Understanding the Root

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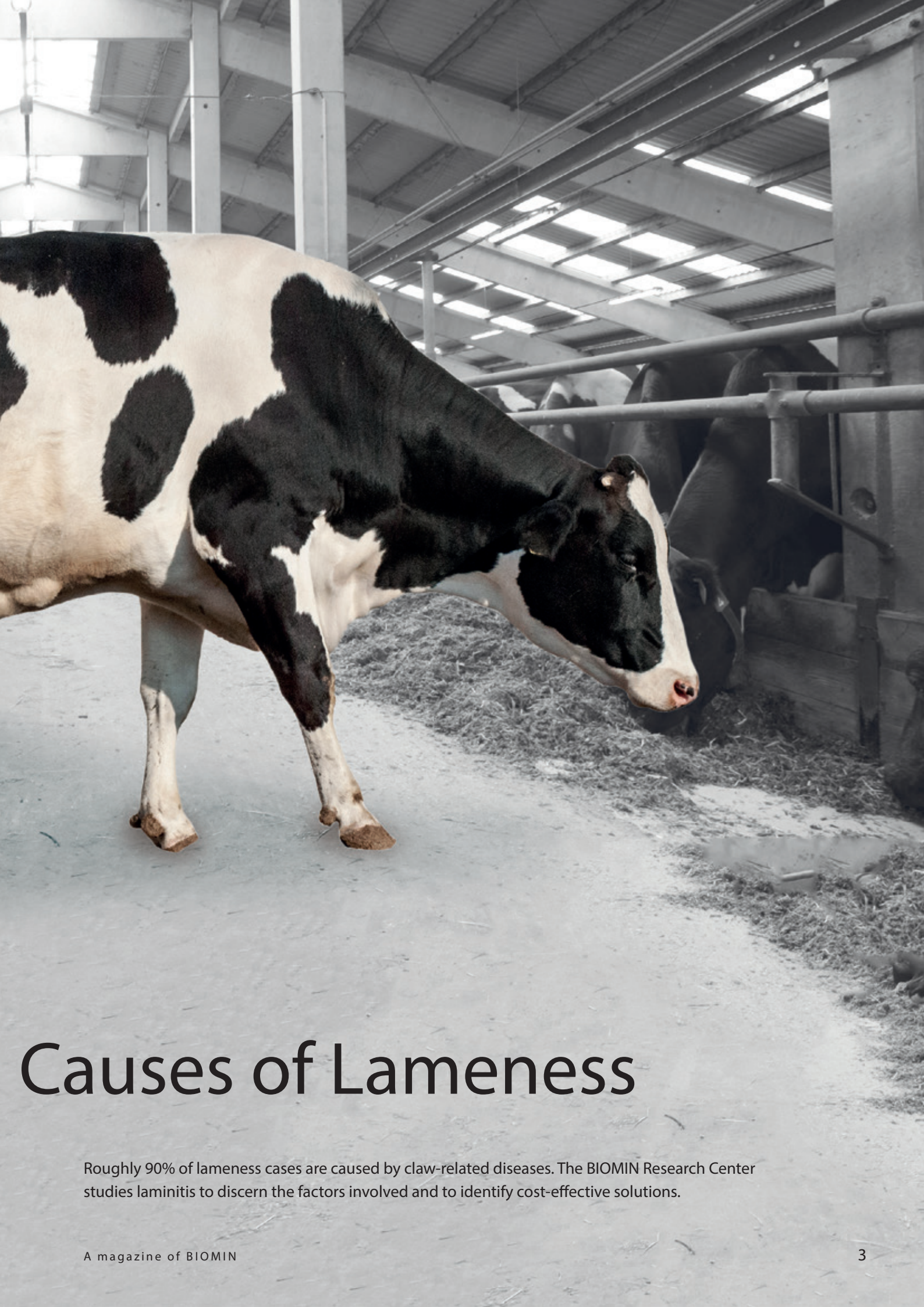


Causes of Lameness

Roughly 90% of lameness cases are caused by claw-related diseases. The BIOMIN Research Center studies laminitis to discern the factors involved and to identify cost-effective solutions.

Endotoxins and fumonisins have the capability

After mastitis and fertility problems, lameness is the third most important source of economic losses in dairy production. Laminitis –a disease characterized by inflammation of the lamella tissue of the claw– leads to pain for animals, greater susceptibility to other diseases, higher treatment costs, lower performance and lameness.

However, the pathology of laminitis is still not fully understood. As it is a multifactorial disease, several substances and toxins such as endotoxins are discussed as possible trigger factors. Endotoxins, or lipopolysaccharides, are cell wall components of Gram-negative bacteria that are released when bacteria multiply, lyse and die.

During bacterial imbalance in the rumen, endotoxin concentration can rapidly increase. Once endotoxins have reached the blood flow through an impaired rumen barrier, they can reach the hoof tissue and have a negative impact on tissue integrity through different mechanisms, e.g. inflammation, in which specific cells activate cytokines (e.g. TNF-alpha, IL-6) and enzymes (e.g. matrix metalloproteinases) that weaken or destroy the tissue.

In severe cases, the connective tissue of the pedal bone completely separates from the lamellar tissue—

Figure 2. Explants of about 5x5 mm contain all three important layers of the hoof/claw: connective tissue to the pedal bone (1), lamellae tissue (2) and the inner hoof/claw wall (3).

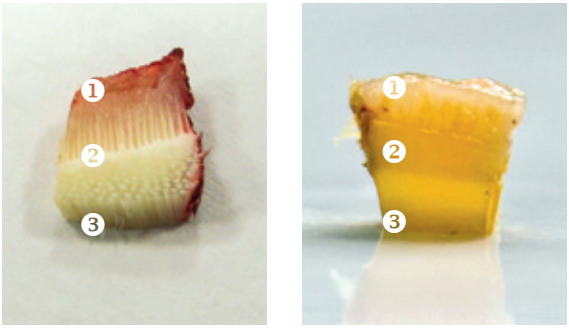


Figure 1. Overview of the dissection process of the equine hoof or bovine claw.

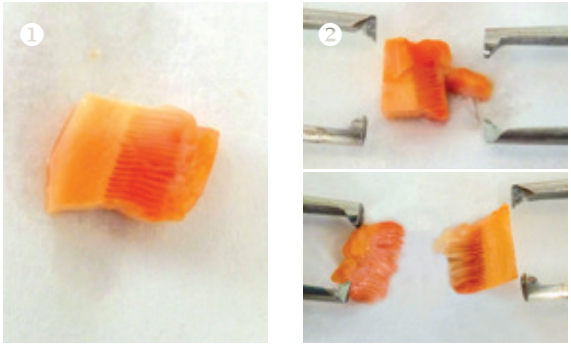
Equine hoof

Bovine claw

to aggravate the severity of laminitis.

Figure 3. Explants are cultured in cell culture plates (24 wells) with culture medium and potential trigger factors.

Figure 4. Manual separation test of explants with forceps: (1) intact explant, (2) separated explants.



causing the rotation and sinking of the pedal bone. This irreversible process causes considerable pain.

Benefits of the *ex vivo/in vitro* laminitis model

Animal experiments are associated with pain and stress for the animal. In addition, they are very time and cost extensive. An *ex vivo/in vitro* model offers an alternative way to investigate the role of different trigger factors during laminitis without the need for animal trials, and at lower costs.

From a research perspective, it allows scientists to evaluate different toxins and concentrations in one trial and to evaluate the interaction of different toxins and other trigger factors.

Furthermore, this model mimics the *in vivo* situation quite well, as all affected tissue layers are involved. The practical application aspect is also important, as it allows for the evaluation of nutrition strategies to prevent laminitis.

How the *ex vivo/in vitro* laminitis model works

Equine hooves and bovine claws are obtained from a local abattoir (horse hooves commonly serve as a model for ruminants in scientific research). The tissue is put on ice and quickly transported to the lab. Next, hooves or claws are carefully washed with a disinfectant. Initial steps of the dissection process (*Figure 1*) are performed with a band saw. Then, surgical instruments are used to prepare explants containing three layers: the inner hoof/claw wall, epidermal lamellae and connective tissue (*Figure 2*).

Finally, prepared explants are cultivated in 24 well plates (1 explant/well) with 1 mL cultivation medium at 37 °C and 5% CO_2 (*Figure 3*). During incubation potential trigger factors, e.g. toxins, can be added to each explant. Explants cultivated in medium only served as negative control.

Two different methods can be applied to evaluate if tested trigger factors have an influence on the tissue:

1. **Evaluation, if explants separate**
   Lamellar separation is tested by fixing the hoof/claw wall and the connective tissue into forceps. Explants are scored as separated, if lamellar is separated from the connective tissue or lamellar are completely destroyed; and scored intact, if not (*Figure 4*).

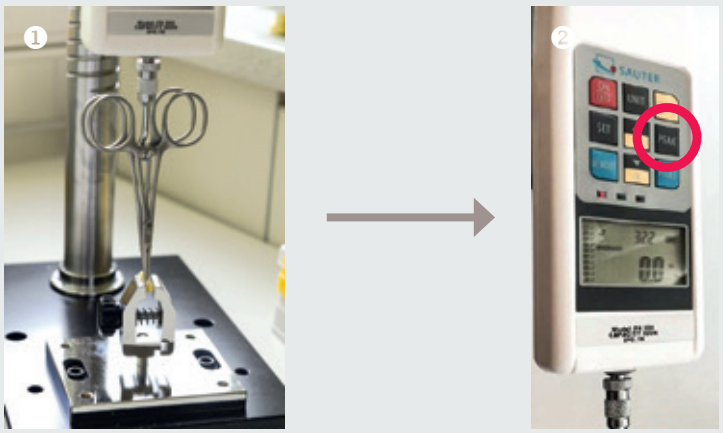
2. **Evaluation of force, which is needed to separate explants**
   The explants are fixed to a calibrated force transducer,

Table 1. Recent BIOMIN Research Center findings on laminitis.

Species	Tested toxins	Effects	Reference
Horse	Endotoxins	Significantly increased number of separated explants after 24 and 48 hours	Reisinger *et al*. 2014
Horse	Endotoxins	Significantly decreased separation force after 24 hours	Reisinger *et al*. 2015
Cow	Endotoxins	Significantly decreased separation force after 24 hours	Reisinger *et al*. 2017
Horse	Mycotoxin Fumonisin	Significantly decreased separation force after 24 hours Increase of fumonisin biomarkers (Sphinganine to sphingosine ratio)	Reisinger *et al*. 2016

Source: BIOMIN

Figure 5. Evaluation of separation force of explants. (1) explants are fixed to a force transducer, (2) maximal force is recorded, which is needed to separate explants.



and the force required for explant separation is measured (*Figure 5*).

Recent results

Recent scientific papers have shown that endotoxins and fumonisins have the capability to aggravate the severity of laminitis (*Table 1*).

Prevention tips

Our understanding of the causes of laminitis continue to advance. Here are several steps you can take to reduce the risk of laminitis in your herds:

- Appropriate feeding management: avoid excessive amounts of carbohydrates
- Proper and sufficient bedding material
- Good hygiene management
- Regular hoof/claw trimming
- Mineral supplementation
- Proper mycotoxin risk management
- Endotoxin prevention and counteracting strategies e.g. binding and bioprotection