectin AUC after subcutaneous injection to lambs infected with a mixture of parasites (nematodes), including the *Ostertagia*, *Trichostrongylus*, and *Cooperia* genera. Similarly, Lespine et al. (2004) observed a lower oral ($P < 0.05$) and subcutaneous ($P < 0.05$) moxidectin AUC after natural infection in sheep. For both routes of administration, a statistically significantly lower MRT was seen in diseased versus healthy sheep. Thus, from the results of these two studies, a direct effect of GI drug absorption can be ruled out as being the sole factor responsible for the infection-associated decrease in drug exposure.

Contrasting results were reported by McKellar et al. (1991), who examined the effects of *Nematodirus battus* (which is associated with intestinal villus atrophy) on the PK of ivermectin. Although, as compared with their healthy counterparts, infected lambs exhibited a decrease in the mean blood levels after the administration of oral ivermectin, they tended to exhibit higher mean blood levels after subcutaneous injection. However, none of these differences was found to be statistically significantly different, an outcome that may reflect the high interanimal variability and small number of animals per treatment group ($n = 6$). Insufficient information is available to ascertain why the diseased animals in the McKellar et al. study (subcutaneous dose) were associated with a trend toward higher systemic ivermectin concentrations rather than the lower exposure observed by Lespine et al. (2004). Pérez et al. (2006) suggested that it could be related to differences in parasites studied and the associated body condition scores of the study subjects.

With regard to the study by Lespine et al. (2004), they compared their results to those reported in other investigations. They noted the similarity between their results and the disease-associated reduction in the OXF AUC (but no change in T_{max} or $t_{1/2}$) after the oral administration of OXF in goats and sheep infected with *H. contortus* and *Teladorsagia circumcincta* (Hennessy et al., 1993). Hennessy et al. (1993) further reported that in contrast to the parent compound, experimental infection of sheep with *H. contortus* and *T. colubriformis* did not lead to disease-associated changes in the T_{max} or AUC of FBZ or FBZ sulphone after OXF intraruminal administration (despite a reduction in total OXF metabolite C_{max} and $t_{1/2}$). Similar outcomes were observed in goats.

The complexity of the influence of infection on drug PK was clearly seen in the investigation by Salam Abdullah and Baggott (1986). In that study, the PK of intravenous imidocarb was examined in control goats ($n = 8$) or in goats with fever induced by LPS ($n = 6$), *T. evansi* ($n = 6$), or infectious bovine rhinotracheitis (IBR) virus ($n = 6$). Salam Abdullah and Baggott observed marked differences in the imidocarb plasma concentration versus time profile across the four treatment groups (decrease in CL and steady state volume of distribution (Vdss) in goats administered LPS or IBR virus, but increase in *T. evansi*–infected animals). They concluded that alterations in the disposition kinetics of imidocarb in the febrile goats are related not only to the febrile reaction but also to the pathophysiology of the disease condition.

In contrast to the trypanosome-associated increase in imidocarb CL seen in goats, mongrel dogs with experimentally induced *T. b. brucei* infection exhibited a significant decrease (rather than increase) in the CL of intravenous diminazene (Anika and Onyeyili, 1989). Corresponding changes in diminazene PK were not seen after intramuscular injection of cattle infected with *T. congolense* (Mamman et al., 1993). Thus, as with other modes of infection and inflammation, whether there will be a disease-induced change in PK and the magnitude of such a change (if it occurs) are dependent upon a wide range of factors, including drug, route of drug administration, patient (species), and inflammatory pathway.

Examples of Discrepancies in Study Results

Several discrepancies can be found in reported relationships between disease or cytokine exposure on transporter or enzyme activity. For example, in a study using isolated rat capillaries (Hartz et al., 2006), P-gp transporter activity in the brain was rapidly reduced (i.e., within 30 minutes) by exposure to low concentrations (0.01–1 ng/ml) of TNFα. These authors also suggested that the effects of LPS on P-gp appeared to be (at least in part) through the TNFα receptor TNF-R1 in the rat brain. On the other hand, exposure of immortalized human brain capillary endothelial cells exhibited a downregulation (mRNA expression) of the BCRP gene after 72 hours of incubation with IL-1β, IL-6, or TNFα, but P-gp gene expression was only slightly downregulated by IL-1β or IL-6 and significantly upregulated by TNFα (Poller et al., 2010). Despite the upregulation of mRNA expression, a corresponding increase in efflux transporter activity was not observed. When comparing their results to those of other published investigations, Poller et al. suggested that some of the apparent dissimilarities may reflect differences attributable to animal species and duration of exposure to these cytokines. Furthermore, they suggest that the factors underlying the cytokine mechanism of action may vary as a function of the experimental design, culture fluid contents, etc.

Another factor that may influence the relationship between disease versus drug PK is breed-associated differences in cytokine up and downregulation that occur in response to a given infecting agent. For example, two breeds of pig (Laiwu vs. Yorkshire $\times$ Landrace) not only exhibited significantly different disease responses when infected with porcine circovirus type 2 but also had very different patterns of disease-induced changes in cytokine release. Furthermore, in response to viral infection, the Laiwu pigs had a significant increase in mRNA expression and protein levels of serpin peptidase inhibitor, clade A, member 1. This change was not observed in the Yorkshire $\times$ Landrace breed of pigs. The importance of this finding is that serpin peptidase inhibitor, clade A, member 1, inhibits the activity of neutrophil enzymes that could result in or at least contribute to inflammatory responses and tissue damage (Li et al., 2016). Thus, the breed of animal being evaluated and, therefore, the population variability that can occur in the changes in cytokine release (and subsequent changes in drug metabolism, transport, and clearance processes) should be considered as part of any cross-study comparison.

One of the frequent observations, irrespective of drug, pathogen, or animal species, is that disease is often associated with a higher variability in drug concentrations (whether they are based upon blood, tissue, milk, or other biologic matrix of interest) as compared with those of healthy animals. A multitude of factors may lead to this variability, including the nature and magnitude of cytokine release, severity of infection, individual expression of organ-associated responses to disease, nutrition, and others (e.g., Rubino et al., 2009; Veiga and Paiva, 2018). The influence that this increase in PK variability may have on drug safety and/or effectiveness in veterinary species is yet to be determined.

Conclusions

The disease-PK relationship is highly complex (time after insult, duration of infection, pathogen, route of pathogen entry into the host, disease site, tissue site considered, animal species, use of mRNA vs. activity, physiologic changes associated with the pathologic state, total vs. free drug concentrations), and the relevance of potential changes needs to be considered on a case-by-case basis. This would be particularly important when the drug has a narrow therapeutic window. Furthermore, for any given PK change, the time of disease onset and the duration of the infection, inflammation, or concomitant stressors remain unaddressed questions.

Therefore, returning to our original questions, is there published evidence that a change in immune state (due to infection, stress, or inflammation) can alter drug PK in veterinary species? The answer to this is clearly yes. However, with regard to the second question regarding the possibility of identifying specific relationships, we could not recognize a rule of thumb that could be applied. Rather, what we found are a multitude of factors that influence whether there will be a change and, if yes, the nature and mechanism of that change.

Clearly, more studies are needed to improve our ability to predict the impact of disease on drug metabolism and transporter function of drugs in veterinary species. Investigators should be encouraged to gather PK information not only in healthy animals but also in animals that reflect the patient population, considering both total and free drug concentrations in the blood.

Authorship Contributions

Performed data analysis: Martinez, Greene, Kenna, Kissel, Kuhn.

Wrote or contributed to the writing of the manuscript: Martinez, Greene, Kenna, Kissel, Kuhn.

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Drug Metabolism and Disposition

The impact of infection and inflammation on drug metabolism, active transport, and systemic drug concentrations in veterinary species

Marilyn N. Martinez, Jonathan Greene, Leslie Kenna, Lindsey Kissell, and Matt Kuhn

SUPPLEMENTAL MATERIAL: Part 1

Expansion of information Table 1. Note that the % change values for a parameter was calculated as 100*[(disease/control) – 1].

For references associated with Supplement Material Part 1, see the main manuscript.

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Influence of induced disease states on the disposition kinetics of imidocarb in goats	Abdullah and Baggot, 1986	*LPS, T. evansi, IBR*	Imidocarb	IV	Goats	LPS and IBR reduced Vd and CL. Infection with *T. evansi* resulted in an increase in Vd and CL.	Total	Not reported	NA	*E. coli* (-53%), *IBR* (-43%) *T. evansi* (+153%)	*E. coli* (-54.9%), *IBR* (-47.8%) *T. evansi* (+163%)	Change not significant	NA	NA
Pharmacokinetics of difloxacin in healthy and *E. coli*-infected broiler chickens	Abo El-Ela et al., 2014	*E. coli*	Difloxacin	IV and oral	Chicken	Following IV administration, disease resulted in an increase in CL and Vd and a decrease in AUC.	Free	IV-(-46%) Oral (-34%)	Oral (- 21.6%)	IV (+73%)	IV (+195%)	IV (+31%) Oral (-27%)	NA	NA
Oxytetracycline concentrations in healthy and diseased calves	Ames et al., 1983	Pneumonia (BVDV)	OTC	IV	Calves	Pneumonia resulted in an increase in Vd, half-life and oxytetracycline lung concentrations.	Total	Not reported	NA	Change not significant	(+55%)	(+25%)	Yes, liver, kidney, and lung	Increased lung residues (+36%), NS change in kidney or liver
Effects of trypanosomal infection on the pharmacokinetics of diminazene aceturate in dogs	Anika and Onyeyili	*T. brucei*	Diminazene	IV	Dogs	Infection with *T. brucei* resulted in a decrease in Vd and CL	Total	(+25%)	NA	(-21%)	(-24%)	Change not significant	NA	NA
Pharmacokinetics of tulathromycin in edible tissues of healthy and experimentally infected pigs with *A. pleuropneumoniae*	Bladek et al., 2015	*A. pleuropneumoniae*	Tulathromycin	IM	Swine	Infection resulted in a change in tulathromycin tissue concentration–time profile, characterized by an increase in elimination half-life and AUC, (plasma concentrations not determined)	Total	NA	NA	NA	NA	NA	Yes, liver, kidney, muscle, skin with fat, and injection site	Higher concentrations in all tissues, with statistics reported at specific times but not summary parameters. Higher residue (µg/kg) in all tissues of infected calves vs healthy calves at 360 hr (0.0280 vs 0.0014) and at 792 hr (0.0242 vs 0.0007)

NA = not available, NS = not statistically significant, OTC = oxytetracycline, %Change = 100×[(Infected-Control)/Control], IMM = intramammary infusion.

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Impact of an experimental ORRSV and *Streptococcus suis* coinfection on the pharmacokinetics of ceftiofur hydrochloride after intramuscular injection in pigs	Day et al., 2015	PRRSV and *S. suis co-infection*	Ceftiofur	IM	Swine	Coinfected pigs had lower AUC and Cmax values, but increased VD and CL values.	Total	(-18%)	(-23%)	(+23%)	(+30%)	Change not significant	NA	NA
Pharmacokinetics of tilmicosin in healthy and experimentally *Pasteurella multocida* infected lactating goats	El-Komy et al., 2016	*P. multocida*	Tilmicosin	SC	Goats lactating	Infection resulted in a decrease in plasma Cmax, AUC and MRT..	Total	(-31%)	(-14%)	NA	NA	Change not significant	NA	NA
Pharmacokinetics of Flunixin after intravenous administration in healthy and endotoxaemic rabbits	Elmas et al., 2006	LPS	Flunixin	IV	Rabbit	LPS resulted in a decrease in CL and an increase in AUC and half-life.	Total	(+44%)	NA	(-31%)	Change not significant	(+60%)	NA	NA
The influence of Actinobacillus pleuropneumoniae infection on tulathromycin pharmacokinetics and lung tissue disposition in pigs	Gajda et al., 2015	*A. pleuropneumoniae*	Tulathromycin	IM	Swine	Greater lung tissue AUCs were observed in pneumonic pigs compared to healthy pigs,	Total	Change not significant	Change not significant	NA	NA	Change not significant	Yes, lung	Greater Lung AUCs (+30%) in pneumonic pigs compared to healthy pigs
Altered plasma pharmacokinetics of ceftiofur hydrochloride in cows affected with severe clinical mastitis	Gorden et al., 2016*	*E. coli* or *Klebsiella* spp.	Ceftiofur	IM	Cattle (lactating dairy)	Mastitic cows had significant increases in VD and CL compared to healthy cows.	Total	(-45%)	(-41%)	(+82%)	(+114%)	Change not significant	NA	NA

* Values reported in this table are based upon steady state estimates.

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
E. coli infection modulates the pharmacokinetics of oral enrofloxacin by targeting P-glycoprotein in small intestine and CYP450 3A in liver and kidney of broilers.	Guo et al., 2014	*E. coli*	Enrofloxacin with or without oral verapamil	Oral	Chicken	By 12 hrs postinfection, significant up-regulation of Abcb1 mRNA in kidney, jejunum and ileum. Expression of Cyp 3a37 mRNA significantly decreased in liver and kidney. Significant decrease in enrofloxacin Cmax and AUC but later Tmax. Disease induced changes in systemic exposure reduced by verapamil.	Total	w/o verap (-50%) verap (-12%)	w/o verap (-65%) verap (-30%)	NA	Values divided by F so difficult to interpret	w/o verap 24.6 hr vs 3.36 hr w/verap 6.92 hr vs 8.43infect vs healthy	NA	NA
Elimination kinetics of ceftiofur hydrochloride in milk after an 8-day extended intramammary administration in healthy and infected cows.	Han et al., 2017	*S. aureus*	Ceftiofur	IMM	Cattle (lactating dairy)	No differences in serum or milk PK	Total	Change not significant	Change not significant	NA	NA	Change not significant	Yes, milk	Quarter production efficiency but not disease influences drug conc in milk
Pharmacokinetic-pharmacodynamic indices of enrofloxacin in *E. coli* O78/H12 infected chickens	Haritova et al., 2011	*E. coli*	Enrofloxacin	Oral	Chicken	Mdr1 mRNA expression was significantly decreased in infected animals but was partially restored with 5 days of oral danofloxacin or enrofloxacin treatment. No blood PK samples were collected.	NA	NA	NA	NA	NA	NA	NA	NA
Comparative kinetic disposition of oxfendazole in sheep and goats before and during infection with *Haemonchus contortus* and *Trichostrongulus colubriformis*	Hennessy et al ., 1993	*H. contortus* *T. colubriformis*	^{14}C-Oxfendazole (OFZ)	Intra-ruminal	Sheep and Goats	NC PK of fenbendazole (FBZ) or FBZ-SO_2, but significant decrease in OFZ Cmax and AUC in both species	Free	OZF Sheep (-32%) Goats (-59%)	OZF Sheep (-26%) Goats (-99%)	NA	NA	Change not significant in either species	NA	NA
Comparative pharmacokinetics of marbofoxacin in healthy and *Mannheimia haemolytica* infected calves	Ismail and El-Kattan, 2007	*M. haemolytica*	Marbofloxacin	IM and IV	Calves	Infection resulted in a decrease in CL (IV), and an increase in half-life (IM and IV), AUC (IM and IV) and Cmax (IM). There were no changes to protein binding.	Total	IM route (+112%) IV route (+113%)	(+66%)	IV route (-57%)	Change not significant	IM route (+70%) IV route (+78%)	NA	NA

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Effect of *Haemonchus contortus* infection on the clearance of antipyrine, sulfobromophthalein, chloramphenicol, and sulfathiazole in lambs	Kawalek and Fetterer, 1990*	*H. contortus*	Antipyrine, sulfobromophthalein, chloramphenicol, sulfathiazole	IV	Lambs	Clearance of sulfobromophthalein and sulfathiazole was unaffected by infection. CL and VD of antipyrine were increased by infection.	Total	Decreased** Sulfathiazole (-32%) Antipyrine (-59%) Chloramphenicol (-29%)	NA	Antipyrine (+84%)	Antipyrine (+12%)	Antipyrine (-31%)	NA	NA
Comparison of pharmacokinetics and milk elimination of flunixin in healthy cows and cows with mastitis	Kissell et al., 2015	Mastitis (*E. coli* or *Klebsiella spp*)	Flunixin	IV	Bovine	Mastitis resulted in a substantial decrease in CL and increase in milk parent flunixin concentrations	Total	(+117%)	NA	(-44%)	Change not significant	Change not significant	Yes	Marked profile change: metabolite -59% (hr 2), -53%% (hrs 12). Mean flunixin conc in milk below 20 ng/mL at 36 hrs in controls and 13.02 at hr 60 in mastitic cows.
Plasma pharmacokinetics and milk levels of ceftriaxone following single intravenous administration in healthy and endometritic cows	Kumar et al., 2010***	Endometritis (unknown)	Ceftriaxone	IV	Bovine	Based upon mean values (no statistical analysis indicated), endometriosis was associated with decreased AUC, increase in CL, increase in Vd, and therefore increase in T½.	Total	(-41%)	NA	(+87%)	(+210%)	(53%)	Yes, milk	Milk drug concentrations were highly variable in control and diseased cows and therefore difficult to compare. Both groups not detected by 48 hrs postdose

* Drugs not mentioned had changes that were not statistically significant. ** Comparisons based upon pre-infection and during infection. It is unclear how after an IV dose, significant differences in AUC could be identified without concomitant significant changes in CL. Parameter and drug values not indicated above imply a lack of statistical significance. *** Although we indicate changes, note that statistical analysis not provided in the manuscript by Kuman et al. (2010).

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
The influence of a heavy infection with sensitive and resistant strains of *Ostertagia circumcincta* and with *Trichostrongylus colubriformis* on the pharmacokinetics of febantel on lambs.	Landuyt et al., 1995	***G1:*** susceptible *O. circumcincta),* ***G2****:T. colubriformis;* ***G3****:* Resist *O. circumcincta*	Febantel (sequential administration, first healthy then after infection)	Oral	Lambs	Statistics not provided: Based upon means, PK changes were dependent on nature of parasite infection. In all cases, there were similar decreases in active metabolites. Primary parasite-associated difference was in impact on Cmax values. Rate but not extent of exposures differed between susceptible and resistant strains of *O. circumcincta*.	Total	**G1**:FBZ -25% OXF -11% $FBZSO_2$ -7%; **G2**: FBZ -34% OXF -13% $FBZSO_2$ +9% **G3**: FBZ -13% OXF -21% $FBZSO_2$ -33%	**G1**:FBZ +57% OXF+24% $FBZSO_2$ +54%; **G2**: FBZ +3% OXF -26% $FBZSO_2$ -33% **G3**: FBZ +5% OXF -14% $FBZSO_2$ -19%	NA	NA	NA	NA	NA
The influence of parasitism on the pharmacokinetics of moxidectin in lambs.	Lespine et al., 2004	*H. contortus* and *T. colubriformis mix* (natural infections	Moxidectin	SC and oral	Lambs	Ignoring inferential statistics (because of high variability and small n per condition), we can at best focus on relative changes in means. From this, we see an increase in mean CL/F, decrease in mean residence time, decrease in T1/2, and a decrease in AUC as a function of disease across both routes. Mean Cmax decreased as a function of disease following oral but increased following SC administration. Tmax was unchanged as a result of disease following oral administration but increased following SC administration. However, given the variability and small sample size, it is equally possible that the apparent changes are within the normal level of variability for some PK parameters	Total	SC (-53%) Oral (-45%)	SC (+52%) PO (-29%)	SC (+81%) PO (+73%)	NA	MRT SC (-66%) PO (-63%)	NA	NA
Pharmacodynamics and pharmacokinetics of carprofen, a non-steroidal anti-inflammatory drug, in healthy cows and cows with *E. coli* endotoxin-induced mastitis	Lohuis et al., 1991	LPS	Carprofen	IV	Bovine	Mastitis resulted in a reduction in carprofen CL , an increase in half-life and greater excretion of carprofen into milk.	Total	(+72%)	NA	(-42%)	Change not significant	(+40%)	Yes	Not detected in milk of healthy cows but detected for more than 45 hrs after IV injection in mastitic cows

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Comparative pharmacokinetics of diminazene in noninfected Boran (Bos indicus) cattle and Boran cattle infected with *Trypanosoma congolense*	Mamman et al., 1993	*Trypanosoma congolense*	Diminazene	IM	Cattle	Drug PK of each animal was determined before and during acute and chronic phases of infection. Infection influenced absorption kinetics and volume of distribution but not drug elimination.	Total	Effectively unchanged	(+73%) for acute, but nearly back to control state in chronic infection	Effectively unchanged	(-26%) during acute phase but similar to control in chronic	Primary effect was the greater variability in infected cows.	NA	NA
Effect of parasitism with *Ostertagia circumcincta* on pharmacokinetics of fenbendazole in sheep	Marriner et al., 1985	*Ostertagia circumcincta*	Fenbendazole	Oral first to infect then same sheep as control	Sheep	Consistently lower blood levels of fenbendazole and its metabolites when animals were infected. This was accompanied by lower drug and metabolite exposures in the abomasum.	Total	Plasma: FBZ (-23%) OxFBZ (-44%) Abomasum FBZ (-38%) OxFBZ (-67%)	Plasma: FBZ (-38%) OxFBZ (-52%)	NA	NA	NA	NA	NA
Effect of parasitism with *Nematodirus battus* on the pharmacokinetics of levamisole, ivermectin and netobimin	McKellar et al., 1991	*Nematodirus battus*	levamisole, Ivermectin, netobimin	Oral and SC	Lambs	No significant differences in PK.	Total	(see note 1 at end of this appendix).	Large variations led to inconsistent results, with varying degrees of difference reported (see note 1).	NA	NA	NA	NA	NA
Effect of Parasitism on the Pharmacokinetic Disposition of Ivermectin in Lambs	Perez et al., 2006	*Ostertagis, Trichostrongylus Cooperia* mix	Ivermectin	SC	Lambs	Parasite infection resulted in a decrease in AUC. CL and VD were not reported	Total	(-44%)	Change not significant	NA	NA	Change not significant	NA	NA
Pharmacokinetics of florfenicol after intravenous administration in *E. coli* lipopolysaccharide-induced endotoxaemic sheep	Perez et al., 2014	LPS	Florfenicol	IV	Sheep	Endotoxemia resulted in higher florfenicol plasma concentrations due to a decrease in CL.	Total	(+35%)	NA	(-23%)	Change not significant	(+63%)	NA	NA
The pharmacokinetics of oxytetracycline following intravenous administration in healthy and diseased pigs	Pijpers et al., 1990	*A. pleuropneumoniae*	OTC	IV	Swine	In diseased pigs, CL, Vd and T½ were significantly decreased when dosed at 10 mg/kg but not different when dosed at 50 mg/kg. Values in table from 10 mg/kg dose	Total	(+12%)	NA	(-11%)	(-22%)	(-13%)	NA	NA

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
The influence of disease on feed and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs	Pijpers et al., 1991	*A. pleuropneumoniae*	OTC	Oral	Swine	In diseased pigs, CL/F was significantly reduce resulting in an increase in AUC and T½.	Total	(+91%)	(-53%)	(-63%)	NA	(+138%)	NA	NA
Influence of porcine *A. pleuropneumoniae* infection and dexamethasone on the pharmacokinetic parameters of enrofloxacin	Post et al., 2002	*A. pleuropneumoniae*	Enrofloxacin	IV	Swine	Disease resulted in a decrease in Vd and half-life, but CL was unaffected. APP did not affect the metabolism of enrofloxacin to ciprofloxacin.	Total	Change not significant	NA	Change not significant	(-50%)	(-32%)	NA	NA
The effect of endotoxin and dexamethasone on enrofloxacin pharmacokinetic parameters in swine	Post et al., 2003	LPS	Enrofloxacin	IV	Swine	Administration of LPS was associated with a decrease in enrofloxacin CL and an increase in AUC and T½.	Total	(+100%)	NA	(-50%)	Change not significant	(+54%)	NA	NA
Effects of endotoxin-induced fever and probenecid on disposition of enrofloxacin and its metabolite ciprofloxacin after intravascular administration of enrofloxacin in goats	Rao et al., 2000	*E. coli*	Enrofloxacin	IV	Goat	Disease reduced the CL of enrofloxacin resulting in an increase in AUC and half-life. Ciprofloxacin plasma concentrations were decreased and T½ was increased.	Total	Enrofloxacin (+74%) Ciprofloxacin (-46%)	Ciprofloxacin (-60%)	Enrofloxacin (-46%)	Change not significant	Enrofloxacin (+62%) Ciprofloxacin (+53%)	NA	NA
The impact of acute phase response on the plasma clearance of antipyrine, theophylline, phenytoin and nifedipine in rabbits	Saitoh et al., 2000	LPS	Antipyrine, theophylline, phenytoin, nifedipine	IV	Rabbits	Decreases CL for antipyrine, theophylline, phenytoin, and nifedipine in infected animals. An increase in Vd was observed for phenytoin and nifedipine. The T½ for all the drugs was increased in diseased animals.	Total	Antipyrine (+30%), Theophylline (+89%), Phenytoin (78%), Nifedipine (+22%)	NA	Antipyrine (-23%), Theophylline (-46%), Phenytoin (-42%), Nifedipine (-19%)	Phenytoin (+32%), Nifedipine (+44%) Other drugs no significant change	Antipyrine (+20%), Theophylline (+158%), Phenytoin (+95%), Nifedipine (+54%)	No	NA

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Tissue distribution and disposition kinetics of enrofloxacin in healthy and E. coli infected broilers	Soliman, 2000	*E. coli*	Enrofloxacin	IV and oral	Chicken	Following IV administration, the CL significantly increased, AUC and T½ significantly decreased but the increase in Vd was not statistically significant when comparing healthy versus diseased chickens. Nine days post-dose enrofloxacin breast muscle concentrations were significantly greater in infected birds. There were no other differences in the other tissues assayed or at other time points.	Total	IV (-34%) Oral (-32%)	Oral (-16%)	IV (+51%)	Change not significant	IV (-24%) Oral (-32%)	Yes, liver, kidney, spleen, lung, heart, brain, breast muscle, thigh muscle, fat, skin	Enrofloxacin tissue concentrations were similar between healthy and infected birds at all time points and tissues, except nine days after the last dose, where enrofloxacin in breast muscle was significantly greater in infected birds compared to healthy.
Pharmacokinetics and efficacy of tilmicosin in the treatment of Pasteurella haemolytica bronchopneumonia in calves	Soliman and Ayad, 2014	*P. haemolytica*	Tilmicosin	IV and SC	Calves	Following IV administration, CL and Vd were significantly decreased in diseased calves.	Free	IV (+13%)	NA	IV (-14%)	IV (-9%)	Change not significant	NA	NA
Pharmacokinetics of ceftiofur hydrochloride in pigs infected with porcine reproductive and respiratory syndrome virus	Tantituvanont et al., 2009	PRRSV	Ceftiofur	IM	Swine	PRRSV infection resulted in an increase in CL and VD and a decrease in AUC, Cmax and T½.	Total	(-70%)	(-53%)	(+240%)	(+116%)	(-38%)	NA	NA
Effect of tick-borne fever and trypanosomiasis on the pharmacokinetics of sulfadimidine and its metabolites in goats	Van Gogh et al., 1989	*Ehrlichia phagocytophila* and *Trypanosoma brucei* 1066	Sulfadimidine	IV	Goats	Parasitic infection resulted in a decrease in CL and increase in AUC and MRT. For Vd, decrease seen after 20 mg/kg but not the 200 mg/kg dose	Total	*200 mg/kg - E. phagocy*tophila (+142%), *T. brucei* (+209%)	NA	*20 mg/kg - E. phagocytophila* (-68%), *T. brucei* (-38%)	*20 mg/kg - E. phagocytophila* (-18%), *T. brucei* (-27%)	*20 mg/kg - E. phagocytophila* (+224%) *T. brucei* Change not significant	NA	NA

Title	Author	Pathogen	Drug	Method of Drug Administration	Species	Consequence	Total or Free Drug	% Change Dis/Healthy AUC	% Change Dis/Healthy Cmax	% Change Dis/Healthy Clearance	% Change Dis/Healthy Vd	% Change Dis/Healthy T½	Any Tissue measured? If yes, which	Tissue Residue Change
Influence of *Escherichia coli* endotoxin-induced fever on the pharmacokinetic behavior of marbofloxacin after intravenous administration in goats.	Waxman et al., 2003	*E. coli*	Marbofloxacin	IV	Goat	Disease resulted in a decrease in CL and VD and an increase in AUC,	Total	(+90%)	NA	(-45%)	(-39%)	Change not significant	NA	NA
Pharmacokinetics of tilmicosin in healthy pigs and in pigs experimentally infected with *Haemophilus parasuis*	Zhang et al., 2017	*H. parasuis*	Tilmicosin	Oral	Swine	No significant differences in tilmicosin pharmacokinetics were observed in healthy and infected pigs.	Total	Change not significant	NA	Change not significant	Change not significant	Change not significant	NA	NA

Note 1: Additional detailed information from McKellar et al., 1991

	ORAL					
	AUC			Cmax		
	Lev	IVM	Net 7.5	Lev	IVM	Net 7.5
Healthy	6.2	1.49	15.23	0.84	0.029	1.14
Diseased	5.38	2.13	16.49	0.82	0.021	1.21
%change	-13.23	42.95	8.27	-2.38	-27.59	
	SC					
	AUC			Cmax		
	Lev	IVM	Net 7.5	Lev	IVM	Net 7.5
Healthy	4.66	2.44	0.28	1.41	0.03	0.17
Diseased	5.97	4.2	1.65	1.67	0.035	0.03
%change	28.11	72.13	489.29	18.44	16.67	-82.35

SUPPLEMENTAL MATERIAL: Part 2 (references at end of Part 2)

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

Description of Methods

The following description of methods serves as a key to the "Marker for Evaluation" column in Table 2. The letter associated with each method in Table 2 corresponds to a method described below.

(A) Total CYP

Difference spectroscopy, a method for quantifying cytochrome CYP content in a sample, compares the sample's absorption spectra before and after an intervention. The resulting difference spectrum is a signature of the molecule. The spectral signal is converted into a concentration using the extinction coefficient of the medium. CYP was originally identified as a pigment in liver microsomes that produces a spectrum with a wavelength maximum at 450 nm when bound to carbon monoxide (Klingenberg, 1958; Garfinkel, 1958). Omura and Sato (1962, 1964 a,b) further developed this method and characterized the enzyme in greater detail**.**

(B) Metabolic Activity

(1) CYP Activity / Monooxygenase assays

Cytochrome CYP isoforms may be characterized by their activity toward high-affinity substrates. Catalytic activity is monitored via the concentration of marker metabolite(s) generated per mg protein in microsomal preparations over a period of time.

SUPPLEMENTAL MATERIAL: Part 2

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

The following table lists substrates used in the publications reviewed here and their associated CYP isoforms.
Note: These are human probes/markers.

Probe Molecule	**Reaction**	**Cytochrome CYP Isoform**
7-Ethoxyresorufin	O-deethylation	1A1 (Bourrié et al., 1996)
Phenacetin	O-deethylation	1A2 (Bourrié et al., 1996)
Caffeine	N-3-demethylation	1A2 (Kot and Daniel, 2008)
Coumarin	7-hydroxylation	2A6 (Bourrié et al., 1996)
Pentoxyresorufin	O-depentylation	2B (Nakajima et al., 1990)
Tolbutamide	4-methylhydroxylation	2C9 (Bourrié et al., 1996)
Aminopyrine	N-demethylation	2C8 (Niwa T and Imagawa Y, 2016)
Dextromethorphan	O-demethylation	2D6 (Bourrié et al., 1996) (In Dogs: 2D15)
Aniline	4-hydroxylation	2E1 (Bourrié et al., 1996)
Chlorzoxazone	Hydroxylase	2E1 (Peter et al., 1990)
Nifedipine	Dehydrogenation	3A4 (Bourrié et al., 1996)
Testosterone	Hydroxylation	3A4 (Krauser et al., 2004)

SUPPLEMENTAL MATERIAL: Part 2

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

Antipyrine is a general marker of CYP activity. Antipyrine is not a probe for specific cytochrome CYP enzymes because it is metabolized by at least six hepatic cytochrome CYP enzymes: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4 (Engel et al., 1996).

Similarly, 4-nitroanisole (synonym: p-nitroanisole) is metabolized by at least two cytochrome CYP isoforms: CYP2A6 and CYP2E1 (Jones and Tyman, 1997).

Theophylline probes the activity of CYP 1A2 and CYP 2E1 (Thorn, 2012).
Phenytoin is metabolized by CYP 2C9 and CYP 2C19 (Thorn et al., 2012).

7-ethoxycoumarin is metabolized by CYP lA2 and CYP 2El (Yamazaki H et al., 1996).

7-methoxy-4-trifluoromethyl coumarin was initially thought to be a specific probe for CYP 2C9, but has been shown to be metabolized by CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 (Porrogi et al., 2008).

Ethylmorphine is metabolized by CYP2D6 and CYP3A4 (Liu et al., 1995).

SUPPLEMENTAL MATERIAL: Part 2

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

(2) UDP-Glucuronyltransferase activity

UDP-Glucuronyltransferase activity was measured by assaying the glucuronidation rates of 1-napthol, morphine, chloramphenicol, and paracetamol.

(C) RNA Hybridization

The dot hybridization method separates RNA without using electrophoresis. RNA samples are spotted onto a matrix and hybridized to cDNA probes (White and Bancroft, 1982).

(D) Cytokine assays

Cytokines are signaling molecules of the immune system; a change in their concentration indicates an immune response to stimulus.

IL-6 is a cytokine that often acts as a growth factor, therefore, one assay for IL-6 measures cellular proliferation. IL-6 is essential for hybridoma growth and monitoring hybridoma cell growth after exposure to IL-6 provides information about the concentration of IL-6. A murine hybridoma cell line (B9) which is sensitive to IL-6 was used to quantify IL-6 in serum samples.

Tumor necrosis factor alpha (TNFα) causes cell death, thus, one assay for TNFα concentration measures cell killing. A murine fibrosarcoma cell line, WEHI 164 clone 13, was used to determine TNFα in serum samples. TNF mediates high cytotoxicity towards WEHI 164 clone 13 cells. The high sensitivity of WEHI 164 clone 13 cells to TNF has made it possible to detect TNF in different biological fluids where the TNF concentration is very low.

SUPPLEMENTAL MATERIAL: Part 2

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

(E) Western Blot

The Western Blot assay is a method for determining protein sequence. First, protein fragments are separated by electrophoresis. Then the separated protein fragments are transferred to a filter membrane and detection by probe hybridization.

(F) Plasma concentration

Drug concentration in plasma was determined using a high-performance liquid chromatography (HPLC) method.

(G) Liver tissue activity / microsomes

Metabolism was studied by incubating substrates of interest with the 9000 × g supernatant fraction from liver tissue.

(H) Quantitative Polymerase Chain Reaction (qPCR)

Quantitative PCR, also referred to real-time PCR, monitors the amplification of a targeted DNA molecule during PCR, i.e. in real-time, and not at its end, as in conventional PCR. Monitoring occurs in real time by incorporating fluorescent labels in the DNA. RNA is amplified with single stranded cDNA molecules.

SUPPLEMENTAL MATERIAL: Part 2

SUMMARY OF MECHANISMS OF PK METABOLISM AND TRANSPORTER CHANGES OBSERVED IN VETERINARY SPECIES:

(I) Immunohistochemistry for P-glycoprotein (P-gp)

Tissue was incubated overnight with an antibody to P-glycoprotein at 37ºC for 1 hour with the secondary antibody. P-gp immunoreactivity was visualized with 3,3'-Diaminobenzidine staining. Sections that were not incubated with the primary antibody served as negative controls.

(J) RNA-Seq Analysis

This is a method of screening a transcriptome for upregulated genes, with the aim of relating the impact of a disease state on gene expression. In this case, the disease was mycotoxin-induced stress. The workflow was to extract RNA from a sample and sequence it. Sequences were mapped to genes. Those gene counts were normalized and compared between conditions to generate a list of differentially-expressed genes (DEGs). DEGs were mapped to pathway annotations and pathways enriched for DEGs were selected.

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