A NEW CASE OF TOXIN INDUCED HEPATIC PROBLEMS?

Labeo, Ph. Pitel, V. Kientz F., Duncombe, 14053 Caen cedex 4

Irish Equine Center, U. Fogarty, Johnstown, Co. Kildare, Irlande

Reverdy Nutrition Équine, C. David, 50520 Juvigny-le-Tertre

RESPE, C. Marcillaud Pitel, 3 rue Mandela, 14280 Saint Contest

After detecting a hepatic abnormality, usually by measuring specific liver enzymes, determining the aetiology is always complex. Mostly, after having eliminated metabolic and infectious (virus, bacteria or parasites) possibilities, dietary causes, and therefore the toxin (poison) one is regularly explored. In the latter case, the problem is mostly double: sampling methodology and analytical aspects. Over the last few years, the arrival on the market of new analytical technologies have permitted new approaches to screening rather than targeted ones.

This presentation intends to expose the discovery of a molecule responsible for liver problems and related to the presence of red fescue.

I. BACKGROUND

In a training yard (Y1) certain horses showed elevated liver enzyme counts from 2010 onwards. Using diet analysis and implementing exclusion diets, the hay was incriminated. Forage analysis excluded mycotoxins as a cause. Despite having removed a number of ragwort, meadow vetchling and vetch plants growing on part of the fields the problem reappeared at the end of 2012.

At the same period a similar problem occurred on a large Normandy stud farm (F2) notably in yearlings, although they were without symptoms. A number of investigations on feed and possible infections were undertaken to find a cause and did not allow a precise aetiology even if there was a leaning towards the feed.

At the beginning of the summer in 2013, a botanical inventory on F2 showed a wide scale presence of a dense grass sward unusual in the floristic of Lower Normandy stud farms. This same grass was found in a more diffuse way in one of the fields used to make hay for Y1. This grass was identified as being red fescue. Fescues are known to host endophytic fungi. Fescues like their endophytics can produce different toxins notably alkaloids. The best known are responsible for problems during gestation and the neonatal period in the equine species.

II. METHOD

After drying and grinding the hay and fresh grasses were extracted before being injected into the LC/MS-QTOF. The serums were themselves directly injected. This equipment measures the time of flight of molecules and from this time, calculates the precise mass and deduces the empirical formula.

Comparisons between the formulas and the mass of ragwort alkaloids as well as the main alkaloids of fescue endophytics (loline, hordenine…) were carried out.

Mycological searches were also carried out on samples of red fescue. The molecule was also looked for in colonies of fungi.

III. RESULTS AND FOLLOWING UP OF CASES

In our case, analysis using the LC/MS-QTOF permitted the identification of a molecule with the formula $C_{18}H_{28}N_2O_6$. This formula corresponds to 130 molecules identified in different data bases but none of them suggested being potentially pathogenic. The empirical formula perhaps corresponding to an alkaloid formula.

This molecule is almost systematically found in samples of red fescue collected from the two structures as well as in the serum of horses having grazed in paddocks contaminated by red fescue or in hay made in these paddocks. $C_{18}H_{28}N_2O_6$ was also found to be present in hay bought from a merchant in order to complete the requirements at F2.

Control feeds based on red fescue and exclusion diets also served to show a rapid elevation in liver enzymes (particularly GLDH), as well as the appearance (2 to 5 days), and the rapid disappearance of the molecule if the contaminated feed was withdrawn. Liver biopsies were carried out for histological analysis. No profound and irreversible lesions could be detected. Test groups of yearlings were also formed in order to evaluate all the paddocks, at the same time a floristic inventory of the fields took place.

The majority of red fescue samples led to the identification of fungi. However from isolated colonies the molecule was unable to be identified. This does not allow to exclude that one or more of these fungi are responsible for the production of the toxin. Indeed in-vitro growth conditions are in no way comparable to conditions on the ground (hydrometric, temperature, stress…). It's interesting to note that most of the time the molecule became practically undetectable after the first frosts (to be confirmed).

At Y1 the situation was managed by reorganising hay making with an agreement with the farmer who made it. Only forage produced in fields without fescue was used for feeding horses.

On F2, every animal was transferred to another farm abroad. A number of trials in the fields and paddocks were carried out : grazing by cattle, by sheep, spreading Bordeaux mixture. None of them eliminated the molecule nor the presence of fescue. In order to avoid massive use of herbicide on the stud farm, mechanical treatment - discs, burying, then reseeding with seed mixes tested as being exempt of the molecule – was undertaken. Tests with the yearling groups were done and showed effectiveness. The paddock borders were also treated in the same manner.

In addition to these two cases, the incriminating molecule has been found in a number of structures in the West of France, belonging to both the one-horse owner and professionals. It was notably detected in forage fed to animals in a pre-training centre. The latter showing very elevated liver enzyme levels upon their arrival at their training yard. It was proved that the hay at the pre-training centre came from a forage merchant who had previously supplied F2 and in which the molecule had been identified.

TO SUM UP

When a number of liver problems are detected on the same yard, looking for the toxic molecule related to red fescue must now become part of the differential diagnosis. It can complement searches for other toxic molecules such as alkaloids from ragwort or the mycotoxins traditionally looked for. Normalisation of hepatic parameters, notably GLDH is rapid after with drawing the incriminated forage (fresh grass, hay…). This normalisation confirms itself by recent group winners who were amongst horses bred and raised during the acute crisis at F2.

Work carried out, notably at F2 showed that amongst the non-chemical treatments, only mechanical treatment and reseeding with seeds controlled as being exempt from endophytics including the unidentified molecule eliminated the molecule from fields and paddocks (biochemical tests and tests using batches of yearlings).