



Analytical tools to detect feed fraud: Case study of the TSE Feed ban

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Introduction: Use of Animal proteins in Feed

1986

First BSE case in UK



2001:

Total Feed ban: farmed animals



2017

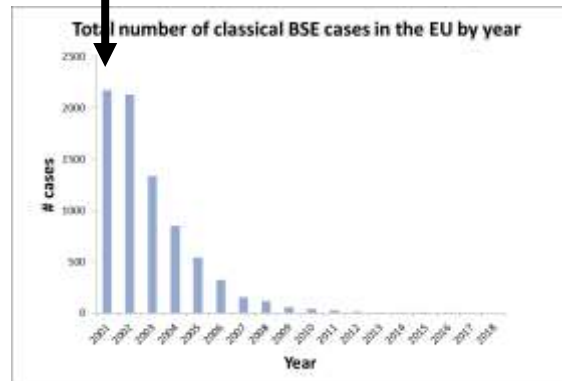


1994

Partial Feed ban: ruminant feed



Feed ban



2013

2021



Introduction: Current regulation

		Destination					
		Feed intended for farmed					
		Rum.	Pigs	Poultry	Others	Fish	Pets and fur animals
Ruminant	PAP	Red	Red	Red	Red	Red	Yellow
	Blood meal	Red	Red	Red	Red	Red	Yellow
	Blood products	Red	Red	Red	Red	Red	Yellow
	Gelatine and collagen	Red	2021				Yellow
	Hydrolysed proteins other than those derived from hides/skins	Red	Red	Red	Red	Red	Yellow
	Hydrolysed proteins derived from hides/skins	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Milk, milk products, colostrum	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Fish	Fishmeal	Red	Red	Red	Red	Red	Yellow
Pig	PAP	Red	Red	2021	Red	2013	Yellow
	Blood meal	Red	Yellow	Yellow	Yellow	Yellow	Yellow
	Blood products	Red	Yellow	Yellow	Yellow	Yellow	Yellow
	Gelatine and collagen	Red	Yellow	Yellow	Yellow	Yellow	Yellow
	Hydrolysed proteins other than those derived from hides/skins	Red	Red	Red	Red	Red	Yellow
	Hydrolysed proteins derived from hides and skins	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Poultry	PAP	Red	2021	Red	Red	2013	Yellow
	Blood meal	Red	2021	Red	Red	2013	Yellow
	Blood products	Red	2021	Red	Red	2013	Yellow
	Egg, egg products	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Hydrolysed proteins other than those derived from hides/skins	Red	Red	Red	Red	Red	Yellow
	Hydrolysed proteins derived from hides/skins	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Other non-ruminant	PAP	Red	Red	Red	Red	2013	Yellow
	Blood meal	Red	Red	Red	Red	2013	Yellow
	Blood products	Red	Red	Red	Red	2013	Yellow
	Gelatine and collagen	Red	Yellow	Yellow	Yellow	Yellow	Yellow
	Hydrolysed proteins other than those derived from hides/skins	Red	Red	Red	Red	Red	Yellow
	Hydrolysed proteins derived from hides/skins	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Insect	PAP	Red	2021		Red	2017	Yellow
Di and tricalcium phosphate of animal protein		Red	Yellow	Yellow	Yellow	Yellow	Yellow
Animal proteins other than those mentioned		Red	Yellow	Yellow	Yellow	Yellow	Yellow

Animal by-products of Category 3

Ruminant	PAP
	Blood meal
	Blood products
	Gelatine and collagen
	Hydrolysed proteins other than those derived from hides/skins
	Hydrolysed proteins derived from hides/skins
	Milk, milk products, colostrum
Fish	Fishmeal
Pig	PAP
	Blood meal
	Blood products
	Gelatine and collagen
	Hydrolysed proteins other than those derived from hides/skins
	Hydrolysed proteins derived from hides and skins
Poultry	PAP
	Blood meal
	Blood products
	Egg, egg products
	Hydrolysed proteins other than those derived from hides/skins
	Hydrolysed proteins derived from hides/skins
Other non-ruminant	PAP
	Blood meal
	Blood products
	Gelatine and collagen
	Hydrolysed proteins other than those derived from hides/skins
	Hydrolysed proteins derived from hides/skins
Insect	PAP
Di and tricalcium phosphate of animal protein	
Animal proteins other than those mentioned	

By-product nature:

- Species of origin
- By-product type

+except in milk replacer

Introduction: Belgian feed chain situation

RASFF – the Rapid Alert System for Food and Feed (system for reporting food/feed safety issues within the European Union) <https://webgate.ec.europa.eu/rasff-window/screen/search>

reference	category	subject	In EU (2021-2022): 12 notifications	date	notifying_country	risk_decision
2022.4586	feed materials	Ruminant DNA in fish feed		05-08-2022	Cyprus	not serious
2022.3882	feed materials	Ruminant protein DNA in pork hemoglobin		04-07-2022	Poland	undecided
2022.3761	feed materials	ruminant DNA in fish feed from		28-06-2022	Cyprus	not serious
2022.1664	feed materials	Presence of ruminant DNA		22-03-2022	Cyprus	serious
2022.1456	feed materials	Ruminant DNA in poultry		11-03-2022	Austria	not serious
2022.1217	feed materials	Ruminant DNA in feather meal and poultry protein concentrate		02-03-2022	Austria	not serious
2022.1207	feed materials	Ruminant DNA in poultry meal and pig haemoglobin powder		01-03-2022	Austria	undecided
2022.0571	compound feeds	Ruminant DNA in complete feed for carp from Austria		28-01-2022	Slovenia	undecided
2021.6943	compound feeds	Ruminant DNA in animal feed for aquaculture from Spain		16-12-2021	Cyprus	undecided
2021.6759	compound feeds	Ruminant DNA in compound feed (fish feed) from Lithuania		09-12-2021	Latvia	not serious
2021.4904	compound feeds	PRESENCE OF RUMINANT DNA IN FISH FEED FROM SPAIN		14-09-2021	Cyprus	not serious
2021.3201	compound feeds	Ruminant DNA in feed for trout from Belgium		18-06-2021	Belgium	not serious

Regulation respected
 OR
 Frauds not detected ?

Reference methods for the detection of PAPs

➤ Light microscopy



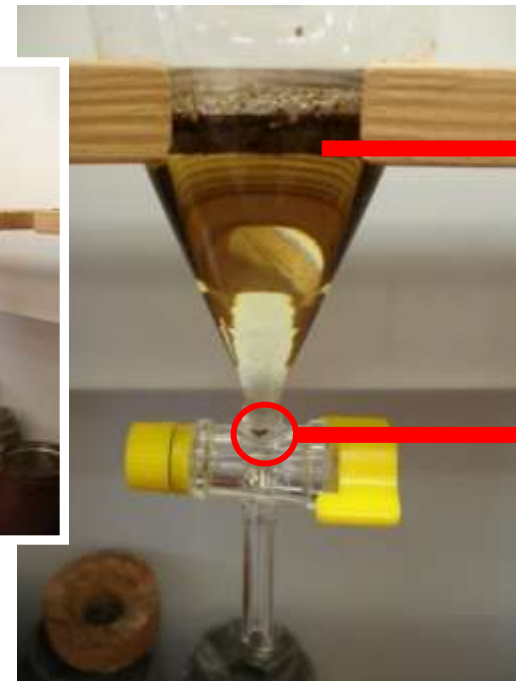
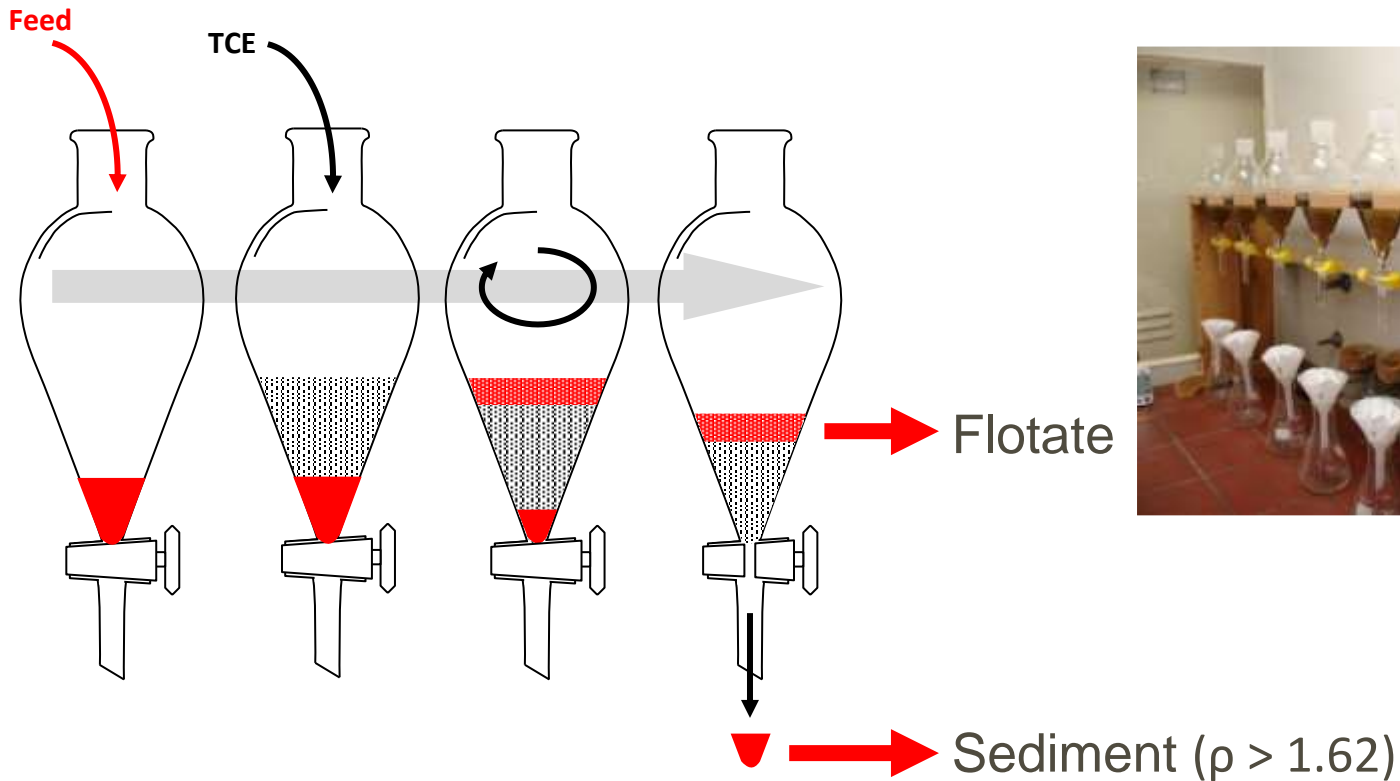
➤ Real time Polymerase Chain Reaction (qPCR)



Light microscopy

Principle

- Observation of identifiable structures on # fractions obtained by sedimentation(s)



Cereals, ...
Muscle fibres, feathers,
hairs, insect particles, blood
meal, plasma, milk,...

Bones, cartilage, minerals,...

Light microscopy

Principle

- Observation of identifiable structures on # fractions obtained by sedimentation(s)



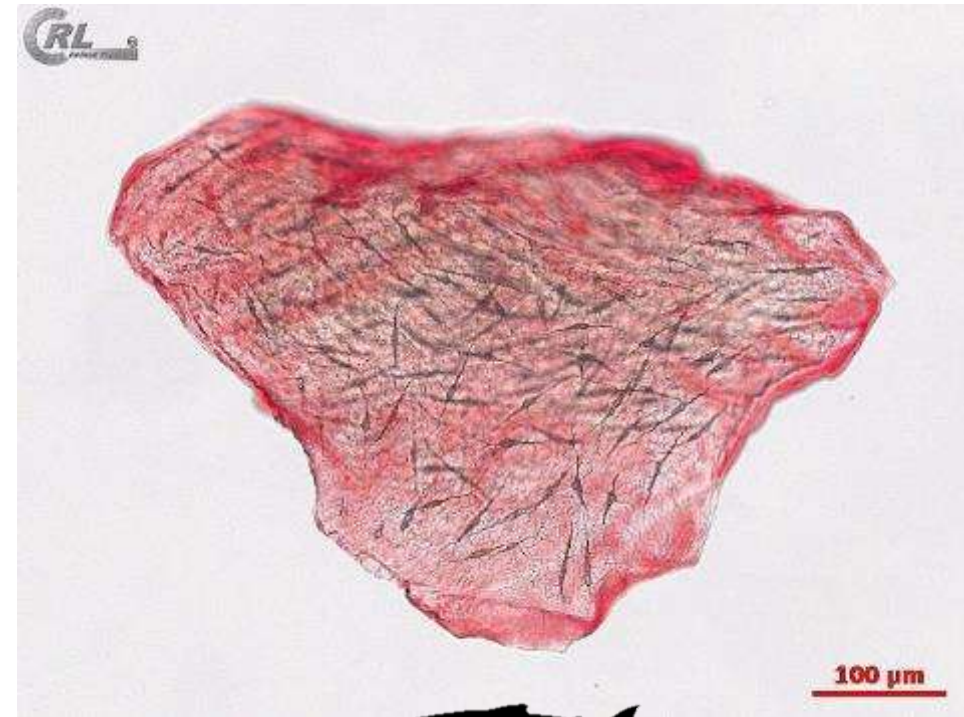
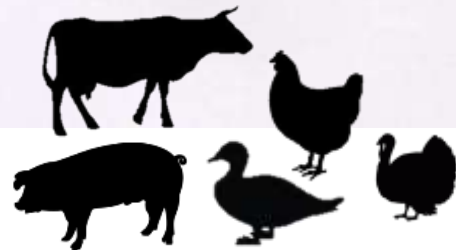
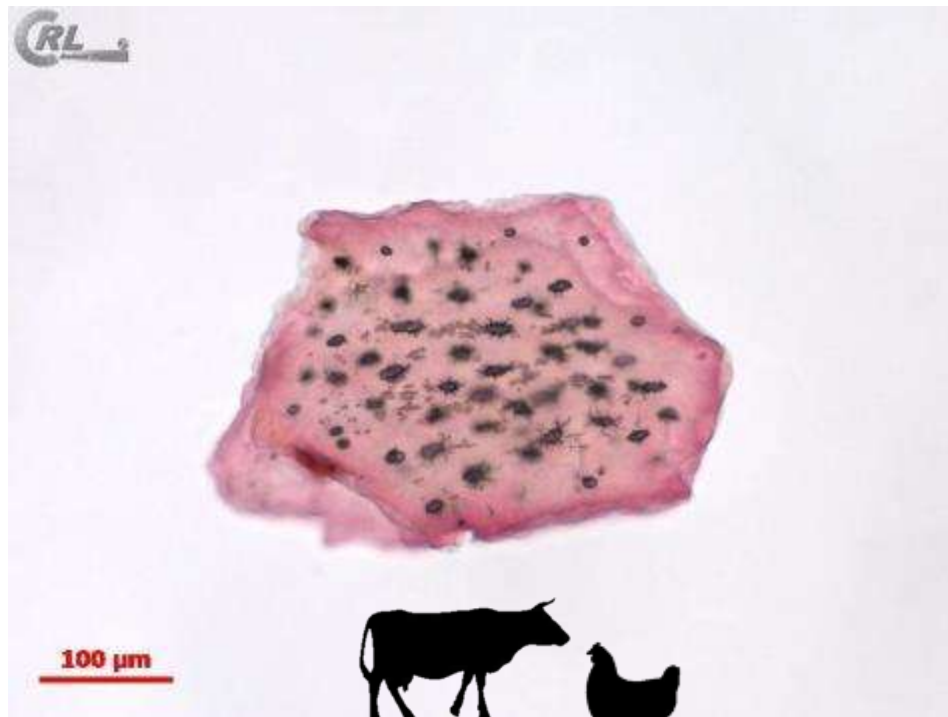
Staining can be used :

- Alizarin Red → bones, scales
- Fehling → Muscle fibres
- Cystine reagent → hairs, feathers
-

=> Distinction of PAPs from **terrestrial vertebrates**, **fish** and **terrestrial invertebrates**

Light microscopy

Identification : terrestrial vertebrates ↔ fish



Light microscopy

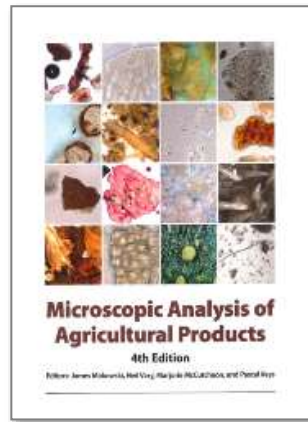
Expertise... (plant or animal ?)



Light microscopy: Advantages and drawbacks



- Ease of use
- Cheap
- Very sensitive (<0.01%)
- References



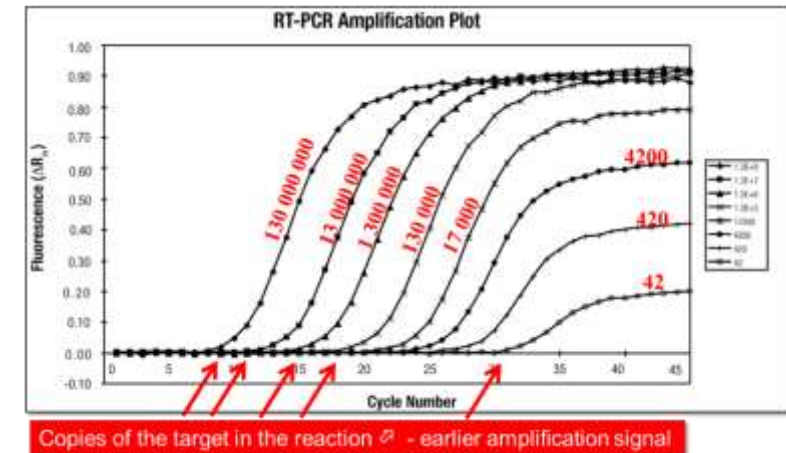
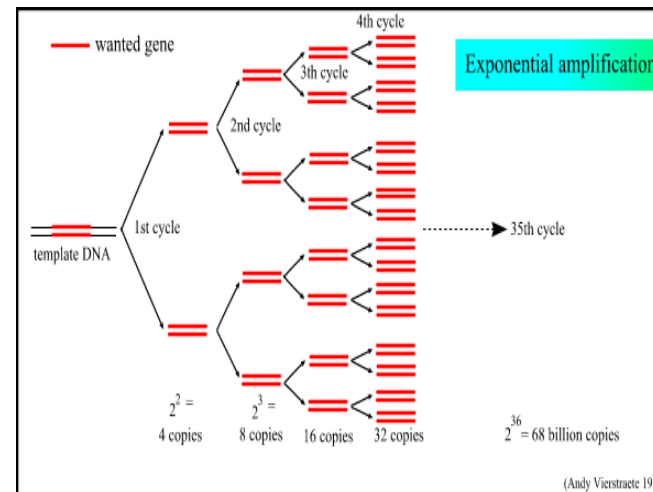
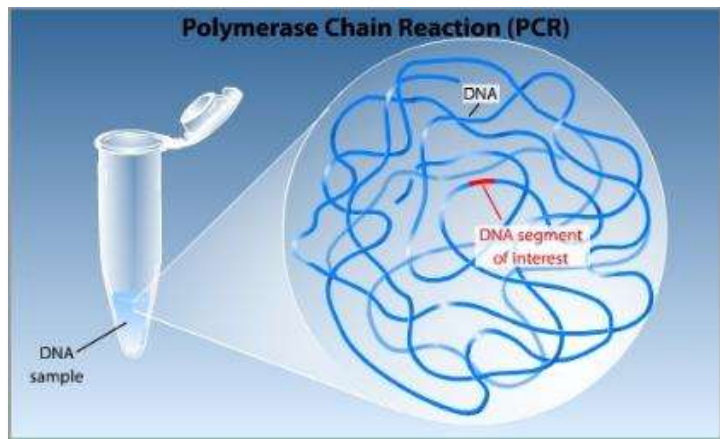
- **Skilled** people, real microscopists
 - Continuous training to keep skills at the top
 - new feed compounds and by products
- **No species** identification
- Based on particle detection only, some ingredients are **not always visible**
- Only **qualitative**...!

Real time Polymerase Chain Reaction (qPCR)



Principle

- Monitoring of the amplification of a targeted DNA molecule
- The greater the quantity of targeted DNA in the material, the earlier the fluorescent signal is above the fluorescence threshold



Real time Polymerase Chain Reaction (qPCR)



- Targeted DNA is specific to

- a species (e.g. pig),



- a group of species (e.g. poultry),



- a taxon (e.g. ruminant)



qPCR: Advantages and drawbacks



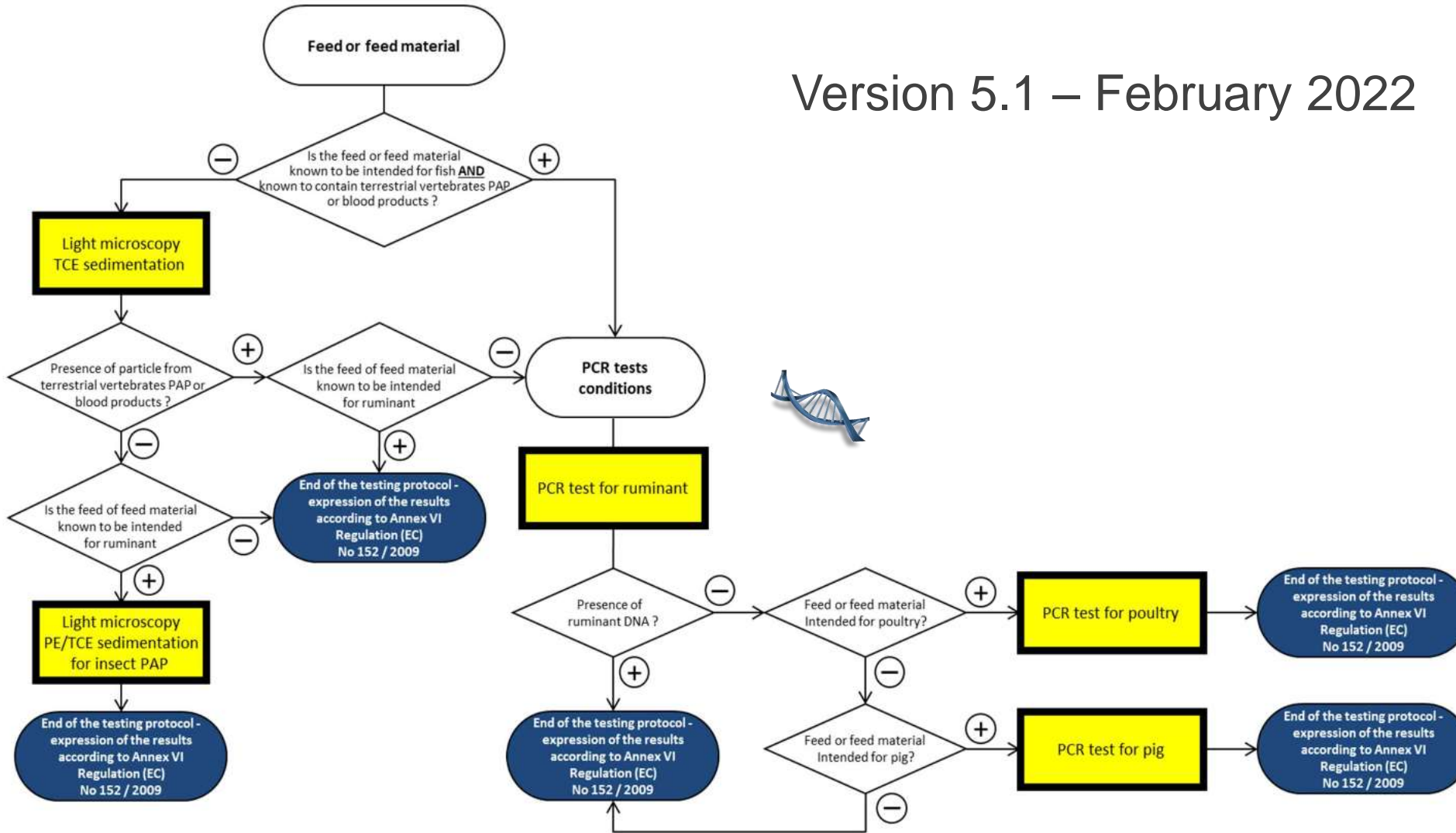
- Species or taxa identification (e.g. ruminant, pig,...)
- Very sensitive (~ 0.1%)
- Common technique
- Able to detect DNA degraded by heating processes



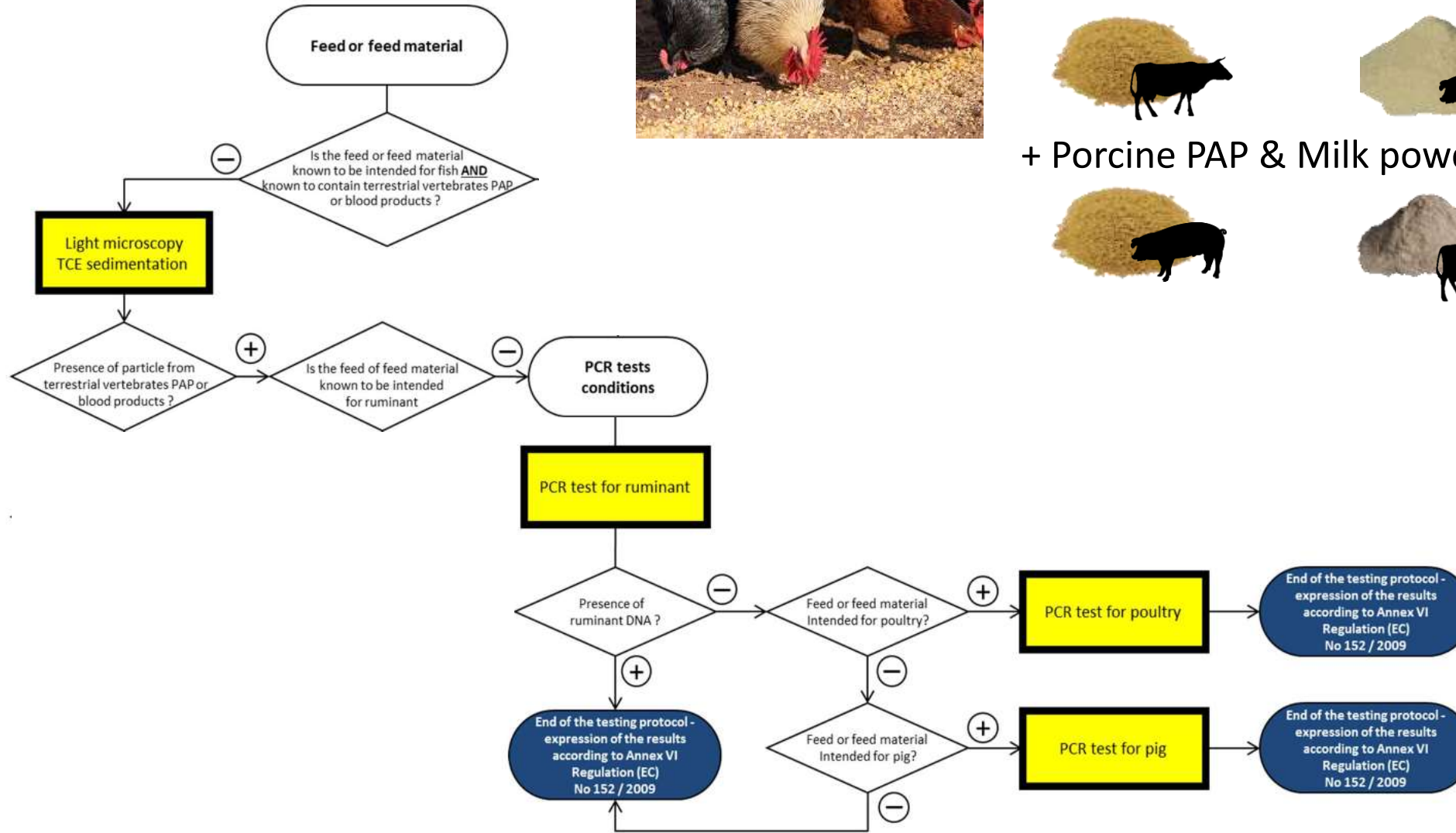
- Not able to determine the **source** of the DNA (e.g. milk vs bovine PAP)
- Trained people
- **Specific and costly equipment**
- Only **qualitative...!**

Combination of methods

Version 5.1 – February 2022



Combination of methods



+ Bovine PAP & Porcine plasma powder



+ Porcine PAP & Milk powder



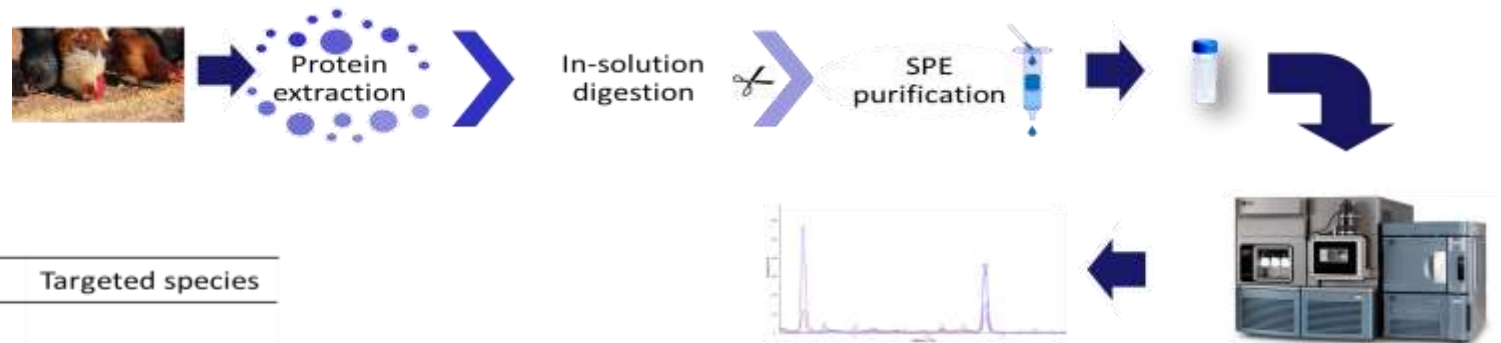
Complementary method



Mass spectrometry (MS)-based proteomics

Principle:

- **Proteomics:** the study of an organism's proteome (>< genomics for its genome)
- **MS:** identification of ionised molecules according to their mass-to-charge ratio (m/z)
- Provides information about the tissue and species of origin



Targeted by-products	Proteins:	Targeted species
Milk	Casein Beta-lactoglobulin	
Blood, PAP	Hemoglobin	
Plasma	Serotransferrin Apolipoprotein	
PAP, Gelatine	Collagen	

Conclusion

Today : Is everything under control ? Can all fraud be detected?

...No ☹️

Ex : porcine blood products vs blood meal



		Rum.	Pigs
Animal by-products of Category 3			
Ruminant	PAP		
	Blood meal		
	Blood products		
	Gelatine and collagen		
	Hydrolysed proteins other than those derived from hides/skins		
	Hydrolysed proteins derived from hides/skins		
Milk, milk products, colostrum			
Fish	Fishmeal		
Pig	PAP		
	Blood meal		
	Blood products		
	Gelatine and collagen		

Tomorrow?

What is the next relaxation of the feed ban???

=> Need of techniques to anticipate policy regulation in the matter?

Ex : bovine blood products vs blood meal



Take-home message

- Each method has its pros and cons.

➔ Combination of methods 😊 **BUT** Higher complexity for fraud detection ☹️
e.g. technical skills, LOD, ...

- Revision of Food and Feed regulations for (novel) protein :



Thanks for your attention Question?



More info about AP-analytical tools:
@mail: secretary@eurl.craw.eu

More info about
CRA-W:



References

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- Lecrenier M.-C. et al., 2020. Official Feed Control Linked to the Detection of Animal Byproducts: Past, Present, and Future. J. Agric. Food Chem., 68, 8093-8103. doi: 10.1021/acs.jafc.0c02718.