UNIVERSITY OF VETERINARY MEDICINE

Institute for Animal breeding, Nutrition and Laboratory Animal Science Department for Veterinary Genetics and Animal Breeding

A Study into Photosensitivity in Cattle: Aetiology, Diagnosis and Management

Review of literature

Diploma work

Written by: Deirdre Cremins

Supervisor: Prof. Könyves László

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Abstract

This Thesis aims to give a general overview into what we know today about photosensitivity. It ranges from the simple to the complex and can go from being relatively easy to manage to a no-hope case very quickly. This is why is it important to be able to identify the signs as soon as possible and better yet alleviate all potential risks before the disease can cause harm. Many farms are already familiar with the signs however there are a substantial number of farmers who have never seen a case and are likely to dismiss signs until it is too late. With concerns ever growing over climate change and global warming showing no signs of stopping, this is one disease that we must give focus to going into the future, particularly in Ireland where we are fortunate enough to have mild climates and often not enough sun to give photosensitivity a high importance status. Over the years veterinarians are beginning to see more and more cases, to such an extent that it can no longer be classed as rare problem and it is of vital importance that vets and farmers work together to both prevent it from happening and to treat cases as soon as possible.

I decided to look at photosensitivity in cattle through the stages, first from an aetiological view point, then at the clinical signs to pathological findings, giving a closer look at diagnostics and ultimately treatments. My hope is that this literature review on photosensitivity in cattle will be almost a handbook in how to deal with one of the most up and coming diseases of our time. While it might not be the most significant problem veterinarians face it is no excuse not to know how to deal with and treat photosensitivity. I was recently told, by a seasoned veterinarian, that large animal practice going forward will be more about herd health and herd management, rather than day to day treatment of sick livestock. Therefore being able to help and give advice to farmers concerning the best practices in and on their farms will undoubtably make you an invaluable asset in this ever changing profession.

Abbreviations

UV	Ultraviolet
PS	Photosensitivity
BCEPP	Bovine congenital erythropoietic protoporphyria
PDA	Photodiode Array
SM	Secondary Metabolites
SPP	Secondary Plant Products
AST	Aspartate Transaminase
GGT	Gamma-Glutamyltransferase
LDH	Lactate Dehydrogenase
AP	Alkaline Phosphate

Glossary of Terms

Photosensitivity	an extreme sensitivity to ultraviolet (UV) rays from the sun and other light sources
Ultraviolet	a form of electromagnetic radiation with wavelength from 10 to 400 nm, shorter than that of visible light, but longer than X-rays
photodynamic	of, relating to, or having the property of intensifying or inducing a toxic reaction to light in a living system
aberrant	diverging from the normal type
Idiopathic	relating to or denoting any disease or condition which arises spontaneously or for which the cause is unknown.
Paramecia	single-celled protists that are naturally found in aquatic habitats
Apoptosis	Apoptosis is a form of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes and death.

Introduction

Photosensitivity is when ultraviolet (UV) light increases the sensitivity of the skin as a result of photodynamic agents that exist in the skin. This is why animals that are on pasture, particularly in the summer months, need to be protected. Spring and summer seasons are the most dangerous times for photosensitivity as animals are exposed to longer and more intense hours of sunlight. White haired cattle or cattle with sparce hair coverage are most at risk. This is why less pigmented and bald areas are more sensitive, such as the eyes, teats and muzzle. Photosensitivity is often confused with both sunburn and photodermatitis however neither of these ailments require the presence of a photodynamic agent. A simple pane of glass can protect from sunburn however the harmful UV rays involved in photosensitivity are not so easily avoided.

There are 4 different types or causes of Photosensitization. Primary, Aberrant Pigment Metabolism (congenital), Hepatogenous (Secondary) and Idiopathic photosensitivity.

Type 1: Primary Photosensitivity

Primary Photosensitivity is via a direct interaction with a photodynamic agent, by ingestion or absorption. The photodynamic agents causes the UV rays in direct sunlight to interact with and damage the skin.

Type 2: Aberrant Pigment Metabolism

Enzymes ordinarily present in haem synthesis develop an inherited functional defect and as a result protoporphyrin, a photodynamic agent, builds up in the blood stream.

Type 3: Hepatogenous (Secondary) Photosensitivity

Hepatic photosensitivity is by far the most common type of photosensitivity found in cattle and livestock and presents as a secondary problem due to liver disfunction. Phytoporphyrin, a phototoxic agent accumulates in the body.

Type 4: Idiopathic Photosensitivity

Either the cause is unknown or the photodynamic agent has not been identified, even after a source ‘hunt’.

History

Almost 150km from the earth, the suns thermonuclear reactions produce energy that is converted to heat on its surface. The light that we receive on earth comes from the radiant energy emitted from this heat. As a result 'sunlight' is made up of huge amounts of energy and a complete range of wavelengths from "cosmic, gamma rays, x-rays, UVB and UVA radiation, visible radiation and infrared radiation". (Holick, 2016)

The Ozone layer absorbs all of the UV rays below 290nm(UVC), approximately 0.1% of UVB(290-320nm) rays reach the earths' surface and 5% of UVA(320-420nm) rays reach the earths' surface. Therefore most of the solar energies reaching the earth are visible and longer, infrared wavelengths. Exposure to the small amounts of UVA and UVB radiation can produce skin lesions. One of the ways it does this is by reacting with photosensitive agents in living organisms.

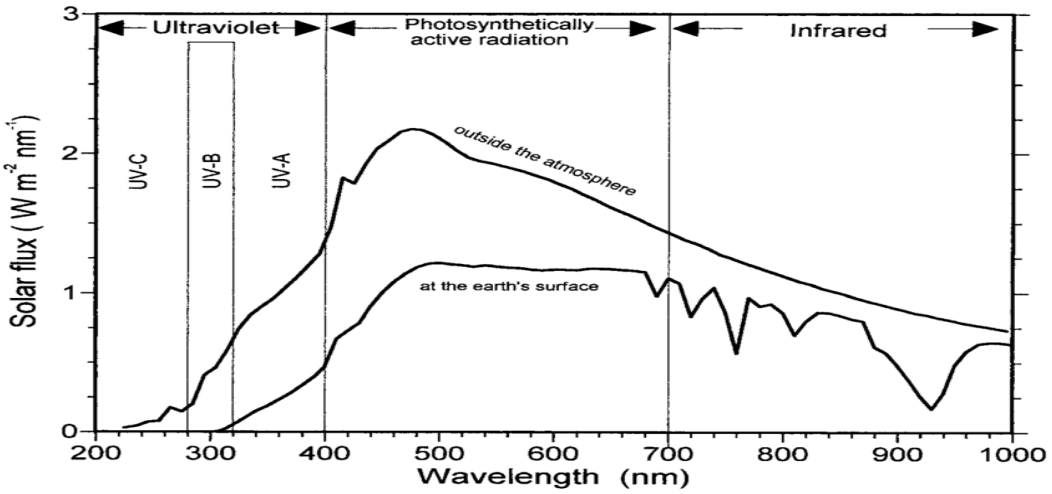


Figure 1. General characteristics of solar radiation outside the atmosphere and at the Earths' surface. (Whitehead, De Mora and Demers, 2020)

Photosensitivity first came to the attention of the veterinary community in the early 1900's when work by scientists began to show that hypersensitivity to light does occur. The disease is produced by any single wavelength or combination of wavelengths in the 290 to 790 nm spectrum.

Oscar Raab has been credited with the discovery of photosensitivity due to his work involving the toxicity of acridine towards paramecia. Raab discovered that close to midday toxicity was highest and that paramecia was inactivated more effectively if the acridine solution was kept close to a bright window than if it was in the dark (Raab, 1900). Hermann Von Tappeiner was Raabs Mentor, and it was he who coined the term 'Photodynamic' to reference photosensitization, differentiated from the photosensitive effects that could be seen in photographic plates. (*Photosensitization - What Stopped the Wiggling?*, 1990).

Figure 2. A cow with photosensitivity lesions on its Muzzle. (Bríd Lenihan, 2020)

Mechanism of Action

The First Law of Photochemistry in photobiology states that the light (photons) absorbed must have an effect and the molecule that absorbs the photon ultimately alters another molecule in the system. In general, it is the photon-absorbing molecule that is the photosensitiser and the altered one is the accepter which reacts with proteins, lipids and DNA leading to photosensitivity. The skins increased susceptibility to UV rays causes the production of free radicals which damage the cell and lysosomal membranes. Phototoxic agents are unstable, high energy molecules when exposed to UV rays (280-790nm).
But why does photosensitisation occur? A number of hypothesis are held, and all support cell membrane permeability which depends on the placement of the photodynamic agent within the cell. Secondary metabolites(SM), some of which are photodynamic agents (Appendix 1 and 2), connect with the cell superficially and alter its properties through the loss of cellular potassium and cytoplasmic extrusion. When the lysosomal membrane is damaged, lytic enzymes are released in the cell and sun rays penetrate the cell leading to the release of histamine. (Hussain *et al.*, 2018) This is turn gives rise to skin ulcers, necrosis, oedema, irritation and cell death. (Kessel and Smith, 1989).
Just how long it takes for clinical signs to appear after exposure to a photodynamic agent all depends on the type of agent, how long the animal was exposed for, how intense the sun was and how much of the agent they were exposed too.

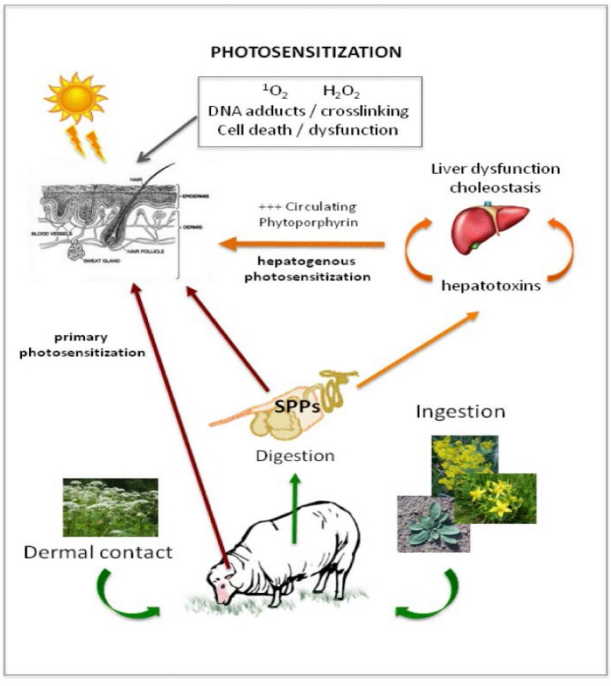


Figure 3. Mode of action for PS. (Quinn, Kessell and Weston, 2014)

Aetiology

Type 1: Primary Photosensitivity

When a photodynamic agent is ingested, injected or absorbed via the skin it enters into the bloodstream and damages the skin cell membrane in the presence of UV light. Poisonous plants are the most common cause of Type 1 photosensitization, namely Hypericum perforatum (more commonly St. John's Wort) and Fagopyrum esculentum (more commonly Buckwheat).

When the grazing animal ingests one of these toxic plants the photodynamic agents they contain are quickly absorbed from the gut then filtered through the liver and settle in the skin throughout the entire body. Here, they are activated by the sunlight and cause photosensitisation. The photodynamic agents are also responsible for causing certain liver problems such as cholestasis because there is not enough time for the normal biochemical reactions to take place. The abnormal, metabolic by-products of porphyrin, a photodynamic agent, can cause gene mutations in the animals skin such as altering enzyme activity in haem production. (Hussain *et al.*, 2018)

Other, lesser responsible photodynamic agents include phenothiazine anthelmintics, which are worming agents administered to cattle and coal tar derivatives like polycyclic aromatic hydrocarbons, tetracyclines and sulfonamides. Coal tar is used in conjunction with UV light as an alternative treatment for severe psoriasis in humans for this very reason.

There are a number of plants that have either been confirmed or are highly suspected of causing photosensitivity. (Appendix 3)

- Hypericum perforatum (Klamath weed/St. John's wort) (Araya and Ford, 1981)
- Fagopyrum esculentum (Buckwheat)
- Heracleum mentagazzianum (Giant hogweed)
- Cymopterus watsonii (Spring-Parsley)
- Brassica rapa
- Erodium

- Indigofera linnaei (Birdsville indigo)
- Medicago polymorpha (Burr trefoil)
- Polygonum
- Enterolobium contotisiliquum
- Visnaga seeds
- Trifolium pretense
- Vicia sp.
- Froelichia humboldtiana (Souza *et al.*, 2012)

Figure 4. Buckwheat. (*Guide to Poisonous Plants – College of Veterinary Medicine and Biomedical Sciences – Colorado State University*, 2020)

Figure 5. St. John's Wort. (*The Benefits and Side Effects of St. John's Wort - Health Juices - Healthy Drinks*, 2020)

Type 2: Aberrant Pigment Metabolism

In this type of photosensitivity, enzymes that usually take part in haem synthesis have a functional defect that is predominantly congenital or inherited. Photosensitizing porphyrins in the form of endogenous pigments are produced as a result of these malfunctioning enzymes. Bovine Congenital Erythropoietic Protoporphyria or BCEPP is one of the most common diseases found in cattle with Type 2 photosensitivity.

BCEPP is most commonly found in Limousin cattle however it can also be seen in Blonde d'Aquitane cattle. The enzyme affected in this particular form of aberrant pigment metabolism is ferrochelatase. Ferrochelatase is active in the final stage of the haem biosynthesis pathway. It aids in the transition of chelation (of the ferrous iron) to protoporphyrin.

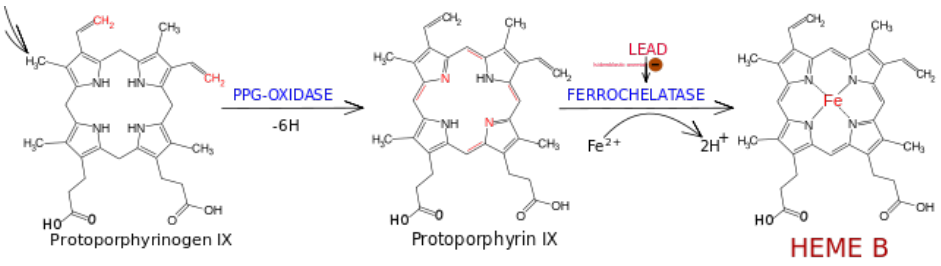


Figure 6. Ferrochelatase catalysing chelation to protoporphyrin.

Too much protoporphyrin accumulates in cellular membranes as it is lipophilic. Protoporphyrin absorbs the UV rays and energy and transfers it into a reactive oxygen species that in turn interacts with proteins, lipids and DNA. BCEPP is believed to be inherited via an autosomal recessive pattern. Photosensitivity is the obvious clinical expression of BCEPP and forms due to the build-up of reactive oxygen species. (McAloon *et al.*, 2015)

Figure 7.. "Erosion and moist exudative dermatitis in the lumbosacral area of a calf with bovine congenital erythropoietic protoporphyria". (McAloon *et al.*, 2015)

Type 3: Hepatogenous (Secondary) Photosensitivity

This is, without doubt, the most common type of photosensitivity found in cattle and livestock in general. It is referred to as 'secondary' photosensitization because it occurs as a result of liver insufficiency and is often found alongside an onset of other conditions. Phylloerythrin is the normal metabolite of chlorophyll and ordinarily, it is reabsorbed from the gut, conjugated in the lever and excreted in the bile. However, it is a photosensitising porphyrin agent and it can build up in the plasma as a result of poor hepatobiliary excretion due to cholestasis and ultimately it gets activated by UV light to cause complications in the body.

Unfortunately this hepatogenous form has the poorest prognosis as it does involve problems in the liver and by the time clinical signs of photosensitivity are seen it may have progressed too far. Primary hepatic lesions and defects that lead to phylloerythrin (also known as phytoporphyrin) build up vary however the resulting photosensitivity is primarily brought on by eating plants containing hepatoxic agents. (Glenn, Monlux and Panciera, 1964) The exact mode of action of phytoporphyrin is not known, however it does bind to the Golgi apparatus and mitochondria. Type 3 photosensitivity is most common in grazing animals, but it can occur in livestock fed silage or hays containing chlorophyll or its derivatives as well. It is also possible for other plant species to be toxic also if they are exposed to and altered by the sun rays and humidity in the surrounding environment. (Hussain *et al.*, 2018)

A small number of secondary photosensitivity cases can occur as a result of physical and mechanical blocking of the bile duct by gallstones, chemical hepatotoxicity or perhaps due to a leptospirosis infection which may significantly disturb hepatic function.
Some plant species containing hepatotoxins such as saponins, terpenes and mycotoxins are: (Appendix 3 and 4)

- Tribulis terrestris (puncture vine)
- Lippia rehmanni
- Amsinckia menziesii (Fiddleneck)
- Amsinckia intermedia (tarweed)

- Narthecium ossifragum (bog asphodel)
- Panicum coloratum (Kleingrass)
- Brassica napus
- Clrotolaria spp. (rattlebox)
- Medicago sativa
- Pterodon emarginatus
- Brachiaria brizantha
- Brachiaria decumbens
- Cynodon dactylon
- Stryphnodendron obvovatum
- Stryphnodendron fissuratum
- Trifolium pretense
- Myoporum laetum (ngaio)

Figure 8. Cynodon dactylon. (Hussain *et al.*, 2018)

Type 4: Idiopathic Photosensitivity

This type of photosensitivity is when the cause is unknown or when the photodynamic agent has not been identified, even after a source 'hunt'. Some mouldy straw or water-damaged hay has been reported to cause photosensitivity but it has not been confirmed and is presumed to be hepatogenous. Heavy rainfall may be a factor in cases like these.

Clinical and Pathological Signs

Clinical Signs

The clinical signs of photosensitivity are generally the same regardless of the type or cause. In experimentally induced cases, signs of PS occurred at 48 hours after exposure to the toxin, however, clinical signs in naturally occurring cases begin from 4 days to several weeks after exposure to mouldy feed or toxic plants. Early nonspecific signs sometimes go unnoticed, such as reduced feed and water intake as well as depression. Unfortunately, the clinical signs for photosensitivity are not pathognomonic, however, a combination of erythema and a few other symptoms will help in gaining an accurate diagnosis.

A list of some of the most commonly seen clinical signs and what they mean:

Pruritus:
Defined as an unpleasant sensation within the skin that provokes the desire to scratch. It is the most common dermatologic problem in both small and large animals, meaning you cannot make a definitive diagnosis when it is present.

Photophobia:
Also known as light sensitivity, is an intolerance of light. Animals will try to seek shade as the sun irritates the skin.

Hyperesthesia:
Excessive physical sensitivity, especially of the skin. The cattle suffering from photosensitivity will be extremely sensitive to touch and their behaviour will be classed as strange and they will appear agitated and uncomfortable.

Erythema:
Redness of the skin or mucous membranes, caused by hyperaemia (increased blood flow) in superficial capillaries. An early sign of photosensitivity but develops rapidly.

Oedema:

The medical term for fluid retention in the body. The build-up of fluid causes affected tissue to become swollen. This symptom quickly follows signs of erythema. However, if the animal is put in a dark shed and exposure to light is limited at this stage the symptoms can quickly resolve.

Serum Exudation:

Produced from fluid that has leaked out of blood vessels and closely resembles blood plasma. Fluid leaks from capillaries into tissue at a rate that is determined by the permeability of the capillaries and the hydrostatic and osmotic pressures across the capillary walls. (Weller, 2009) Nasal and ocular exudates begin as serous discharges and progress to a thick yellow consistency. This can lead to infections and swelling particularly in the eye and blindness is not an uncommon finding, particularly seen in the congenital form, BCEPP.

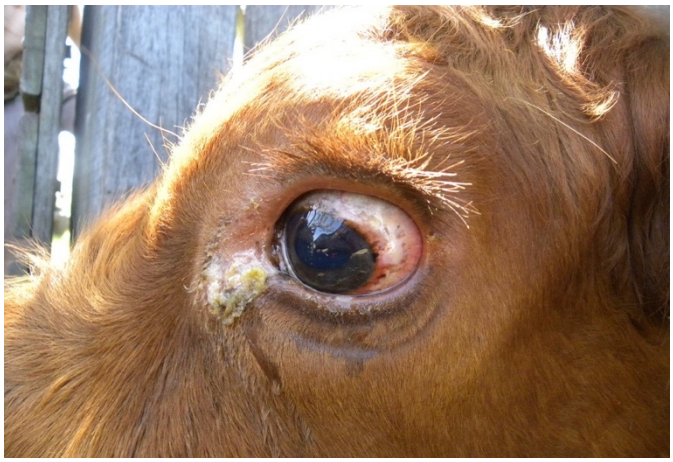


Figure 9. Cow seen with ocular discharge. (Giaretta *et al.*, 2014)

Ulceration:

The formation of a blister like formation, a break on the skin or on the surface of an organ. An ulcer forms when the surface cells die and are cast off. Skin damaged due to excessive scratching and irritation due to the photodynamic reactions will develop ulcers. More severe lesions develop over the dorsolateral trunk and the face. In solid black or dark-coloured cattle, lesions may be present on the lips of the vulva, edges of the eyelids, the sclera, and the cornea

Ill-thrift:

a term used to describe when stock grow at a slower growth rate than expected, given their feed allocation. Generally when cattle have more than 30% slower growth rates than expected. (‘Fact-Sheet-177—Ill-thrift.pdf’, 2020).

Exfoliation:

The shedding of dead and necrotised skin. As the ulcers form and the cells die they fall from the infected areas.

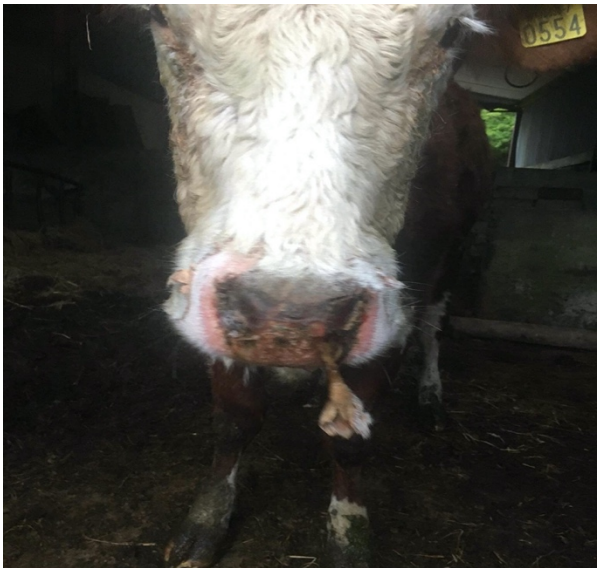
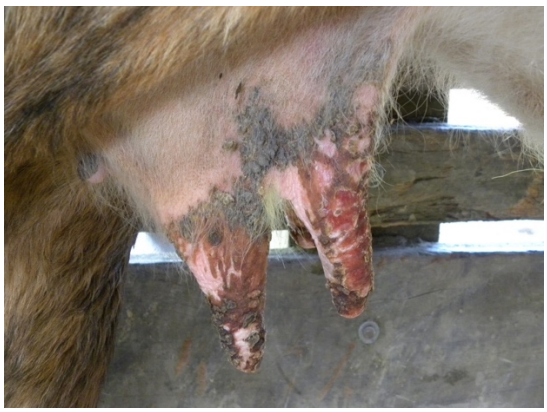
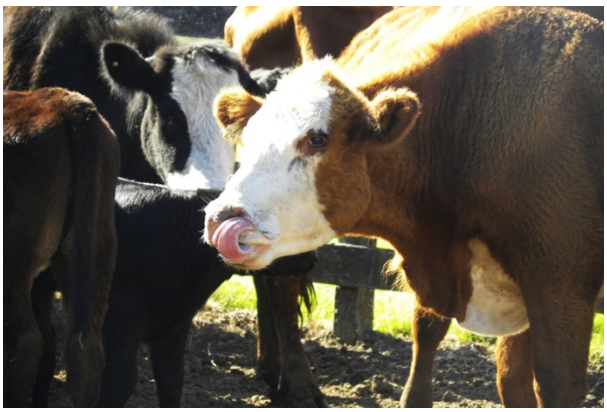


Figure 10. Exfoliation of dead skin from the muzzle of a cow with photosensitivity. (Bríd Lenihan, 2020)

As a result of all the aforementioned symptoms, other clinical signs can manifest. For example, the rectal temperature of the cow will be around 41 °C (normal 37.8-39.2) and you will see the cow licking his nose/muzzle where the erythema is causing irritation. The animal will be seen to swish their tails and kick at their stomachs in an aim to relive the agitation caused by the photodynamic processes taking place in their skin. Hair loss at the site of infected and irritated areas will be substantial. In lactating cows you will see a sharp drop in milk production levels and a cessation in both nursing and breeding because the teats and vulva, as identifiably sensitive areas will be affected.

Figures 11. & 12. Ulceration and crusts on the teats of a cow and licking of nose due to irritation and nasal discharge. (Giaretta *et al., 2014*)

As a result of ulcers forming in the mouth and on the tongue, the animal will find it difficult to eat because of pain and discomfort caused. This lack of appetite as well as depressed attitude of the cow leads to anorexia, the lack or loss of appetite for food. Weight tends to be dramatic and many untreated animals develop a foul odour and die.

Photosensitisation with hepatic malfunction will cause a redish discolouration of the urine and faeces due to a build-up of porphyrin metabolites that have built up in the urinary and digestive system such as uroporphyrin and coproporphyrin. Swelling of the legs can also been seen in rarer cases due to the build-up of cutaneous porphyrins. (Appendix 5)

To put it simply, if you see the skin becoming red, weepy or swollen, if you see a decrease in appetite and depression, the eyelid and ears begin to droop, oedema under the jaw and extremities, difficulty breathing due to excessive swelling and exudation at nostrils and throat, cracked skin that has both ulceration and exfoliation, itching and kicking and/or a drop in milk production then photosensitivity should be you diagnosis.

If the liver has been affected by/ or whose malfunction has caused photosensitisation then the animal may develop jaundice around the eyes, gums and skin. In many cases of photosensitisation where the animal has not be treated early enough they may go into shock and if the symptoms are even more severe the animal may die.

Example 1: Hypericin: (Hypericum perforatum/ St. John's Wort)

Hypericin a photodynamic agent contained in St. John's Wort is responsible for the generation of a reactive oxygen species which gives rise to cell loss in the skin by apoptosis and necrosis. These processes, causing cell damage, rely on the reactive oxygen species generated by hypericin and their cellular targets to be in the same cell because the diffusion of these reactive oxygen species can only cover short distances as a result of their unstable nature. Hypericin has been shown to go to the same cells as organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes. This suggests that all of these essential intracellular organelles are perhaps the primary targets for this phototoxic compound. Therefore the presence of hypericin and occurrence light exposure results in degradation of stratum basale layer of cells in the dermis by 'reactive-oxygen species-mediated cell death', resulting in the significant clinical photosensitization observed in affected livestock.(Jendželovská *et al.*, 2016)

Example 2: Froelichia humboldtiana

The photodynamic agent in F. Humboldtiana is unknown, however it is believed to be a naphthodianthrones or similar as ocular lesions were not found to be present in examined cases. Ocular lesions are commonly seen in plants containing furocoumarins. Clinical signs included alopecia, oedema, crusts and skin necrosis in the flank, lumbosacral area and teats. Unfortunately F. humboldtiana is a highly palatable plant and the animals who graze on it consume large quantities. Farmers in Brazil, where this plant is problematic said that clinical signs of cattle brought in were far more severe than those seen on cattle born on the farm.(Souza *et al.*, 2012)

Pathological Findings

As the skin lesions are common for all forms of PS, narrowing down the correct type of PS helps to limit the number of choices on the differential diagnosis list and by ruling in or out liver involvement you can cater your treatment plan accordingly. If you have more than one animal presenting signs of PS after one or more animals has died you can determine liver involvement by means of a post mortem. Primary necropsy findings indicative of PS include exfoliative dermatitis, Jaundice, an orange-coloured liver, and enlarged superficial lymph nodes. When carrying of a histopathological exam you can see the bile duct necrosis as well as its proliferation, centrilobular degeneration, and necrosis of hepatocytes. In situations where no fatality has occurred, liver biopsies can be taken for histopathologic examination. (Step and Smith, 2006)

The lesions that are typically seen are cholangitis (Figure 13.) and pericholangitis. These issues are characterised by interlobular bile duct proliferation and obstruction, periportal fibrosis, and bile stasis. However it is only in extreme cases that hepatic parenchymal lesions are generally described. One study showed that abnormal pathological results were indicative of renal damage in bovines that consumed foxtail-or- chardgrass hay. (Witte and Curry, 1993)

Pathological exams in animal carcasses that have died from PS, apart from signs of jaundice, show no major lesions on or in internal organs other than the liver and biliary systems, meaning, visible lesions are not frequently observed in the livers samples that had mild or transient symptoms. The earliest, most obviously detectable alterations seen in severely affected animals are minor thickenings of the portal spaces and yellow staining of the parenchyma. The lobular architecture is more easily observed on cut surface as a result. As the symptoms progress, the liver develops a stronger yellowish tint which is highly suggestive of growing bile retention. It has also been noted that as a result of these disturbances there is a slightly increased resistance to cutting in the liver.

Animals that are most affected by PS showed sufficient thickening of the portal tracts and proliferation of the interlobular connective tissue. Bile staining of the parenchyma is intense with an irregular and patchy distribution which created a somewhat mottled effect to the cut surface of the liver. The worst hepatic and biliary pathological signs are mostly

seen at about 6 weeks into the illness. Oedema can be seen in the mucosa and sub- mucosa of the gall bladder in some animals. (Glenn, Panciera and Monlux, 1965)

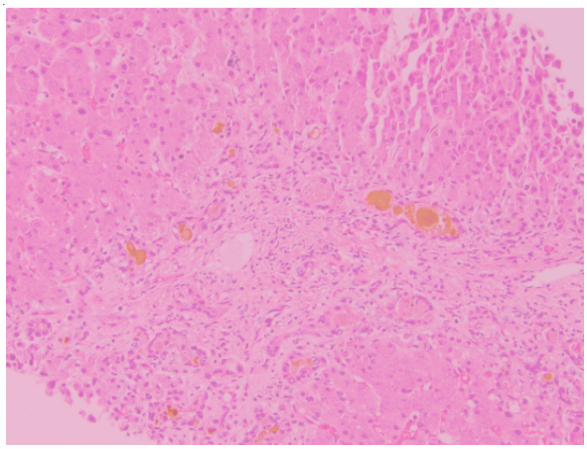


Figure 13. Liver, bile duct cholestasis (Gonzalez, 2019)

In a study carried out into *Senecio* spp. poisoning in cattle, the main necroscopy findings were a hard liver, distended gall bladder, oedema of the mesentery and abomasum. Spongiosis was also detected in the brain of necropsied cows which is characteristic of hepatic encephalopathy. (Giaretta *et al.*, 2014)

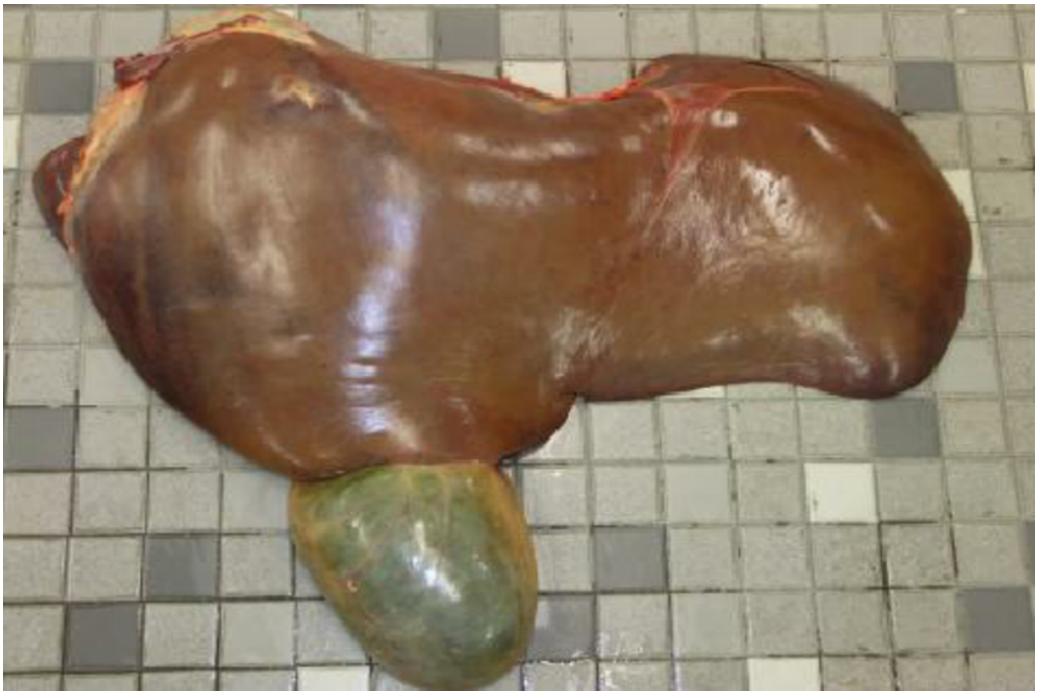


Figure 18. Diffusely enlarged and pale-brown liver of a cow (Collett and Matthews, 2014)

Diagnostics

Diagnosis of photosensitisation is generally made on the basis of the location and appearance of the skin lesions and abnormal behaviour of the animal. The condition is diagnosed after consideration of clinical signs and after a search for toxic plants nearby is udertaken. Blood tests can be carried out for liver function and a post- mortem examination can be undertaken to confirm photosensitisation due to liver disease.

When making a diagnosis for photosensitisation, your differential diagnosis will include:

- Sunburn
- Bluetongue
- Mange (Sarcoptes scabiei var. bovis, Psoroptes ovis and Chorioptes bovis)
- Hypotrichosis
- Alopecia Anemia
- Lice (Sucking and Biting)
- Dermatitis as a result of poisonous plants (Hairy Vetch)

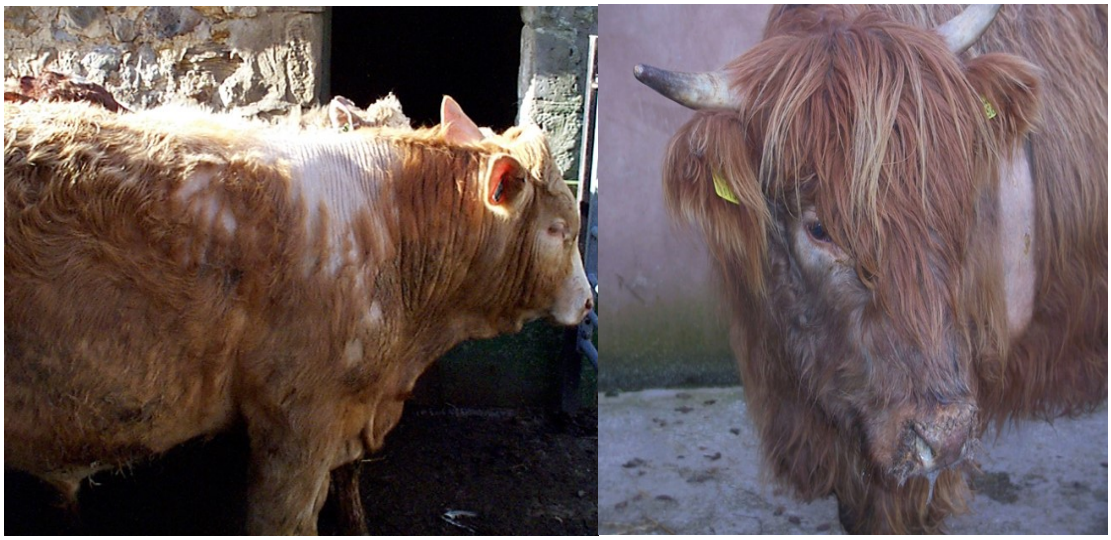


Figure 15. and 16. A cow with a louse infestation, showing similar hair loss patterns to photosensitisation and a cow with inflamed nostrils with nasal discharge as a result of Bluetongue, again similar to photosensitisation. (*NADIS - National Animal Disease Information Service*, 2020)

Detection of Phytoporphyrin (phylloerythrin) in the Blood

Where liver damage is also found, the pigment phylloerythrin may be responsible. This is where we are in luck, as it is possible to determine the levels of phylloerythrin in the blood.(Perrin, 1958) In 2009 WM Campbell *et al.* carried out a study into a spectrofluorometric method for measuring chlorophyll *a* metabolites, specifically phytoporphyrin (phylloerythrin), as well as the chlorins, pheophorbide a and pyropheophorbide *a*, in the blood of photosensitive cattle and sheep (Figure 7.3). I have given a brief outline of this method of diagnosing below.

Method

Reagents

1. Pheophorbide a
2. Pyropheophorbide a
3. Phytoporphyrin
4. Methanol(99.8%)
5. Chromatography grade methanol
6. Ammonium Acetate(98%)
7. Acetone
8. Ultra-pure Milli-Q water
9. Venous blood samples

Equipment

1. HPLC system (Separations module, PDA detector and mass spectrometer)
2. Splitting device (Between the PDA and mass spectrometer)
3. 5µun Sphericlone octadecasilyl-2 column (with ODS guard column)
4. Syringe filters
5. Scanning spectrophotometer
6. Fluorometer (Luminescence spectrophotometer)
7. Ultrasonicator

Results

Pheophorbide a and pyropheophobide a had an excitation/ emission max of 408/669nm and 409/669nm respectively. Phytoporphyrin exuded a typical porphyrin fluorescence

spectrum with excitation/ emission max of 425/644nm. In serum from photosensitive animals, the emission of 644nm was shown to come only from phytoporphyrin and no other chlorophyll a metabolite.

Conclusion

A spectrofluorometric method to determine the quantity of phytoporphyrin in blood of photosensitive animals has been validated. This will prove to be a very necessary tool into the cause of photosensitivities in farm animals. (Campbell *et al.*, 2010)

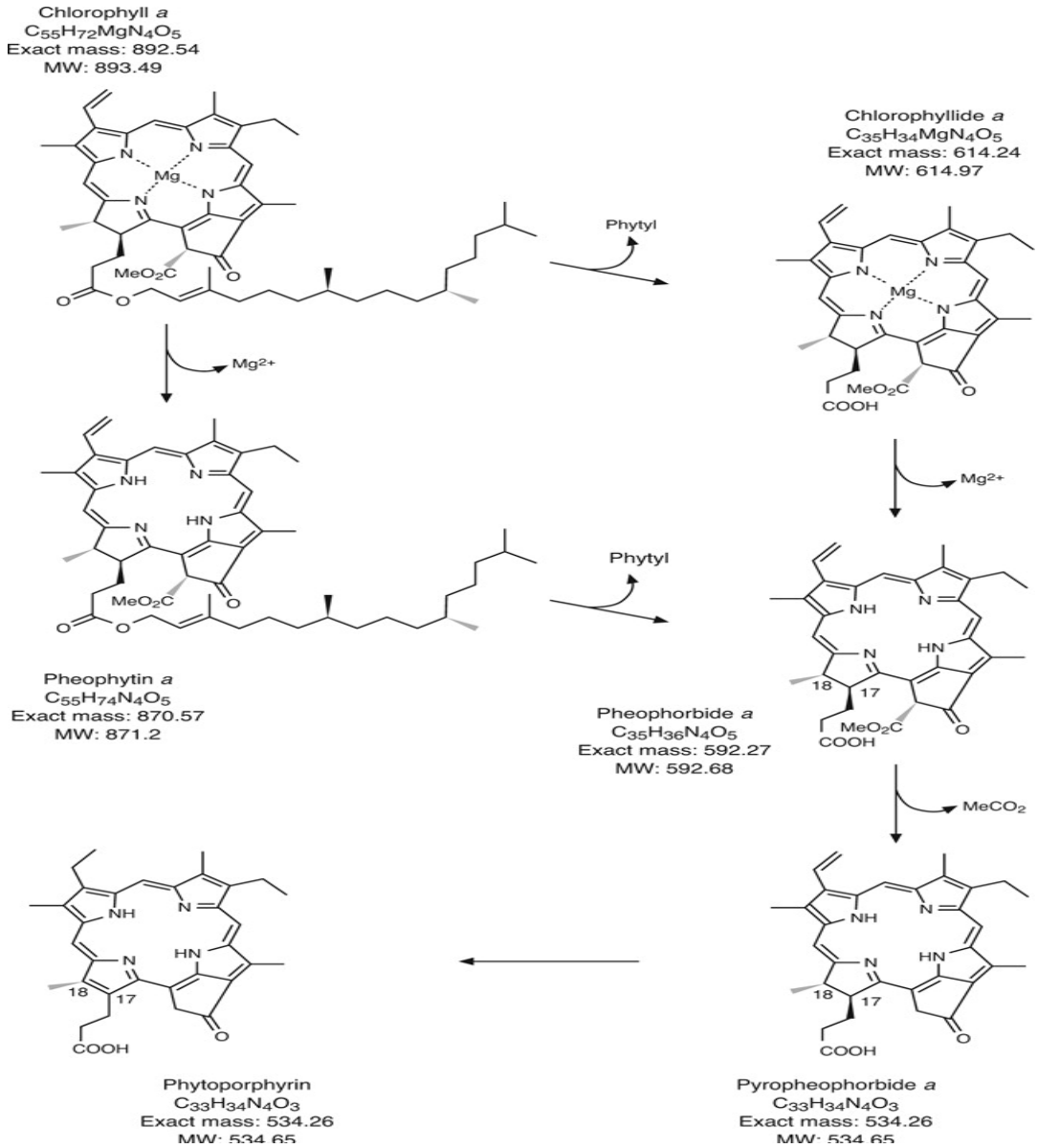


Figure 17. The formation of Phytoporphyrin from Chlorophyll and some intermediate metabolites. (Campbell *et al.*, 2010)

It is also possible to carry out liver function tests in the case of hepatogenous photosensitivity. Generally in these tests a sample of blood is taken from the animal with suspected photosensitivity and tested for Serum aspartate amino transferase (AST), alkaline phosphatase (AP), Serum gamma glutamyl transferase (GGT), Serum total proteins and Serum total bilirubin.(Sharma and Sridhar, 2007) Hepatic biopsy can also help to identify the reason for the hepatic disease and determine how bad the damage is. Many inflammatory diseases such as cholangiohepatitis respond well to therapy.

Due to developments in detection of metabolic compounds using both gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) we have the ability to identify these bioactive photosensitizing compounds in serum, urine, and skin of cattle and other livestock, not only in the plants that produce them. This provides hope that many more photodynamic agents will be structurally identified in the not too distant future. (Quinn, Kessell and Weston, 2014)

For many vets and farmers, unless the problem reaches an epidemiological level, the hopes of them paying for and taking the time to carry out these tests are not likely. Therefore, the diagnosis of photosensitization is carried out by careful pasture analysis, a detailed anamnesis, observed clinical signs and ultimate necropsy. These methods are the most cost effective ways to determine cases of PS, but of course over time, as cases increase and photosensitivity becomes more prevalent, we can hope that the use of spore counting, pathological examination and various laboratory tests such as serum biochemical reactions will become more prevalent and readily used so that we can come to better understand what specific methods of action are behind this deadly disease.

Treatments

In the case of PS, the prevention is more important than a cure. With a little understanding of the topic and good herd management then prevention can be almost 100% effective, particularly in type 1 PS cases. For type 2 or congenital photosensitivity then common sense should be used and animals that present with this type of PS or a diagnosis of BCEPP should not be used for breeding purposes and should be removed from the herd if possible. Unfortunately for type 3 PS the prognosis is poor, therefore early detection and treatment is vital. Once signs are detected, treat the symptoms of photosensitisation and they may survive unless the liver has already been too badly damaged, but it is important to remember that most often treatment involves mostly palliative measures. While recovery from mild symptoms may only take a few days, in cases where the liver is effected, recovery, if possible, could take weeks. While clinical signs of photosensitivity continue, animals should be shaded fully or preferably, housed and allowed to graze only during darkness. The intense stress of photosensitization and extensive skin necrosis can be extremely debilitating. This alone could increase mortality rates. If left untreated an outbreak of PS could see casualties within 5 days.

Prevention:

Farmers should only bale hay that has been dried properly and it should be stored with other feedstuffs in a way that mould cannot grow in dry, clean well ventilated areas. If it is a persistent problem in a farm, the stockman should select cattle with dark hair coats if they will graze on high-risk pastures. Unfortunately, selecting cattle for hair colour alone fails to address the issue with mould or fungi growth in feedstuffs. While darker coated cattle will show fewer and less severe skin lesions, liver damage can still persist and cause extensive damage. One of the most important things farmers can do is to always provide access to alternate feed sources for animals in case of feed scarcity so that they will not eat toxic plants. Animals generally know not to eat plants that will harm them so often it is at times when there is a scarcity in the food supply that they resort to eating toxic plants. When a herd is moved to new pastures they must be observed while they are adapting and their feed should be supplemented for a few weeks. It goes without saying that animals should not graze in places where chemicals (herbicide, pesticide or fertilizer) have been applied.

Treatment Procedure:

If it can be located then the dietary cause should be completely removed. While this is being done all stock should be removed from the pasture and placed somewhere known to be clear of any toxic plants. Any animals that are already infected should ideally be placed in a darkened shed and provided with water and cereal hay or lower quality pasture hay with no green colour (Less chlorophyll intake). (Robson, 2007) It is crucial that attending veterinarians understand that this condition is not sunburn. The animals infected are extremely allergic to sunlight. This means that they need more than just some shade, they need complete darkness for at least a week. Unfortunately, euthanasia may need to be considered in some instances.

Medications:

Animals with only mild symptoms and lesions can usually avoid any sort of medical therapy as long and they are adequately housed away from sunlight.. However, badly affected animals should be treated with systemic antibiotics, like penicillin, to prevent septicaemia along with the use of anti-inflammatory drugs. Corticosteroids, such as dexamethasone, if administered in the early stages might be helpful. Antihistamines can also be administered if the symptoms are severe enough but the efficacy of them is unknown. Photodermatitis lesions should be treated with topical antimicrobial ointments and lotions., such as paraffin oil or sunscreen.

Figure 18. The same cow from figure 2. and 10. after successful treatment. Bríd Lenihan, 2020)

Conclusion

Throughout my time, researching, learning and writing about photosensitivity I have learned many things. The most important bit of information is that there are four, distinct types of photosensitivity: Primary, Aberrant pigment metabolism, Hepatic and idiopathic. From my perspective and I'm sure from the perspective of anyone reading this review or any of the reports mentioned in it, that without doubt the most important photosensitivities are Type 1 and Type 3.

The symptoms for all 4 types of photosensitivity are the same. Most important clinical signs include erythema, exudation and ulceration. Post mortem lesions are most often found in Type 3 or hepatogenous photosensitivity. While Jaundice can be seen in almost all types it is rare to see necropsy findings, like hepatic or biliary lesions in animals showing mild or early stage symptoms. Even in Type 3 PS, the necropsy findings are primarily limited to the liver and bile duct. Sometimes, after prolonged infection, necropsy findings can be seen in other organs such as the kidney, but it is unusual.

Early detection is key. Animals diagnosed and treated within the first few days of infection have the potential to recover within a week, however severe cases or cases involving hepatic disturbances can take weeks or even months to heal. It is my belief that many animals reach the severe stage as a result of many peoples lack of awareness of photosensitivity and how to treat it. It cannot be confused with simple sunburn as shade is not enough to protect the animal from the harmful UV rays, they need complete darkness and shelter from the sun. Understanding the different energies produced by the sun and the radiation in the waves is very important to fully grasp the seriousness of the disease if it persists.

It is clear that trying to determine exactly what type of photosensitivity is presented is difficult as a result of the clinical signs being so similar across the board. However, new research into detecting phytoporphyrin and other secondary metabolites causing photosensitivity in the blood are promising. Detection of liver function via a blood sample or by taking a liver biopsy are the most efficient ways of detecting hepatogenous photosensitivity.

Farmers and veterinarians will need to keep up to date with latest studies informing them about which plants contain photodynamic agents. With new species of plants being imported and accidently brought in from foreign countries every year it is impossible to know what could be growing on your pasture. That being said, for now it is hugely important to be aware of the most troublesome plants in your country (St. John's Wort in Ireland). Being a vigilant stockman and walking your land regularly is vital in the prevention of photosensitisation.

The treatment of diagnosed cases of PS is simple and effective, which is why it is such a pity if cases are not caught in time. Apart from removal to a dark shed, administration of antibiotics to prevent subsequent infection, corticosteroids to reduce inflammation and application of a topical ointment to alleviate irritation is all that is required for a successful recovery.

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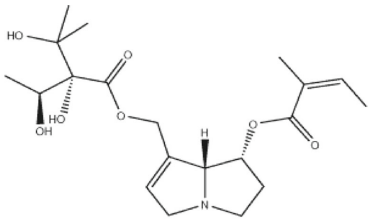
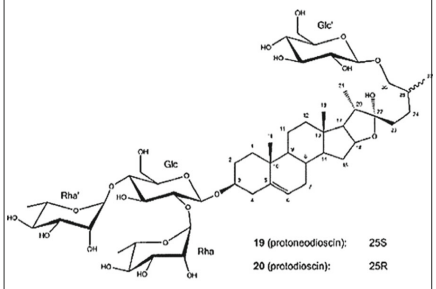
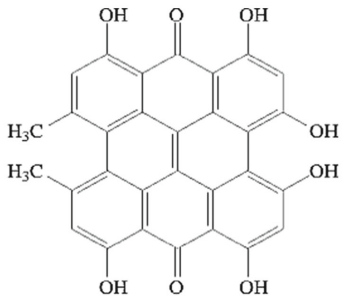
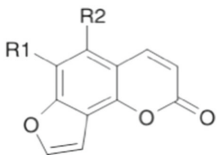
List of Tables and Figures

Appendices

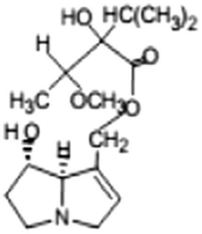
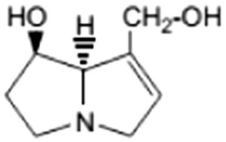
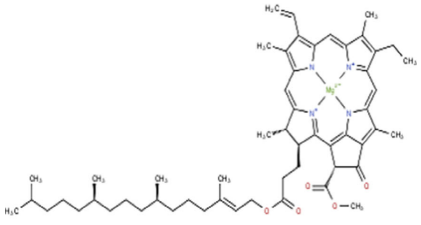
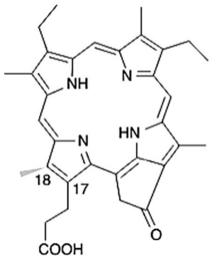
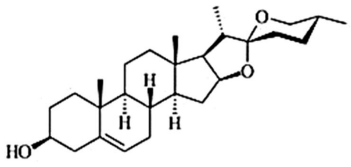
Appendix 1

Table 1 Secondary metabolic compounds

Chemical compounds	Reference	Molecular structure
Echimidine	Cao et al., 2013	
Protodioscin and protoneodioscin	Perez et al., 2016	
Hypericin	EU SCF (2001)	
Angelicin	Wink, 2010. Atta ur Rehman, 2003	

(Hussain *et al.*, 2018)

Appendix 2

Heliotrine	Peter et al., 2004.	
Dehydroretronecine	Peter et al., 2004, Stegelmeier et al., 2016.	
Chlorophyll –A	Chenyu et al., 2017. Campbell et al., 2010 (reproduced, with permission)	
Phytoporphyrin	Campbell et al., 2010 (reproduced, with permission)	
Diosgenin	Tao et al., 2014 (reproduced, with permission)	

(Hussain *et al.*, 2018)

Appendix 3

Table 1. Extracted photosensitisation outbreak data from the peer-reviewed literature by aetiological agent. Percentage morbidity and mortality are included where reported.

Aetiological agent	Country	Type of photosensitisation	Year	Species	Flock/Herd size	Morbidity (%)	Mortality (%)	Reference
Alfalfa hay (predominantly *Medicago sativa*)	US	Primary	2013	Horse	116	6.9	N/A	[34]
Alfalfa hay (predominantly *M. sativa*)	US	Primary	2004	Horse	70	100.0	1.4	[34]
Alfalfa hay (predominantly *M. sativa*)	US	Primary	2008	Horse	N/A	N/A	N/A	[34]
Alternanthera philoxeroides **(Alligator weed)**	AU	**Hepatogenous**	1998	**Cattle**	70	82.9	N/A	[35]
Ammi majus (Bishop's weed)	US	primary	1978	Sheep	N/A	N/A	N/A	[36]
Biserrula pelecinus **vars. Casbah and Mauro** (Biserrula)	AU	Primary	2015	Sheep	167	100	N/A	[37]
B. pelecinus **cv Casbah** (Biserrula)	AU	Primary	2013	Sheep	120	25.0	N/A	[38]
Brachiaria brizantha (Palisade grass)	BR	Hepatogenous	2010	Sheep	80	16.3	12.5	[39]
B. brizantha (Palisade grass)	BR	Hepatogenous	2010	Sheep	113	43.4	35.4	[39]
Brachiaria decumbens **(Signal grass)**	CO	**Hepatogenous**	2015	**Cattle**	N/A	N/A	N/A	[20]
B. decumbens (Signal grass)	MY	Hepatogenous	1985	Goat	12	25.0	N/A	[40]
B. decumbens (Signal grass)	BR	Hepatogenous	2003	Goat	118	14.4	N/A	[41]
B. decumbens (Signal grass)	BR	Hepatogenous	2003	llama	1	N/A	N/A	[42]
B. decumbens (Signal grass)	BR	Hepatogenous	2009	Sheep	24	45.8	N/A	[21]
B. decumbens (Signal grass)	NG	Hepatogenous	1982	Sheep	36	N/A	38.9	[43]
B. decumbens (Signal grass)	BR	Hepatogenous	2009	Buffalo	17	52.9	N/A	[44]
B. decumbens (Signal grass)	BR	Hepatogenous	2003	Sheep	28	25.0	21.4	[45]
Brassica rapa **(Turnip)**	NZ	**Hepatogenous**	2014	**Cattle**	N/A	N/A	N/A	[46]
Copper	BR	Hepatogenous	2006	Buffalo	4	100.0	N/A	[47]
Dicrocoelium dendriticum	UK	Hepatogenous	2011	Sheep	65	49.2	3.1	[48]
Enterolobium contortiliquum **(Pacara earpod tree)**	BR	**Hepatogenous**	2014	**Cattle**	62	22.6	3.2	[49]
E. contortiliquum **(Pacara earpod tree)**	BR	**Hepatogenous**	2002	**Cattle**	N/A	N/A	N/A	[50]
Flood damaged alfalfa hay (predominantly *Medicago sativa*)	US	**Hepatogenous**	1957	**Cattle**	40	N/A	N/A	[51]
Foxtail-or- chardgrass mixture cut hay	US	**Hepatogenous**	1991	**Cattle**	8	100.0	12.5	[16]
Froelichia humboldtiana **(Ervanço)**	BR	**Primary**	2014	**Cattle**	70	38.6	N/A	[52]
F. humboldtiana (Ervanço)	BR	Primary	2014	Donkey	N/A	N/A	N/A	[53]
F. humboldtiana (Ervanço)	BR	Primary	2014	Goat	15	100.0	N/A	[54]
F. humboldtiana (Ervanço)	BR	Primary	2014	Mule	N/A	N/A	N/A	[53]
F. humboldtiana (Ervanço)	BR	Primary	2006	Sheep	5	100.0	N/A	[55]
F. humboldtiana (Ervanço)	BR	Primary	2014	Horse	N/A	N/A	N/A	[23] [53]
Heliotropium europaeum (Common heliotrope)	AU	Hepatogenous	1985	Sheep	120	4.2	N/A	[13]
Heracleum sphondylium (Hogweed)	UK	Primary	2010	Horse	N/A	N/A	N/A	[56]
Hypericum erectum **(St. John's wort)**	JP	**Primary**	1980	**Cattle**	5	100.0	N/A	[57]
H. erectum (St. John's wort)	TN	Primary	1999	Horse	34	N/A	N/A	[58]
Jamesdicksonia dactylidis	AU	**Hepatogenous**	2017	**Cattle**	678	24.3	2.8	[59]
Liver fluke	AT	**Hepatogenous**	2003	**Cattle**	N/A	N/A	N/A	[60]
Lotus corniculatus (Birdsfoot trefoil)	NZ	Primary	1992	Sheep	40	7.5	N/A	[61]
L. corniculatus (Birdsfoot trefoil)	NZ	Primary	1993	Sheep	56	26.8	N/A	[61]
L. corniculatus (Birdsfoot trefoil)	NZ	Primary	1991	Sheep	30	33.3	N/A	[61]
Malachra fasciata (Malachra)	BR	Primary	2016	Sheep	3	100	N/A	[62]
Medicago sativa (Lucerne, alfafa)	ES	Primary	2004	Sheep	1850	24.3	N/A	[63]
Microcystis aeruginosa	SA	**Hepatogenous**	1993	**Cattle**	N/A	N/A	N/A	[64]
Myoporum insulare **(Common boobialla)**	AU	**Hepatogenous**	1980	**Cattle**	177	14.1	6.2	[65]

(*Continued*)

(Chen *et al.*, 2019)

Appendix 4

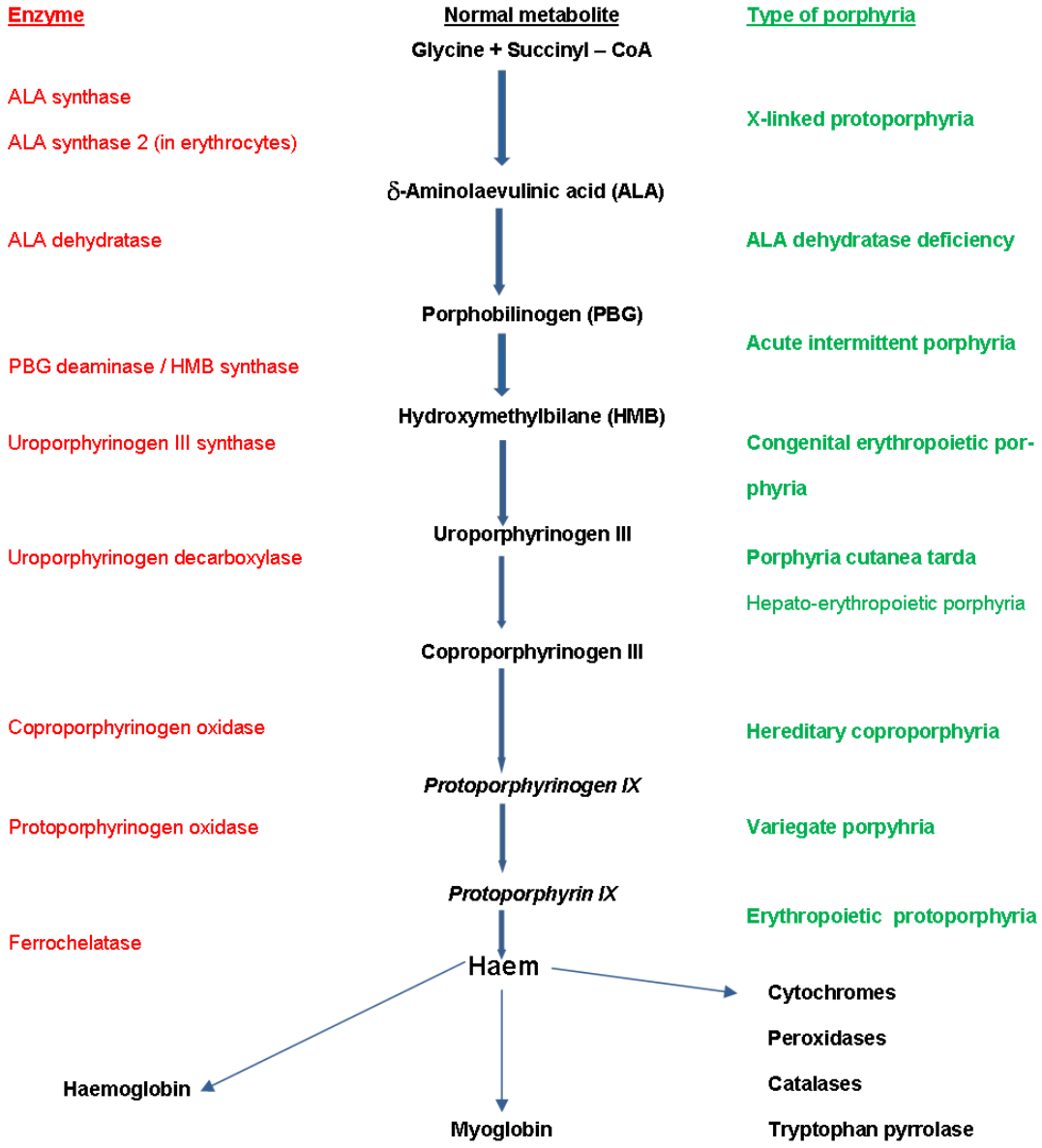
Table 1. (Continued)

Aetiological agent	Country	Type of photosensitisation	Year	Species	Flock/Herd size	Morbidity (%)	Mortality (%)	Reference
Narthecium ossifragum (Bog asphodel)	NO	Hepatogenous	1999	Sheep	165	9.7	N/A	[66]
N. ossifragum (Bog asphodel)	NO	Hepatogenous	1990	Sheep	28	17.9	N/A	[18]
Nodularia spumigena	SA	Hepatogenous	1993	Cattle	N/A	N/A	N/A	[64]
N. spumigena	SA	Hepatogenous	1993	Sheep	N/A	N/A	N/A	[64]
Pangola grass	TW	Hepatogenous	1978	Cattle	8428	4.9	1.4	[67]
Panicum coloratum (Klein grass)	US	Hepatogenous	1987	Sheep	24	100.0	N/A	[68]
P. coloratum (Klein grass)	AU	Hepatogenous	1989	Sheep	2000	N/A	N/A	[69]
Panicum dichotomiflorum (Fall panicum)	BR	Hepatogenous	2009	Sheep	365	22.2	10.7	[70]
P. dichotomiflorum (Fall panicum)	US	Hepatogenous	2006	Horse	14	100.0	35.7	[71]
Panicum miliaceum (Proso millet)	IR	Hepatogenous	2008	Sheep	10	10.0	N/A	[72]
P. miliaceum (Proso millet)	IR	Hepatogenous	2008	Sheep	253	32.8	16.2	[73]
Panicum schinzii (Sweet grass)	AU	Hepatogenous	1986	Sheep	200	30.0	20.0	[74]
P. schinzii (Sweet grass)	AU	Hepatogenous	1991	Sheep	70	28.6	21.4	[75]
P. schinzii (Sweet grass)	AU	Hepatogenous	1986	Sheep	200	25.0	15.0	[74]
Panicum virgatum (Switch grass)	US	Hepatogenous	1991	Sheep	104	16.4	N/A	[76]
Persicaria lapathifolia (Pale knotweed) *and P. orientalis*	AU	Hepatogenous	2009	Cattle	50	4.0	20.0	[15]
Petroselinum crispum (Parsley)	UK	Hepatogenous	1997	Pig	18	88.9	N/A	[77]
Phytolacca octandra (Inkweed)	NZ	Hepatogenous	2006	Cattle	400	5.0	N/A	[78]
Pithomyces chartarum	NZ	Hepatogenous	1997	Fallow deer	20	60.0	30.0	[79]
P. chartarum	AU	Hepatogenous	1985	Sheep	200	15.0	N/A	[80]
P. chartarum	SA	Hepatogenous	1970	Sheep	N/A	N/A	N/A	[81]
P. chartarum	TR	Hepatogenous	2005	Sheep	1000	2.2	N/A	[82]
P. chartarum	US	Hepatogenous	1994	Sheep	N/A	N/A	N/A	[83]
P. chartarum	AU	Hepatogenous	1978	Sheep	22698	10.7	4.1	[84]
Polygonum lapathifolium (Pale persicaria)	AU	Hepatogenous	1986	Cattle	380	N/A	1.6	[85]
Porphyrins	UK	CEP	2008	Cattle	N/A	N/A	N/A	[86]
Porphyrins	UK	CEP	1956	Cattle	N/A	N/A	N/A	[87]
Protoporphyrin	FR	CEPP	1991	Cattle	N/A	N/A	N/A	[88]
Protoporphyrin	IE	CEPP	2015	Cattle	20	5.0	N/A	[89]
Protoporphyrin	NZ	CEPP	2011	Cattle	N/A	N/A	N/A	[90]
Protoporphyrin	UK	CEPP	2000	Cattle	20	5.0	N/A	[91]
Protoporphyrin	UK	CEPP	2013	Cattle	26	7.7	N/A	[92]
Protoporphyrin	US	CEPP	1999	Cattle	70	1.4	N/A	[93]
Senecio brasiliensis (Flor-das-almas)	BR	Hepatogenous	2013	Cattle	162	51.2	N/A	[94]
Senecio spp	BR	Hepatogenous	2014	Sheep	860	0.9	1.2	[95]
Tribulus terrestris (Goat's-head, puncture vine)	AU	Hepatogenous	1983	Goat	35	17.1	5.7	[96]
T. terrestris (Goat's-head, puncture vine)	AU	Hepatogenous	1982	Sheep	1200	20.8	14.7	[11]
T. terrestris (Goat's-head, puncture vine)	IR	Hepatogenous	1998	Sheep	11	100.0	N/A	[22]
T. terrestris (Goat's-head, puncture vine)	IR	Hepatogenous	1975	Sheep	700	8.5	4.3	[97]
T. terrestris (Goat's-head, puncture vine)	TR	Hepatogenous	2013	Sheep	24	100.0	N/A	[98]
T. terrestris (Goat's-head, puncture vine)	AU	Hepatogenous	1982	Sheep	190	36.8	24.2	[11]
Trifolium alexandrinum (Berseem)	IN	Hepatogenous	2013	Cattle	N/A	N/A	N/A	[99]
Unidentified	MY	Hepatogenous	2012	Cattle	N/A	N/A	N/A	[100]
Unidentified	AU	Hepatogenous	1985	Sheep	35	42.9	28.6	[74]
Unidentified	AU	Hepatogenous	1986	Sheep	100	7.0	N/A	[74]

(*Continued*)

(Chen *et al.*, 2019)

Appendix 5



(Dawe, 2017)

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Állatorvostudományi Egyetem
Állatgyógyászati Állomány-egészségtani
Tanszék és Mobilklinika
1078 Budapest, István u. 2.
1400 Budapest, Pf.: 2.

Departement

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Name: Deirdre Cremins

Contact information (e-mail): deirdrecremins@hotmail.com

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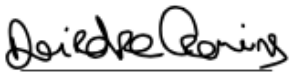

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