

Contamination of untreated livestock with veterinary drugs

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- A UK National Reference Laboratory for veterinary drug residues -antibiotics, anthelmintics, coccidiostats, steroids, Bagonists, non-steroidal anti-inflammatories
- Licensed drugs will have MRL maximum residue levels
- Unlicensed drugs Method will have sensitivity requirement minimum required performance limit (MRPL)



Two-tier testing approach

- Screen by inexpensive, rapid, easy to use methods
 - Microbiological
 - Immunoassay
- Confirm by sophisticated physicochemical methods
 - LC-MS/MS
 - HPLC





Investigation of non compliance

So what happens next.....?

Investigation of non compliance - The first step.....investigate on farm

RES-3

DEPARTMENT OF AGRICULTURE, ENVIRONMENT AND RURAL AFFAIRS

FIELD INVESTIGATION REPORT - CATTLE or SHEEP - AUTHORISED SUBSTANCES (This form should be returned through field DVO to DVO residues)

POSITIVE SAMPLE DETAILS

Herd/Flock owner	Herd/Flock number
Residue substance	
Level detected ug/kg	MRL ug/kg
APHIS test ID Lab re	f number
Sampling Date	

POSITIVE ANIMAL DETAILS

Age Source (born on site/purchased)
If purchased date of arrival and time on farm pre sampling
Transport to slaughter (own/haulier)
Peer group kept separate on lorry or mixed with those from another herd

MOVEMENT RECORDS

Are records kept in accordance with the legislation

DETAILS OF DRUG ADMINISTERED TO POSITIVE ANIMAL

Product name
r toutet name
Formulation (oral in feed or water/injectable)
Batch number
Source
Withdrawal period Last date of administration
Was the medicine administered by the correct route in accordance with manufacturer's instructions
and by the correct method i.e. if multiple site injections were recommended was this done?
RES-33

- Majority of cases
 - Withdrawal not adhered to
- Wrong dose
- Administered incorrectly

However from time to time

- "I used exactly as per manufacturer's instructions"
- "I did not administer any drugs"
- In Northern Ireland, 2008 2015, 0.77% of bovines tested were non-compliant for phenylbutazone
- "No treatment given" in number of cases
- Worthy of further investigation



What is phenylbutazone?

- A non-steroidal anti-inflammatory drug (NSAID)
 - Abbreviated to PBZ; often called "Bute"



- Poses health risks to man so no longer used in human medicine
- Choice of NSAID for equines; substantial clinical history of efficacy and safety
- Problem is that horse meat is consumed in many countries
- The CVMP assessed PBZ in 1997:
 - No thresholds identified so maximum residue limits could not be established
 - Therefore PBZ is not permitted for use in any food producing animals
 - Findings confirmed by EFSA and EMA 2013
- From 2005-2013, 1.6% of equines tested in EU were non-compliant
- EFSA reported 0.18% of bovines tested between 2008 and 2014 as non-compliant for NSAIDs with PBZ responsible for 28% of these
- Represents significant non compliance for an unauthorised drug



On farm investigation of two non compliant samples

Farm 1

- Plasma sample taken from single animal at slaughter
- PBZ confirmed by LC-MS/MS at 0.4 μg L⁻¹
- Intentionally fed to bulls due to issues with lameness
- Animal sampled was a cow!

Farm 2

- Plasma sample taken from single animal at slaughter
- PBZ confirmed by LC-MS/MS at 0.32 $\mu g \ L^{-1}$
- No intentional misuse
- Horse on farm fed PBZ in diet however housed separately from cattle
- The dirty bucket theory

Is it possible that the bovine problem could be largely due to cross contamination from misuse or from horses?





On farm investigation of non compliance in bovines in NI

- i. Admission of illegal treatment in one case
- ii. Remaining cases no treatment given
- iii. Noted the presence of horses on farm

Is it possible that contamination from legal treatment of horses may be the cause of much of the NI bute issue?

1. Could contaminated feeding vessels give rise to detectable residues of the drug in the plasma of untreated cattle?



2. Could association with treated animals (either legally treated horses or illegally treated cattle) be a source of PBZ residues?

3. Contaminated pasture - can pasture be sufficiently contaminated by treated animals to give rise to detectable PBZ concentrations





1. Contaminated vessel





Animal was non-compliant for 49 days after last feeding from the contaminated vessel, with concentrations in plasma peaking at 990 $\mu g \ L^{-1}$

2. Housing with treated animals





Three animals housed with a treated animal were all non-compliant within 24hours of association. Concentrations peaked at 2.85 μ g L⁻¹ and remained for 14 days

3. Contaminated Pasture



Graze for 3 days







Grazed 20 days after U5-U7 were removed

First three untreated animals were all non-compliant, concentrations peaking at 11.95 μ g L⁻¹ Further three animals grazed 20 days after previous three were removed were also noncompliant, levels peaking at 1.69 μ g L⁻¹

Is contaminated pasture really a risk.....??

- Studies unusual in that untreated animals very quickly follow treated onto pasture
- Study more closely mimicking normal farming practice
- 5 animals housed over the winter period
- Extended treatment with PBZ
- Manure stored as per normal
- Spread onto field mid February
- Field remained empty for 70 days
- Five animals allowed onto grass







Is contaminated pasture really a risk.....??

Days after introduction	Plasma				
to field			µg/L⁻¹		
to held	U11	U12	U13	U14	U15
0	<0.28	<0.28	<0.28	<0.28	<0.28
5	2.54	5.32	4.24	6.46	2.42
8	3.42	5.21	9.91	10.34	4.85
12	4.46	3.68	10.67	7.28	4.67
15	1.46	3.34	9.42	4.65	2.33
19	2.7	4.27	4.99	3.52	3.33
22	1.69	4.34	6.03	2.61	2.59
27	1.81	4.26	6.37	3.02	2.60

Normal farming practice may also give rise to PBZ residues



Is contaminated pasture really a risk.....??

- Trial stopped after 27 days due to lack of grass
- Field remained empty for 23 days to permit re-growth
- Introduced five new cattle
- Sampled at various intervals over 44 days
- Only one bovine sample showed detectable concentrations
- 0.29 µg L⁻¹ PBZ



Phenylbutazone - summary of findings

Contamination of untreated animals through a number of sources is likely to be playing a key role in the non compliance detected during statutory surveillance.

Fodey T. L., Smyth W.G., Barnes P., Traynor I.M., Kennedy D.G., Crooks S.R.H. (2015) Investigation into sources of contamination of cattle with phenylbutazone. *Veterinary Record* 176(3) 74-76

 A further study showed non compliance through manure from treated animals being spread onto fields

Barnes P., Fodey T. L., Smyth W.G., Crooks S.R.H. (2017) Investigation into the role of environmental contamination in the occurrence of residues of the veterinary drug phenylbutazone. *Food additives and Contaminants: Part A*, 34(4), 520-524

Untreated animals can be non compliant!

So the obvious questions......

Could this be an explanation for more than PBZ ?

Any other non compliance regularly unexplained?

Is this only an issue when there is no MRL??



Liver fluke and closantel

- Liver fluke in cattle and sheep is a significant and increasing issue in the UK and Ireland due to milder winters and increased rainfall
- Estimated cost to UK farmers of £300 million per year
- Closantel is one of a number of anthelmintic salicylanides introduced in the 1960's and 70s.



- Most widely used of the group which also includes rafoxanide and oxyclosanide
- Highly effective against adult and immature liver fluke (Fasciola hepatica)
- Closantel analysis performed in liver using a multi-residue UPLC-MS/MS method
- MRLs established in both cattle (1000 μ g kg⁻¹) and sheep (1500 μ g kg⁻¹)



Closantel in bovine liver

- In period Jan 2013 December 2016 a total of 2896 bovine livers analysed
- Of these 43 (1.5%) were found to contain non-compliant residues
- On-farm follow up investigations showed widespread closantel use however farmers claimed to have followed dose and withdrawal......
- Series of small studies employing a pour-on formulation of closantel which is widely employed by NI farmers
- Applied along the midline of the back in a narrow strip at a dose of 20 mg/kg bodyweight
- Withdrawal period prior to human consumption of 28 days





Mixing of treated and untreated cattle

- Could mixing of treated and untreated cattle be a problem?
- Occurring on farm though manufacturer had highlighted significant likelihood of cross contamination of non treated animals due to grooming behaviour.
- Three animals were treated with the pour-on application
- Mixed with 3 untreated cattle 4 hours later
- Plasma samples taken throughout the withdrawal period
- Animals slaughtered after 28 days and liver samples taken
- All samples analysed by UPLC-MS/MS





Closantel plasma concentration





Mixing of treated and untreated cattle

Days after	Concentration of closantel in liver (µg kg ⁻¹)						
treatment	Treated 1	Treated 2	Treated 3	Untreated 1	Untreated 2	Untreated 3	
28	916	881	447	965	54	179	

All compliant however a non-treated animal showed the highest liver concentrations!

Would untreated cattle be non compliant earlier in withdrawal?

Study 2: Untreated group slaughtered at day 10 withdrawal

Are high concentrations associated with grooming behaviour?

Days after treatment	Concentration of closantel in liver (µg kg ⁻¹)				
	Untreated 4	Untreated 5	Untreated 6		
10	77	1499	176		
Grooming Behaviour	X	YYY	Y		

Closantel - summary of investigations

- Closantel plasma concentrations in untreated animals can exceed those in treated animals
- Untreated animals may have closantel residue concentrations in their liver which exceed the MRL
- Problem appears to be related to the grooming behaviour demonstrated by cattle
- Provision of advice to farmers re treatment regimes etc. may play a key role in reducing the residue problem



Summary

- 1. Non compliant residues of both MRL and unlicensed/unauthorised substances in untreated cattle
- 2. Non compliant residues of a non-steroidal, an antiparasitic (and an antimicrobial in untreated cattle)
- 3. Non compliant residues in plasma and liver in untreated cattle

More questions

A person shall not sell for human consumption any animal product which contains

- i. An unauthorised substance, an unlicensed substance; or
- ii. An authorised substance at a concentration exceeding the maximum residue limit

However.....

What if the animal that contains the substance has never been treated?

Could the 'person' really be held liable?



Thank you to my colleagues



Dermot Faulkner



Paul Barnes



Steven Crooks



Imelda Traynor



Colin Thompson



Wesley Smyth

Thank you for your attention

