Drug Metabolism and Disposition

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

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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.

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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.

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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

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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).

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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

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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

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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

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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

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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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