

Title Page

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

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Running Title Page:

Impact of Infection and Inflammation on Animal Drug PK ^B

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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 (CL) 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 assess 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 down- regulation of the various cytochrome (CYP) 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 (Morgan, 2009; Renton, 2005). 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 down-regulation of cytochrome 1A2 (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 vs diseased animals. There is ample evidence that such an assumption can be incorrect.

On the other hand, while 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 alpha₁ acid-glycoproteins (Huang and Ung , 2013). Either of these changes could potentially affect the relationship between total vs 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 vs tissues, there are very few situations where such changes will affect free

(unbound) drug exposure. Generally, it is the free drug concentrations that are clinically relevant (Schmidt et al., 2010; Gonzalez et al., 2013; Benet and Hoerner, 2002, Stern et al., 2016; Heuberger et al., 2013). 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 of *in vivo* disease model studies conducted in veterinary species where 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:

- 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 Figure 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-Conquey (2002) who describe the cytokine cascade, and Morgan (2017) who discusses the relationship between inflammation and acute inflammatory responses.

For any given CYP, 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 down-regulation of murine hepatic Cyp2a5 (orthologue of human

CYP2A6/13) when administered low doses of lipopolysaccharide (LPS), the component of the cell wall of Gram-negative bacteria that act as endotoxin, but not by higher doses of LPS. In contrast, other cytochromes (Cyp1a1/2 and Cyp2b9/10) were down-regulated, but only in the presence of high doses of LPS (De-Oliviera et al., 2015). Similarly, the change in CYP activity is influenced by pathogen, body site and time post-infection. For example, murine schistosomiasis resulted in a significant up-regulation of hepatic Cyp1a2, 2c29, 2e1, 2j5, 3a11, 4f13 and 4f18 at 30 days post-infection (dpi), but a 30-96% down-regulation of most of these same CYPs 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 up-regulation 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 (Ho-Piquette-Miller, 2006; Renton, 2005).

However, this may not always be the case: there can be a disconnect between altered transcription activity vs 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 up-regulation 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 Cyps. Conversely, results seen on 45 dpi showed similar changes in the levels of specific Cyps and their mRNA.

Théron et al., (2003) examined the influence of Tumor Necrosis Factor alpha (TNF α) on the multidrug resistance protein 1 a,b (Mdr-1a,b), mRNA and on 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 TNF α 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., 1995a; Monshouwer et al., 1996a; Li et al., 2016; Monshouwer et al., 1996b), Frisian calves (Facino et al., 1984), broiler chickens (Guo et al., 2014; Bartikova et al., 2009), sheep (Zhang et al., 2014; Saitoh et al., 1999; Klingenberg M 1958), dogs (Lambert et al., 1991), and rabbits (Haritova et al., 2008; Garfinkel, 1958). A variety

of *in vitro* methods have been used to probe drug disposition-related changes following infection (Monshouwer et al., 1995a; Monshouwer et al., 1996a; Facino et al., 1984; Guo et al., 2014; Li et al., 2016; Saitoh et al., 2000; Haritova et al., 2008; Bartikova et al., 2009), exposure to a toxin (Zhang et al., 2014), and disease (Lambert et al., 1991). These include the measurement of total Cyp content, drug metabolism activity (by the Cyps 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.

When interpreting published data from *in vitro* studies, it is important to identify the species-specificity of probes used to assess Cyp 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, due to 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 pro-inflammatory cytokines released depends upon the stimulus (Medzhitov and Horng, 2009; Contreras and Rao, 2012; Muraldiharan et al., 2013), with the most potent cytokines being Interleukin-6 (IL-6), TNF α , IL-1b, and interferon (IFN)-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 pro-inflammatory 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 Cyp 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., 1995b).

As with that associated with bacterial diseases, parasitic infections can lead to substantial increases in total drug exposure in human patients. For example, the 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 Cyps down-regulated (protein levels and mRNA) 45 dpi, reflecting chronic infection. In contrast, the mRNAs at 30 dpi were either slightly upregulated or remained unchanged, with Cyp 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 Cyp effects was also observed in dogs with congestive heart failure, with significant decreases observed in total CyPs and Cyp2b, but not in Cyp3a. Interestingly, congestive heart failure in humans has been linked with an increase in TNF α , IL-6 and IL-1 β (Gullestad et al., 2012), which is consistent with the cytokines associated with inflammation-associated changes (down-regulation) in CyPs. In fact, in humans, congestive heart failure is associated with an increase in the gene expression (measured as mRNA) of several cardiac CYPs and the simultaneous down-regulation in the expression of several hepatic CYPs (Zordoky and El-Kadi, 2008). Thus, some physiological 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 pro-inflammatory cytokines (Lee et al., 2009).

Pro-inflammatory 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 due apparently to a down-regulation of membrane P-gp activity (Duan et al., 2017). In contrast, P-gp up-regulation 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 to 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 following SC injection (Lespine et al., 2004). This increase was (at least in part) 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 if 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 up-regulated 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 vs Haritova et al., 2008. Haritova and colleagues report a down-regulation of P-gp (*Abcb1*

mRNA expression) following *E. coli* infection in broiler chickens. These changes were observed in the duodenum, jejunum, caecum and liver within 24 hrs post-infection. In contrast Guo et al. reported an up-regulation in the expression of *Abcb1* mRNA in the kidney, jejunum and ileum (no significant changes observed in the liver or duodenum) following 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 (CFU)]. In the Guo investigation, inoculation was via pectoral injection (0.5 mL containing 1.5×10^8 CFU). 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 vs 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 following oral administration were lower in diseased broilers within 12 hrs 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 IV enrofloxacin within 48 hrs after *E. coli* administration. Although plasma levels were lower rather than higher in the diseased birds, hepatic *Cyp3a37* mRNA was down-regulated in the infected broilers (Guo, et al., 2014). Simultaneously, there was an up-regulation 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 to the healthy and diseased birds that were not co-administered verapamil. In fact, the verapamil treated infected birds exhibited AUC values similar to that of verapamil-treated healthy birds. While it can be argued that the effects of verapamil likely reflect inhibition both of P-gp and Cyp3a enzymes (Wang et al., 2004), the much larger magnitude of increase in blood levels in the infected birds suggest an important role of disease effects on the drug transporter system.

In terms of Bcrp, Su et al. (2013) observed that both Abcg2 (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 oocysts suspension of *Eimeria necatrix* and *E. tenella* (injected into the pectoral muscles) as compared to 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 vs 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 while 25 report either no change or a decrease in exposure. Table 2 highlights the published studies on cephalosporins, fluoroquinolones, non-steroidal 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 following repeated intramuscular (IM) injections tended to be lower in mastitic vs. 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 while 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 vs. 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 (IV) ceftriaxone administered to healthy and diseased (endometritis) cows (Kumar et al., 2010) and intramammary (IMM) infusion of ceftiofur hydrochloride (HCl) 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 more rapid than that derived from low-production quarters. Conversely, Kumar et al. 2010 reported that ceftriaxone levels were lower and CL higher in diseased vs. healthy cattle. A statistical analysis was not conducted to support this conclusion. The mean milk ceftriaxone concentrations were greater in healthy cows as compared to diseased cows at the hr 12 first post-dose milking. Again, no statistical comparison of these values was provided. At all other sampling points, the biological relevance of any apparent numerical differences in mean milk ceftriaxone was difficult to assess because of the large standard deviation seen particular in the milk of endometritic cows.

In swine, following IM injection of ceftiofur HCl, AUC was lower, CL/F was greater, Cmax was lower, and Vd/F was greater in non-pregnant, non-lactating swine artificially infected with porcine reproductive and/or respiratory syndrome virus (PRRSV) as compared to their healthy counterparts (Day et al., 2015; Tantituvanont et al., 2009). 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 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:

- 1) Altered tissue pH: In this regard, a recent investigation demonstrated that ceftiofur contains several pKa 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 physiological 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 IM inject (Preston 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 worthwhile considering in light of potential changes in urine pH that can occur in the presence of the decreased respiratory efficiency associated with pneumonia (Seifert and Chang 2017).

Fluoroquinolones:

The impact of disease on fluoroquinolone PK was studied in bovine pneumonia (Ismail and El-Kattan, 2007), *Actinobacillus pleuropneumoniae* infection in swine (Post et al., 2002), endotoxin-induced systemic shock in swine (Post et al., 2003) and in 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 vs. healthy cattle following IV marbofloxacin administration. V_d did not differ. In the same study, marbofloxacin was administered IM to cattle, resulting in a disease-associated increase in $T_{1/2}$, C_{max} , and AUC.

After IV enrofloxacin administration to healthy pigs or to pigs experimentally infected with *A. pleuropneumoniae*, Post et al. (2002) observed that V_d and $T_{1/2}$ were lower in the infected pigs, while AUC and CL did not differ. In a similar study, IV injection of enrofloxacin to pigs

challenged with LPS resulted in an increase in AUC and $T_{1/2}$, a lower CL but no change in the V_d of the challenged versus normal pigs (Post et al., 2003).

Soliman (2000) reported that as compared to healthy broilers, diseased birds exhibited higher CL after IV administration, resulting in lower systemic drug concentrations both after a single IV or oral enrofloxacin administrations. They also noted a statistically significant longer absorption $T_{1/2}$ (but not a different mean absorption time), smaller AUC, but no difference in estimates of F (based upon between-bird AUC ratios of mean oral/IV AUC values) after oral administration to diseased vs healthy birds. Following 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 one, three and six days after the last administration, but statistically significant differences were observed at nine days post-administration.

Using a model similar to that of Soliman (2000), Guo et al. (2014) also observed a decrease in broiler plasma enrofloxacin concentrations following oral drug administration to chickens with an induced *E. coli* infection (injected into the pectoral muscle) vs 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 Cyp activity, one would have expected an increase rather than the observed decrease in plasma drug concentrations. Based upon data generated following 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). While 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 IV 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 versus healthy broilers was a consequence of decreased drug absorption). Reason for these disparities is not evident from the publications.

In terms of goats, following IV administration, enrofloxacin $T_{1/2}$ and AUC were greater, and CL was lower, in endotoxin-challenged goats as compared to their healthy counterparts (Rao et al, 2000). V_d did not differ. Following IV marbofloxacin administration, V_d and CL were lower, and AUC was greater in LPS-challenged goats compared to 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. (2016) 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 to that of healthy pigs following a single IM injection. In contrast, Gajda et al., (2015) 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 h and 2 h after administration. No differences were observed at time points beyond 2 h posttreatment, likely due to the high variability observed in the plasma concentrations of both groups. Plasma tulathromycin C_{max} values were greater in healthy vs. 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 h and 792 h 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 to the healthy pigs (Gajda et al., 2015). 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 towards 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 following SC injection. However, they did observe that the V_d/F was higher in infected vs. healthy goats. In muscle, liver, and fat tissues collected at 13 days post-dose, 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 (SC) injection vs IM 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 following 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 to healthy lactating goats after a single SC injection of tilmicosin (El-Komy et al., 2016).

Tilmicosin concentrations in serum were measured following IV and SC injections in experimentally *P. haemolytica* infected vs clinically healthy calves (Soliman and Ayad, 2014). Following IV administration, T_{1/2} did not differ between infected and healthy calves, but V_d 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 following SC administration. In terms of bronchial secretions following IV administration, tilmicosin concentrations were higher in diseased vs healthy lungs (statistically significantly higher AUC values). It is important to note that the Soliman and Ayad (2016) study relied upon a microbiological assay. While 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*), IV 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 and colleagues utilized a sequential study design where pigs were administered oxytetracycline before and then after experimental infection with *A. pleuropneumoniae*. In their 1990 study (Pijpers et al., 1990), disease increased plasma AUC and decreased plasma CL, Vd and T_{1/2} following IV administration of 10 mg oxytetracycline/kg. When dose was increased to 50 mg oxytetracycline/kg, these differences were not observed. The investigators followed a similar approach to examining the impact of disease on orally administered oxytetracycline (50 mg oxytetracycline/kg 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.

Nonsteroidal anti-inflammatory drugs (NSAIDs):

Two IV 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 to healthy animals.

Lohuis et al. (1991) reported that following IV injection, mastitic cows exhibited higher plasma carprofen $T_{1/2}$ and AUC, with a corresponding decrease in plasma CL, as compared to 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 (LOD) in the milk from diseased cows.

Kissell et al. (2015) reported that following IV administration of flunixin meglumine, mastitic cows exhibited greater plasma AUC and reduced CL as compared to healthy cows. Furthermore, the comparative concentrations of 5-hydroxy flunixin (marker residue) in milk of mastitic versus healthy cattle varied as a function of time. They were greater in milk from healthy than mastitic cows at 2 and 12 hours after flunixin administration, no different at 24-hour postdose, but by 36 hours postdose, concentrations of 5-hydroxy flunixin were above the Limit of Quantification (LOQ) in the milk of 8 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 LOQ 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 IM injections of ceftiofur and group-matched intramammary infusions of cephalosporin, 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 that of the healthy controls while others had levels that were substantially higher.

Antiparasitic agents:

Febantel (the administered compound) is metabolized to fenbendazole (FBZ) and oxfendazole (OXF), both 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 vs 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 and the inactive metabolite, following 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 *Trichostrongylus 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 towards a decrease in mean drug exposure as a function of disease. All groups were associated with high intersubject variability (only inter-animal 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 *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 the 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 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 IV arm, it was administered as a third period (four 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 IV injection (solvent used to solubilize the febantel was dimethyl sulfoxide).

An infection-associated decrease in drug exposure was also reported for the avermectins. Perez et al. (2006) observed a significant decrease in ivermectin AUC after SC injection to lambs infected with a mixture of parasites (nematodes), including the *Ostertagia*, *Trichostrongylus* and *Cooperia* genus. Similarly, Lespine et al. (2004) observed a lower oral ($P < 0.05$) and SC ($P < 0.05$) moxidectin AUC following natural infection in sheep. For both routes of administration, a statistically significantly lower MRT was seen in diseased vs 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. While as compared to their healthy counterparts, infected lambs exhibited a decrease in the mean blood levels following the administration of oral ivermectin, they tended to exhibit higher mean blood levels following SC injection. However, none of these differences were found to be statistically significantly different, an outcome that may reflect the high inter-animal 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 (SC dose) was associated with a trend towards higher systemic ivermectin concentrations rather than the lower exposure observed by Lespine et al. (2004). Perez 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 that 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 $T_{1/2}$) following 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 following 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 Abdullah and Baggott (1986). In that study, the PK of IV 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 (IRB) virus (n=6). Abdullah and Baggott observed marked differences in the imidocarb plasma concentration vs time profile across the four treatment groups (decrease CL and V_{dss} in goats administered LPS or IRB 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 IV diminazene (Anika and Onyeyili, 1989). Corresponding changes in diminazene PK were not seen after IM injection of cattle infected with *T. congolense* (Mammon et al., 1993). Thus, as with other modes of infection and inflammation, whether there will be a diseased-induced change in PK and the magnitude of such a change (if it occurs) is 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 to 1 ng/mL) of TNF α . These authors also suggested that the effects of LPS on P-gp appeared to be (at least in part) through the TNF α receptor, TNF-R1, in the rat brain. On the other hand, exposure of immortalized human brain capillary endothelial cells exhibited a down-regulation (mRNA expression) of the BCRP gene following 72 hr of incubation with IL-1 β , IL-6 or TNF α but P-gp gene expression was only slightly down regulated by IL-1 β or IL-6 and significantly upregulated by TNF α (Poller et al., 2010). Despite the up-regulation of mRNA expression, a corresponding increase in efflux transporter activity was not observed. When comparing their results to that 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 vs drug PK is breed-associated differences in cytokine up and down-regulation that occurs 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 (SERPINA 1). This change was

not observed in the Yorkshire × Landrace breed of pigs. The importance of this finding is that SERPINA 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 be based upon blood, tissue, milk or other biological matrix of interest) as compared to that 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 has 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, physiological changes associated with the pathological 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 if 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 an unaddressed question.

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 is a multitude of factors that influence whether or not 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.

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Marilyn Martinez: Performed data analysis. Wrote or contributed to the writing of the manuscript.

Jonathan Greene: Performed data analysis. Wrote or contributed to the writing of the manuscript.

Leslie Kenna: Performed data analysis, Wrote or contributed to the writing of the manuscript.

Lindsey Kissel: Performed data analysis. Wrote or contributed to the writing of the manuscript.

Matt Kuhn: Performed data analysis. Wrote or contributed to the writing of the manuscript.

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Figure 1: Inter-relationships associated with the impact of inflammation and infection on drug pharmacokinetics. Refer to Cavillon and Adip-Conquy (2002), Morgan et al. (20018), and Renton (2005) for additional details regarding activation of the cytokine cascade and its potential physiological/PK consequences.

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 CYP 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 <i>Actinobacillus pleuropneumoniae</i> N=9 pigs inoculated in the bronchia N=6 control pigs	Cyp3a, 1a1, 2b, 2e1	A: Total Cyp B1: Cyp activity •Testosterone (3a) •7-ethoxyresorufin (1a1) •Pentoxyresorufin (2b) •Aniline (2e1) B2: UDP activity •1-naphthol •Morphine •Chloramphenicol •Paracetamol C: RNA hybridization •3A4 cDNA	<u>24 hours post-infection:</u> A: Cyp content ↓40% B1: Cyp activity •Testosterone ↓50% •7-Ethoxyresorufin ↓50% •Pentoxyresorufin ↓60% •Aniline ↓33% B2: UDP activity No change C: RNA hybridization: Cyp3a mRNA ↓	Monshouwer et al., 1995a
Pigs	<i>Escherichia coli</i> LPS-induced acute phase response model N=6 pigs LPS injected 17 mcg/kg every hour for 5 doses	Cyp3a, 1a1, 1a2, 2e1	A: Total Cyp B1: Cyp activity •Testosterone (3a4) •7-ethoxyresorufin (1a1) •Aniline (2e1) •Caffeine (1a2) B2: UDP activity •1-naphthol	<u>Post-infection:</u> A: Total Cyp ↓25% B1: Cyp activity •Testosterone ↓45-80% •7-ethoxyresorufin ↓45% •Aniline ↓35% •Caffeine ↓60% B2: UDP activity	Monshouwer et al., 1996a

	N=6 saline injected pigs		<p>D: Cytokine assays</p> <ul style="list-style-type: none"> •IL-6 •TNFα <p>E: Western Blot</p> <ul style="list-style-type: none"> •Cyp1a •Cyp3a <p>F: Plasma concentration</p> <ul style="list-style-type: none"> •Antipyrine 	<p>No significant change</p> <p>D: Cytokine assays</p> <ul style="list-style-type: none"> •\uparrowIL-6: Tmax 3 hours •\uparrowTNFα: Tmax 1 hour <p>E: Western Blot</p> <ul style="list-style-type: none"> •Cyp1a \downarrow •Cyp3a \downarrow <p>F: Plasma concentration</p> <p>CL \downarrow75%</p> <p>T$\frac{1}{2}$ \uparrow3.6 X</p> <p>AUC\uparrow4.2X</p>	
Cows	<p><i>Fasciola hepatica</i> (parasite)</p> <p>30 Frisian calves</p> <p>Group 1: adult parasites</p> <p>Group 2: flukes</p> <p>Group 3: no infection</p>	Cyp	<p>B1: Cyp activity</p> <ul style="list-style-type: none"> •p-nitroanisole •aminopyrine •aniline <p>G: Liver tissue activity</p> <ul style="list-style-type: none"> •Nitroxynil metabolism 	<p>A: Cyp content \downarrow60%</p> <p>B1: Cyp activity</p> <ul style="list-style-type: none"> •p-nitroanisole \downarrow60% •aminopyrine \downarrow60% •aniline \downarrow60% <p>G: Liver tissue activity</p> <ul style="list-style-type: none"> •Nitroxynil metabolism: \downarrow80% in infected cows <p>Inhibited by</p> <p>Mild disease had milder impact</p>	Facino et al., 1984
Chickens (Broilers)	<i>E. coli</i> from infection with Colibacillosis injected into pectoral muscle	<p>Cyp3a</p> <p>P-gp: Abcb1 gene</p>	<p>F: Plasma concentration</p> <p>In infected and healthy:</p> <ul style="list-style-type: none"> •Enrofloxacin 10 mg/kg •Enrofloxacin 10 mg/kg + Verapamil 15 mg/kg 	<p>F: Plasma concentration</p> <p>PK enrofloxacin infected:</p> <ul style="list-style-type: none"> • Cmax \downarrow66% • AUC₀₋₁₂ \downarrow50% • Tmax \uparrow120%\times 	Guo et al., 2014

	<p>N=5 infected N=5 healthy</p>		<p>H: qPCR •Primers specific for Abcb1, Cyp3a, & β-actin</p> <p>I: Immunohistochemistry for P-glycoprotein •Liver •Small intestine</p>	<p>PK enrofloxacin w/ P-gp inhibitor verapamil:</p> <ul style="list-style-type: none"> • Cmax ↓30% • AUC₀₋₁₂ ↓12% • Tmax ↓9% <p>(personal comparison of means provided in the publication: see comments in text)</p> <p>H: qPCR: disease resulted in</p> <p>Significantly higher Abcb1 mRNA levels in kidney, jejunum, ileum. No change in liver.</p> <p>•Cyp3a37 mRNA significantly decreased in liver and kidney</p> <p>I: Immunohistochemistry for P-gp</p> <p>•Healthy birds: P-gp visualized on bile canicular membrane Kidney: P-gp visualized on apical plasma membranes of proximal tubule cells</p> <p>•Infected birds: Internalized in cytoplasm away from bile canicular membrane.</p>	
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				Kidney: distributed in cytoplasm.	
Sheep	Mycotoxin N=27 sheep	Cyp	J: RNA-Seq Analysis H: qPCR	J: RNA-Seq Analysis Multiple Cyps were identified in the RNA-Seq analysis. H: qPCR Only Cyp2c8 and Cyp1a2 were confirmed by qPCR.	Zhang, 2014
Dogs	Congestive Heart Failure N=14 Mongrel dogs	Cyp2c8, 1a2, 2e1, 3a, 2b	A: Total Cyp B1: Cyp activity •Aminopyrine (2c8) •7-ethoxycoumarin (1a2, 2e1) •Aniline (2e1) E: Western blot •Cyp3a •Cyp2b	A: Total Cyp ↓40% B1: Cyp 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 hours Livers from 3 pigs	Cyp and UDP GT	B1: Cyp activity •Testosterone (3a4) •Ethylmorphine (2d6 and 3a4) B2: UDP activity •1-naphthol •paracetamol •morphine	B1: IL-6 caused significant inhibition of metabolism of all substrates tested: 30-50% decrease. B2: IL-1α and TNFα significantly reduced metabolism of 1-naphthol, paracetamol, and morphine: 20-30%.	Monshouwer et. al., 1996b
Rabbits	<i>E. coli</i> LPS N=20 rabbits	Cyp	A: Total Cyp B1: Cyp activity •Aminopyrine	A: Total Cyp ↓25% B1: Cyp activity For all: no change in Km	Saitoh et al., 1999

			<ul style="list-style-type: none"> •Aniline •Caffeine <p>B2: UDP activity</p> <ul style="list-style-type: none"> •p-nitrophenol <p>E: Western blot</p> <ul style="list-style-type: none"> •anti-Cyp1a1/a2 •anti-Cyp2e1 <p>F: Plasma concentration</p> <ul style="list-style-type: none"> •Antipyrine 	<p>Vmax ↓ 45%</p> <ul style="list-style-type: none"> •Aminopyrine •Aniline •Caffeine <p>B2: UDP activity</p> <ul style="list-style-type: none"> •p-nitrophenol No Δ <p>E: Western blot</p> <ul style="list-style-type: none"> •anti-Cyp1a1/a2 ↓ •anti-Cyp2e1 ↓ <p>F: Plasma concentration</p> <ul style="list-style-type: none"> •Antipyrine AUC ↑1.5X 	
Chickens (Broilers)	Experimentally-induced <i>colibacillosis</i> N = 36 chickens	Mdr1 Mrp2	<p>H: qPCR</p> <ul style="list-style-type: none"> •Mdr1 •Mrp2 	<p>H: qPCR</p> <ul style="list-style-type: none"> •Mdr1 ↓Mdr1 mRNA levels in the duodenum, jejunum, caeca, and liver <p>•Mrp2</p> <p>↓ Mrp2 in liver</p>	Haritova et al., 2008
Sheep	<i>Haemonchus contortus</i> N=12 lambs	Cyp3a	<p>B1: Cyp activity</p> <ul style="list-style-type: none"> •7-ethoxyresorufin (1a) •7-methoxyresorufin (1a) •7-pentoxoresorufin (2b) •7-benzyloxyresorufin (3a) •7-methoxy-4-coumarin demethylase(2c9) •clorzoxazone (2e1) <p>B2: UDP activity</p>	<p>B1: Cyp activity</p> <ul style="list-style-type: none"> •7-ethoxyresorufin (1a) ↓12% •7-methoxyresorufin (1a) ↓20% •7-pentoxoresorufin (2b) ↓10% •7-benzyloxyresorufin (3A) ↓40% •7-methoxy-4-coumarin demethylase(2c9) ↓20% •clorzoxazone (2e1) ↓40% 	Bartikova et al., 2009

			p-nitrophenol •Flavine monooxygenase Thiobenzamide	•Flavine monooxygenase Thiobenzamide ↓50%	
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mRNA: messenger RNA

cDNA: complementary DNA, synthesized from a single stranded RNA template in a reaction catalyzed by a reverse transcriptase.

Mdr1: the multidrug resistance gene that encodes for the efflux transporter, P-glycoprotein

Mrp2: 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.

P-gp: P-glycoprotein

qPCR: quantitative polymerase chain reaction, used for its ability to determine the relative or absolute amounts of amplified DNA in samples.

AUC: area under the concentration vs time curve.

UDP: Uridine diphosphate

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

IL-1 α : interleukin 1 α , which is released from the cell upon death and is a potent inflammatory cytokine (van der Vookvan and Netea, 2013), Di Paolo and Shayakhmetov, 2016)

TNF α : tumor necrosis factor α is a pro-inflammatory cytokine that has a key role in the pathogenesis of chronic immune-mediated diseases (Chu, 2013). It significantly reduced metabolism of 1-naphthol, paracetamol, and morphine: 20-30%.

↓: decrease

↑: increase

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 Admin	Species	Consequence
Influence of induced disease states on the disposition kinetics of imidocarb in goats	Abdullah and Baggott, 1986	LPS, <i>Trypanosoma. evansi</i> , infectious bovine rhinotracheitis (IBR) virus	Imidocarb	IV	Goats	LPS and IBR reduced Vd and CL. Infection with <i>T. evansi</i> resulted in an increase in Vd and CL.
Pharmacokinetics of difloxacin in healthy and <i>E. coli</i> -infected broiler chickens	Abo El-Ela et al., 2014	<i>Escherichia coli</i>	Difloxacin	IV and oral	Chicken	Following IV 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 bovine viral diarrhea virus (BVDV) plus <i>P. haemolytica</i>	Oxytetracycline	IV	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	<i>Trypanosoma brucei</i>	Diminazene	IV	Dogs	Infection with <i>T. brucei</i> resulted in a decrease in Vd and CL.
Pharmacokinetics of tulathromycin in edible tissues of healthy and experimentally infected pigs with <i>Actinobacillus pleuropneumoniae</i>	Bladek et al., 2015	<i>A. pleuropneumoniae</i>	Tulathromycin	IM	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 <i>Streptococcus suis</i> co-infection on the pharmacokinetics of ceftiofur hydrochloride after intramuscular injection in pigs	Day et al., 2015	<i>Porcine reproductive and respiratory syndrome virus (PRRSV) and S. suis</i>	Ceftiofur	IM	Swine	Coinfected pigs had lower AUC and Cmax values, but greater Vd and CL values than that of healthy pigs.
Pharmacokinetics of tilmicosin in healthy and experimentally <i>Pasteurella multocida</i> infected lactating goats	El-Komy et al., 2016	<i>P. multocida</i>	Tilmicosin	SC	Goats (lactating)	Plasma tilmicosin concentrations were substantially lower in <i>P. multocida</i> infected goats.
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 T _{1/2} .
The influence of <i>Actinobacillus pleuropneumoniae</i> infection on tulathromycin pharmacokinetics and lung tissue disposition in pigs	Gajda et al., 2015	<i>A. pleuropneumoniae</i>	Tulathromycin	IM	Swine	Greater tissue AUCs were observed in pneumonic pigs as compared to healthy pigs, but significance not detected
Altered plasma pharmacokinetics of ceftiofur hydrochloride in cows affected with severe clinical mastitis	Gorden et al., 2016	<i>E. coli</i> or <i>Klebsiella</i> spp.	Ceftiofur	IM	Cattle (lactating dairy)	Mastitic cows had significantly higher plasma Vd and CL and lower AUC and Cmax as compared to healthy cows.
<i>E. coli</i> 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	<i>E. coli</i>	Enrofloxacin with or without oral verapamil	Oral	Chicken	By 12 hrs post-infection, 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 Cmax and AUC but later Tmax. Disease induced changes in

						systemic exposure 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	<i>Staphylococcus aureus</i>	Ceftiofur	IMM	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 <i>E. coli</i> O78/H12 infected chickens	Haritova et al., 2011	<i>E. coli</i>	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 <i>Haemonchus contortus</i> and <i>Trichostrongylus colubriformis</i>	Hennessy et al., 1993	<i>H. contortus</i> T. <i>colubriformis</i>	¹⁴ C-Oxfendazole (OFZ)	Intra-ruminal	Goats and Sheep	No change in the PK of fenbendazole (FBZ) or FBZ-SO ₂ , but significant decrease in OFZ C _{max} and AUC in both goats and sheep
Comparative pharmacokinetics of marbofloxacin in healthy and <i>Mannheimia haemolytica</i> infected calves	Ismail and El-Kattan 2007	<i>M. haemolytica</i>	Marbofloxacin	IM and IV	Calves	Infection resulted in a decrease in CL (IV), and an increase in T _{1/2} (IM and IV), AUC (IM and IV) and C _{max} (IM). There were no changes to protein binding.
Effect of <i>Haemonchus contortus</i> infection on the clearance of antipyrine, sulfobromophthalein, chloramphenicol, and sulfathiazole in lambs	Kawalek and Fetterer 1990	<i>H. contortus</i>	Antipyrine, sulfobromophthalein, chloramphenicol, sulfathiazole	IV	Lambs	During infection, significant decreases observed in the AUC of sulfathiazole, antipyrine and chloramphenicol. However, only antipyrine associated with a significant increase in CL. Therefore, the reliability of the conclusions are unclear.

Comparison of pharmacokinetics and milk elimination of flunixin in healthy cows and cows with mastitis	Kissell et al., 2015	Mastitis (<i>E. coli</i> or <i>Klebsiella spp</i>)	Flunixin	IV	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	IV	Bovine	Only mean parameters provided (no statistics). Data suggest increase in CL, Vd, T _{1/2} but decrease in AUC. Ceftriaxone milk excretion initially greater in healthy cows, but some differences in mean values observed at hr 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 (percent coefficient of variation, %CV) observed in milk levels of diseased cows were substantially greater at hrs 24 and 36 (46% and 64% CV, respectively) as compared to that of healthy cows (10.9 %CV and 2.9 %CV at hrs 24 and 36, respectively) (n=8 per group).
The influence of a heavy infection with sensitive and resistant strains of <i>Ostertagia circumcincta</i> and with <i>Trichostrongylus colubriformis</i> on the pharmacokinetics of febantel on lambs.	Landuyt et al., 1995	<i>O. circumcincta</i> , <i>T. colubriformis</i>	Febantel	Oral	Lambs	Authors suggest that PK changes (monitored for febantel metabolites) were dependent on the infecting parasitic species. While there was a consistent decrease in mean AUC (compared to 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	<i>Haemonchus contortus</i> and <i>Trichostrongylus colubriformis</i> mix (natural infections)	Moxidectin	Oral and SC	Sheep	increase in CL/F (oral), decrease in mean residence time (oral and SC), and decrease in AUC (oral). Cmax values were difficult to interpret due to the very large intersubject variability.
Pharmacodynamics and pharmacokinetics of carprofen, a non-steroidal anti-inflammatory drug, in healthy cows and cows with <i>E. coli</i> endotoxin-induced mastitis	Lohuis et al., 1991	LPS	Carprofen	IV	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 <i>Ostertagia circumcincta</i> on pharmacokinetics of fenbendazole in sheep	Marriner et al., 1985	<i>O. circumcincta</i>	Fenbendazole (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 (<i>Bos indicus</i>) cattle and Boran cattle infected with <i>Trypanosoma congolense</i>	Mamman et al., 1993	<i>Trypanosoma congolense</i>	Diminazene	IM	Cattle	Drug PK of each animal was determined before and during acute and chronic phases of infection. Acute infection increase absorption rate and decreased Vdss but did not affect CL/F.
Effect of parasitism with <i>Nematodirus battus</i> on the pharmacokinetics of levamisole, ivermectin and netobimin	McKellar et al., 1991	<i>N. battus</i>	Levamisole, Ivermectin, Netobimin	Oral and SC	Lambs	No differences in PK reported.
Effect of parasitism on the pharmacokinetic disposition of ivermectin in lambs	Perez et al., 2006	<i>Ostertagis</i> , <i>Trichostrongylus</i> , <i>Cooperia</i> mix	Ivermectin	SC	Lambs	Parasite infection resulted in a decrease in AUC. Although Cmax tended to be lower in infected animals, the difference was not significant. CL/F and Vd/F were not reported

Pharmacokinetics of florfenicol after intravenous administration in <i>E. coli</i> 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.
The pharmacokinetics of oxytetracycline following intravenous administration in healthy and diseased pigs	Pijpers et al., 1990	<i>A. pleuropneumoniae</i>	Oxytetracycline	IV	Swine	Significantly lower CL, Vd and T _{1/2} were in diseased vs healthy pigs when dosed at 10 mg/kg but 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	<i>A. pleuropneumoniae</i>	Oxytetracycline	Oral	Swine	CL/F was significantly lower in diseased pigs, resulting in an increase in AUC and T _{1/2} .
Influence of porcine <i>A. pleuropneumoniae</i> infection and dexamethasone on the pharmacokinetic parameters of enrofloxacin	Post et al., 2002	<i>A. pleuropneumoniae</i>	Enrofloxacin	IV	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	IV	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	<i>E. coli</i>	Enrofloxacin	IV	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	IV	Rabbits	Lower CL and a longer T _{1/2} was 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 <i>E. coli</i> infected broilers	Soliman, 2000	<i>E. coli</i>	Enrofloxacin	IV and oral	Chicken	Following IV administration, the CL significantly increased, AUC and T _{1/2} 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.
Pharmacokinetics and efficacy of tilmicosin in the treatment of <i>Pasteurella haemolytica</i> bronchopneumonia in calves	Soliman and Ayad, 2014	<i>P. haemolytica</i>	Tilmicosin	IV and SC	Calves	Following IV administration, CL and Vd was 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	IM	Swine	PRRSV infected pigs had higher CL and Vd and lower AUC, C _{max} and T _{1/2} compared to 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	<i>E. phagocytophila</i> and <i>T. brucei</i> 1066	Sulfadimidine	IV	Goats	Both parasitic infections resulted in lower in CL and Vd and larger AUC and T _{1/2} values.

Influence of <i>Escherichia coli</i> endotoxin-induced fever on the pharmacokinetic behavior of marbofloxacin after intravenous administration in goats.	Waxman et al., 2003	<i>E. coli</i>	Marbofloxacin	IV	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 <i>Haemophilus parasuis</i>	Zhang et al., 2017	<i>H. parasuis</i>	Tilmicosin	Oral	Swine	No significant differences in tilmicosin PKs were observed in healthy vs infected pigs.

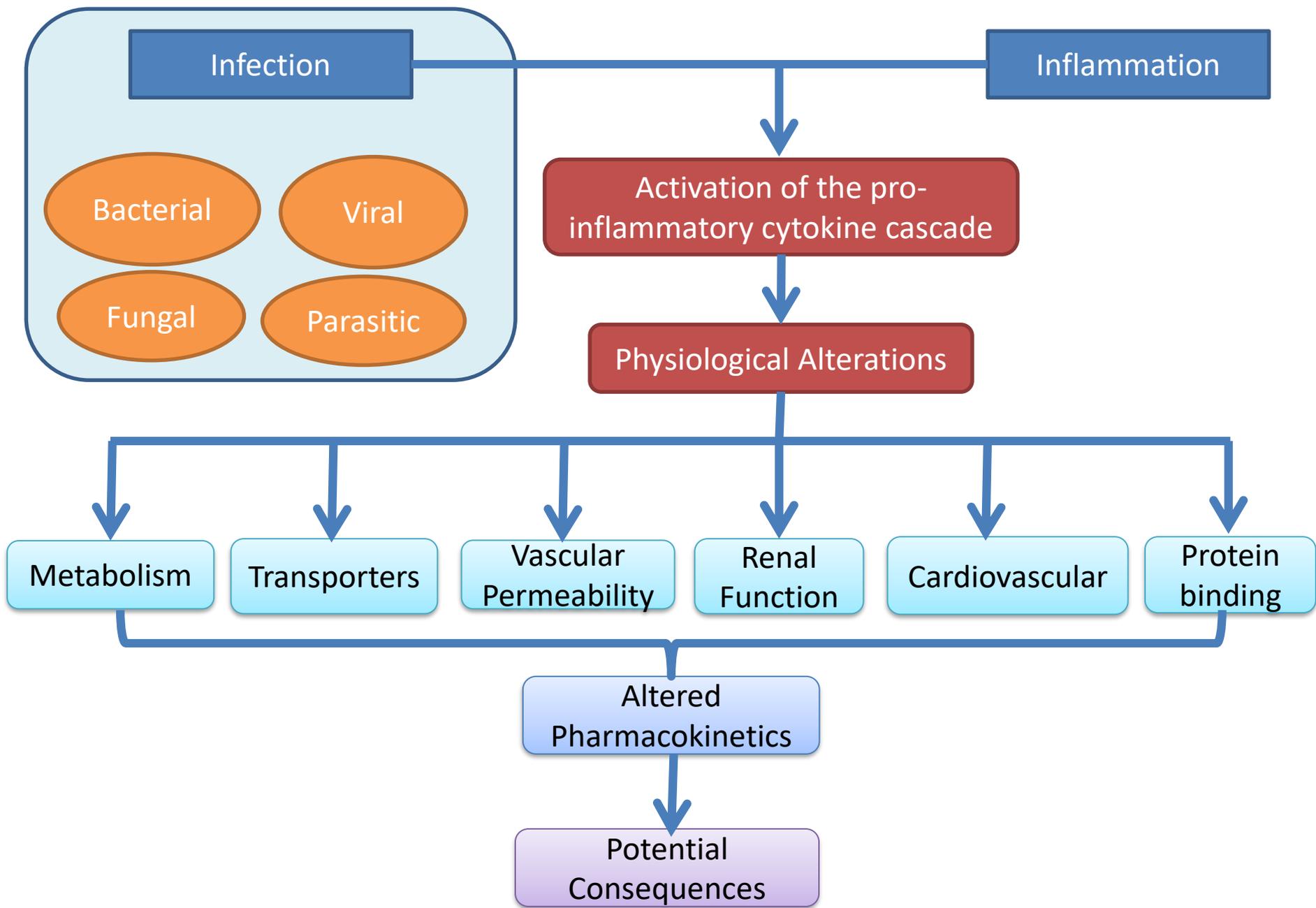


Figure 1