



**One Health**  
Student Conference  
USAMV București



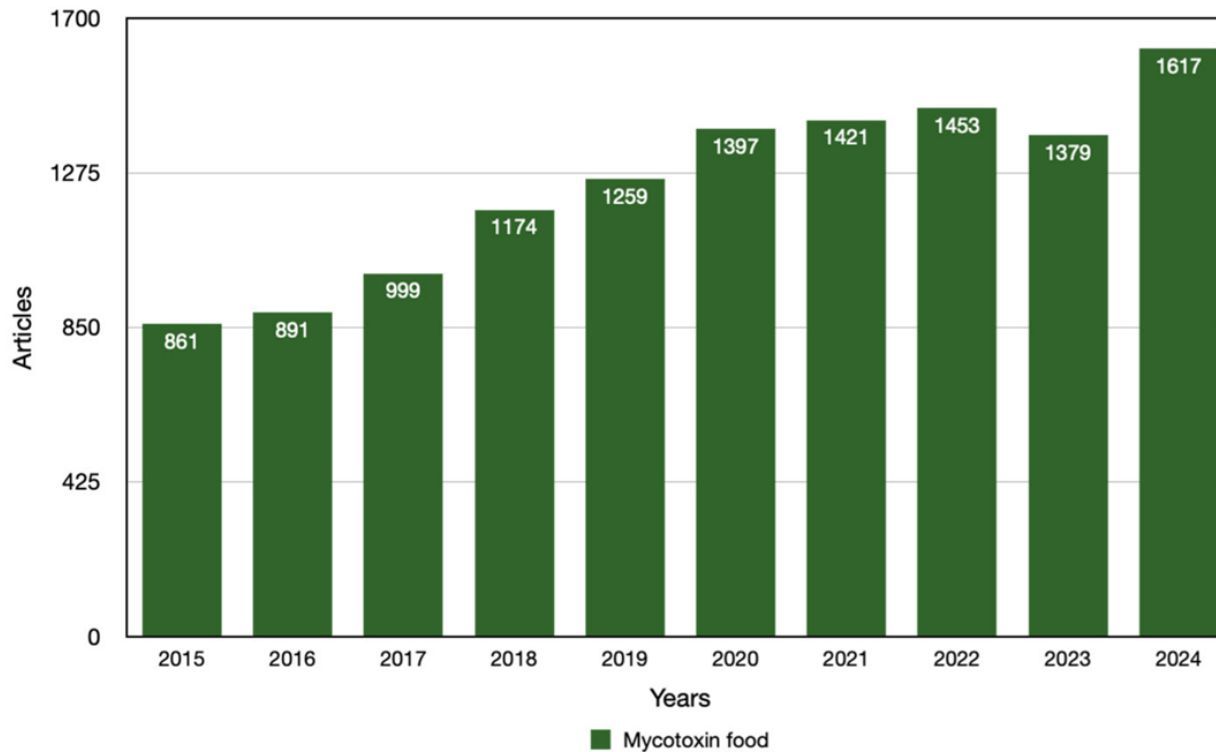
# **MILK SAFETY UNDER EMERGING MYCOTOXIN THREATS: CURRENT KNOWLEDGE AND FUTURE CHALLENGES FROM ONE HEALTH PERSPECTIVE**

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December 3-5, 2025, București



# Why mycotoxins?



Articles published per year between 2015 and 2024, focusing on mycotoxins in food, found on PubMed using the keywords “mycotoxin food”

*Di Salvo et al. (2025): Mycotoxins in Ready-to-Eat Foods: Regulatory Challenges and Modern Detection Methods, Toxics*



# What are mycotoxins?

Toxic secondary metabolites produced by fungi. • Found in:

*Aspergillus*



*Fusarium*



*Penicillium*



**Stable!** – they resist heat and many food processing methods.



➤ Grains



➤ Silage



➤ Animal feed

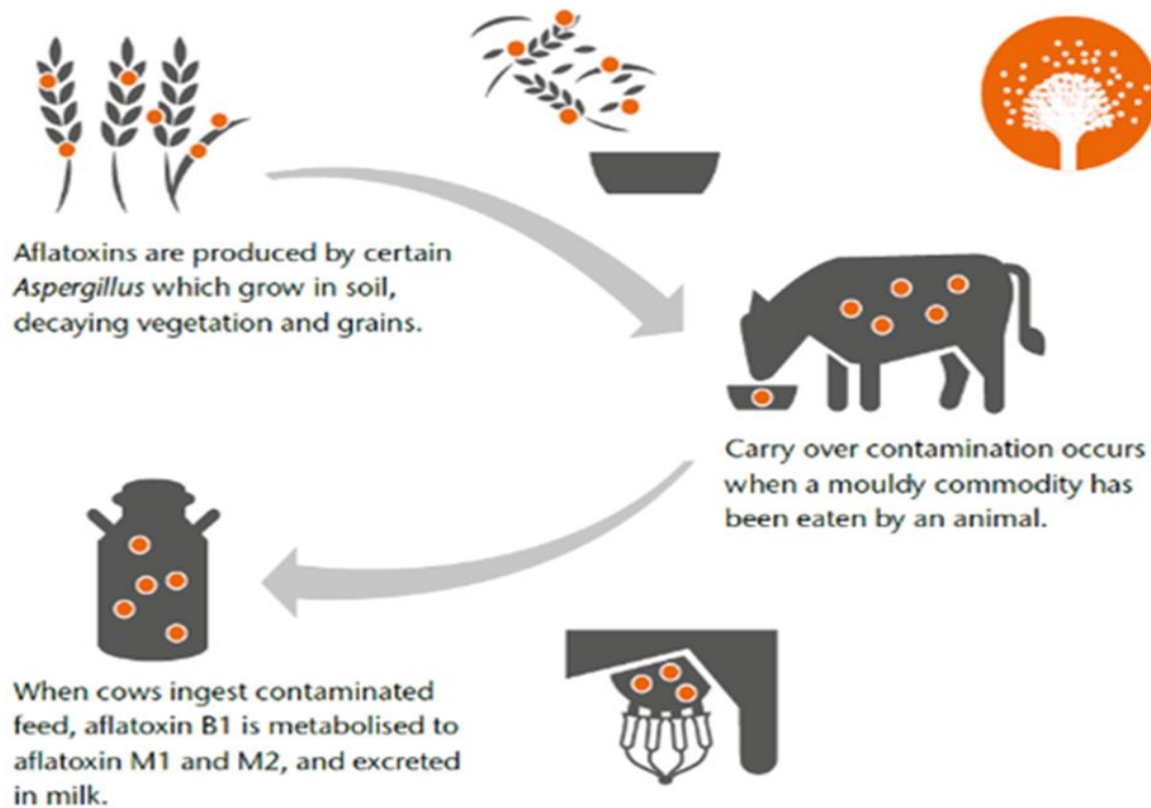


<b>Regulated</b> ( <u>afatoxins</u> , ochratoxin A, Zearalenone, DON (deoxynivalenol), T-2, HT-2, fumonisins...)
<b>Non-regulated</b> (roquefortine C, etc.)
<b>Emerging</b> (BEA, ENNs, etc.)



# Pathway of Mycotoxin Contamination in Milk

## Transfer of aflatoxin from feed to milk

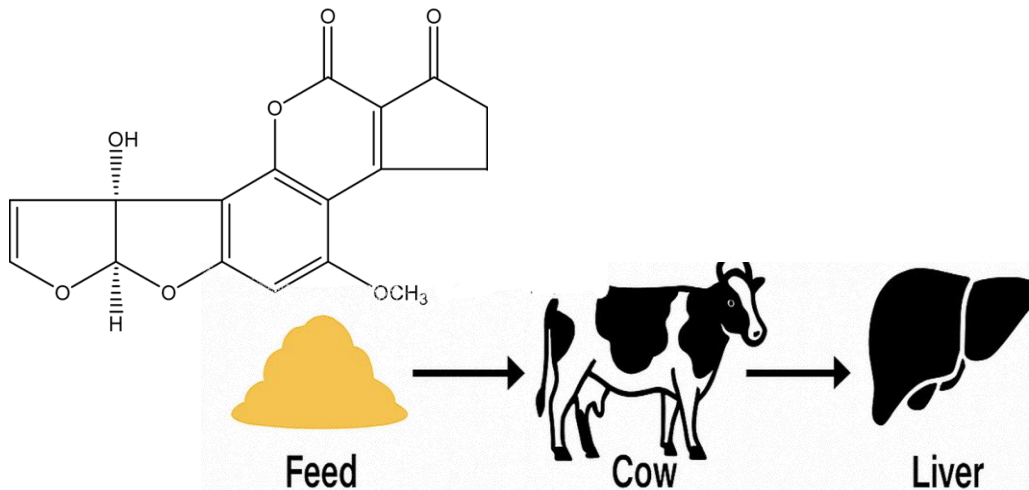




# Aflatoxin M1 – the Most Studied Mycotoxin in Milk

## Aflatoxin M1 (AFM1)

- Metabolite of Aflatoxin B1 – appears in milk after ingestion of contaminated feed
- Produced by *Aspergillus flavus* and *A. parasiticus*
- Highly stable – survives pasteurization and processing
- Classified as **Group 1 carcinogen** (IARC)
- Strictly regulated in milk



### Regulatory Limits for AFM<sub>1</sub> (EC No 1881/2006)

Milk Category	Maximum Level of AFM <sub>1</sub>
Raw milk	0.05 µg/kg
Heat-treated milk	0.05 µg/kg
Milk intended for manufacture of dairy products	0.05 µg/kg
Infant formulae	0.025 µg/kg
Follow-on formulae	0.025 µg/kg



# Emerging Mycotoxins

## Mycotoxins Relevant for Milk Safety

- Common in feed components (maize silage, cereals, concentrates)
  - Fumonisin (FBs)
  - Ochratoxin A (OTA)
  - Deoxynivalenol (DON)
  - Zearalenone (ZEA)
  - Citrinin (CIT)

- Increasingly detected in animal feeds
- Not routinely monitored
  - Beauvericin (BEA)
  - Enniatins (ENNs)
  - Moniliformin (MON)
  - Tenuazonic acid (TEA)
- Key concerns:

- Insufficiently characterized toxicological profiles
- Limited data on:
  - Occurrence patterns
  - Co-occurrence with regulated mycotoxins
  - Human health relevance

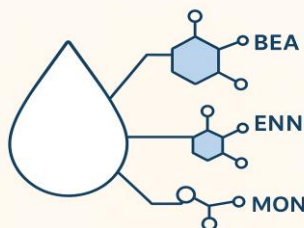
### Regulated

(aflatoxins, ochratoxin A, Zearalenone, DON (deoxynivalenol), T-2, HT-2, fumonisins...)

### Non-regulated

(roquefortine C, etc.)

**Emerging** (BEA, ENNs, etc.)



**Emerging Mycotoxins**  
NEW & UNDER-RESEARCHED  
CONTAMINANTS



## Materials and methods

- Identification of scientific publications on regulated and emerging mycotoxins in milk and dairy production systems
- Focus period: 2010–2025
- Databases searched: Scopus, PubMed, Google Scholar, Web of Science, ScienceDirect
- Authoritative sources: EFSA – risk assessments & scientific opinions  
FAO – food safety and agricultural guidance  
WHO – public health frameworks  
IARC – carcinogenicity classifications

### Scope of the Review:

- Peer-reviewed articles
- Review papers
- Official reports & regulatory documents
- Data relevant to human, animal, and environmental health within the One Health concept



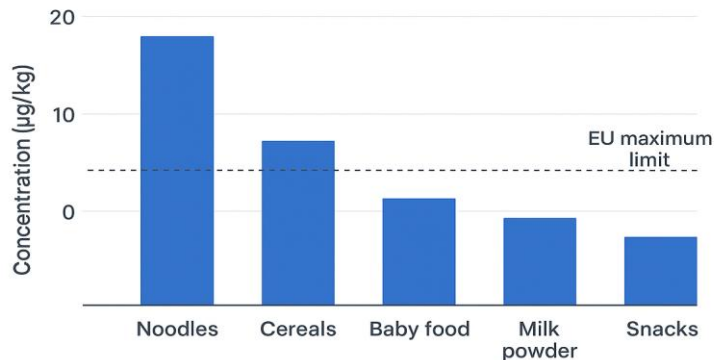


# High Prevalence of Mycotoxins in Foods and Milk: Risks for Infants

- *Flores-Flores & González-Peñas (2017)*: Approximately 10% of worldwide milk samples contain AFM<sub>1</sub> in levels above the maximum set by the EC
- *Mushtaq et al. (2012)*: Aflatoxins detected in all tested RTE foods
  - All contaminated samples exceeded EU maximum limits



**High Prevalence of Mycotoxins in Foods and Milk:  
Risks for Infants**



All contaminated samples exceeded EU limits (Mushtaq et al., 2012)

Country / Region	Maximum Level for AFM <sub>1</sub> in Milk (µg/kg)
EU	0.05
USA	0.5
Serbia	0.25
China	0.5
Canada	0.05
India	0.5

Early-life exposure → strict monitoring of infant foods and dairy ingredients



# Multi-mycotoxin exposure in feed → carryover of toxins and metabolites into milk

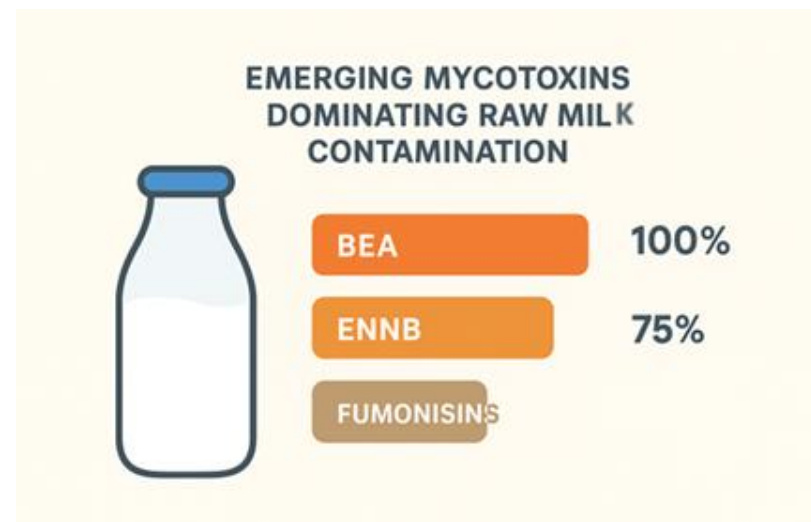
Mycotoxins in milk and their effects in humans and animals (*Ushkalov et al., 2020*)

Aflatoxin B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> , M <sub>1</sub>	<ul style="list-style-type: none"><li>· Genotoxic carcinogenic compounds. In the liver, B<sub>1</sub> is metabolized to M<sub>1</sub> by cytochrome P450.</li><li>· B<sub>1</sub> and M<sub>1</sub> are found in milk</li></ul>
Deoxynivalenol (DON)	<ul style="list-style-type: none"><li>· Inhibition of protein synthesis and redox reactions in mitochondria</li><li>· Contained in milk in low concentrations</li></ul>
Ochratoxin (A, B, C)	<ul style="list-style-type: none"><li>· Inhibits protein synthesis, carcinogen. Capable of accumulation in tissues</li><li>· Excreted with milk</li></ul>
Zearalenone	<ul style="list-style-type: none"><li>· Binds to estrogen receptors. Mycoestrogen</li><li>· Contained in milk in low concentrations</li></ul>
Gliotoxin	<ul style="list-style-type: none"><li>· Cytotoxic immunosuppressant. Used as a fungicide and antibiotic</li><li>· Contained in milk in low concentrations</li></ul>
T-2 toxin	<ul style="list-style-type: none"><li>· Inhibition of protein and nucleic acid synthesis, oxidative cell damage and apoptosis. Genotoxic and cytotoxic effects</li><li>· Contained in milk in low concentrations</li></ul>
Fumonisin (B <sub>1</sub> , B <sub>2</sub> )	<ul style="list-style-type: none"><li>· Similar in structure to sphingolipids, embedded in biological membranes, changing their structure</li><li>· Contained in milk in low concentrations</li></ul>



# Emerging Mycotoxins Dominating Raw Milk Contamination

- Recent evidence shows a shift in contamination patterns
- *Leite et al. (2023)*: 20 raw milk samples (Portugal, bulk cooling tanks)
  - ✓ BEA, ENNB, Fumonisin
- No aflatoxins detected, including AFM<sub>1</sub>



*Leite et al. (2023): Regulated and Emerging Mycotoxins in Bulk Raw Milk: What is the Human Risk?*



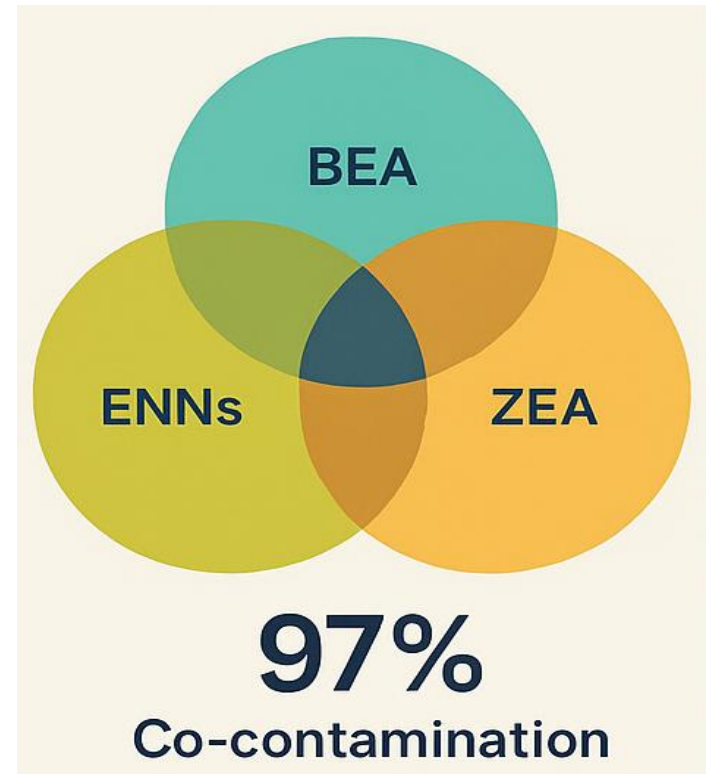
# Emerging Mycotoxins Dominating Raw Milk Contamination

*Leite et al. (2023b)*: Large-scale screening of 100 raw bulk milk samples

- 97% of samples contained  $\geq 1$  mycotoxin
- Only 3 samples were completely uncontaminated
- AFM<sub>1</sub> rare

Most frequent mycotoxins - primarily produced by *Aspergillus* & *Fusarium* spp.:

- Beauvericin (BEA)
- Enniatins (ENNs)
- Zearalenone (ZEA)





# Mycotoxin Occurrence in Raw Milk

*González-Jartín et al. (2021)*

Samples: 31 raw milk samples

Mycotoxins tested: 40

Detected: T-2 toxin, Roquefortine C, ENNs, BEA

High prevalence: ENNs  $\approx$  68%  
BEA  $\approx$  90%

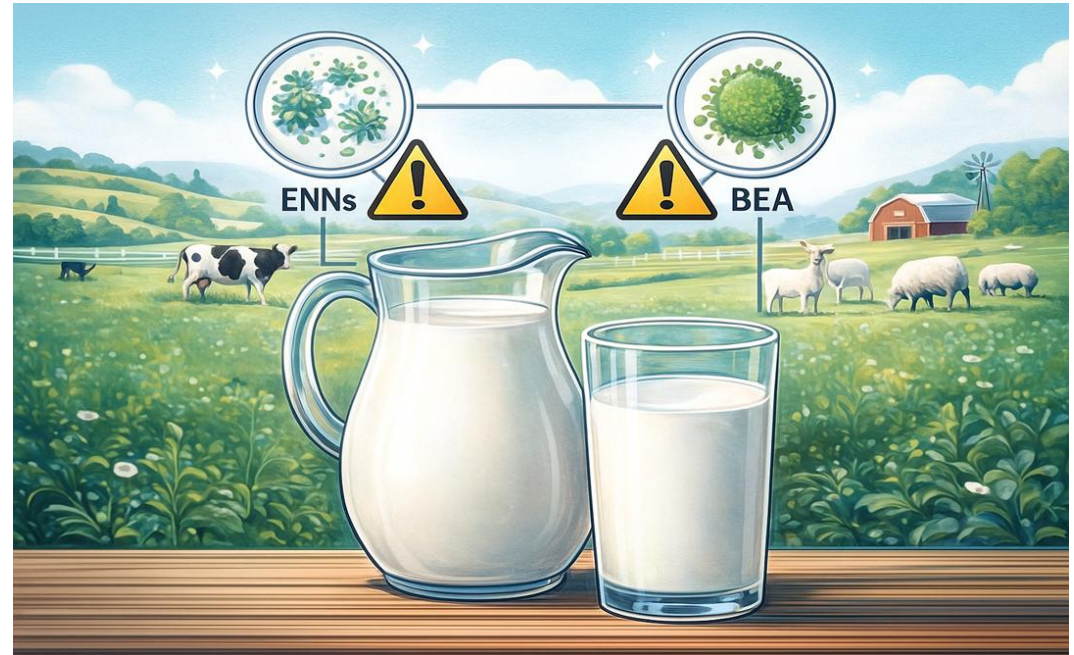
*Akinyemi et al. (2022)*

Samples: 135 milk samples (3 species)

Detected: BEA & ENNB

Prevalence: BEA: 87.4% overall;  
(100% in 23 cow milk samples!)

ENNB: 48.2%



**Clear emerging dairy safety concern!**



# Key Insights on Mycotoxin Contamination in Milk

## 1. Emerging Mycotoxins

- ✓ BEA & ENNs frequently detected in raw milk
- ✓ Often co-exist with regulated mycotoxins → complex exposure patterns

## 2. AFM<sub>1</sub> Status

- ✓ Not always the dominant contaminant
- ✓ Some studies report low or undetectable AFM<sub>1</sub>, while emerging mycotoxins are widespread

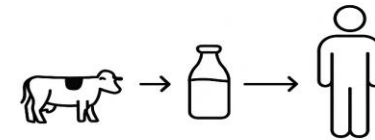
## 3. Co-Contamination

- ✓ Highly prevalent
- ✓ Complicates risk assessment due to synergistic/additive effects
- ✓ Particular concern for infants and children





# One Health Perspective & Health Risks



- ✓ Milk contamination arises from feed quality, environmental factors, and fungal ecology
- ✓ Frequent co-occurrence of multiple mycotoxins in feed & milk
- ✓ Human health risks: immunosuppression, GI disorders, hepatotoxicity, nephrotoxicity, carcinogenic potential
- ✓ Particular concern for vulnerable populations





# Emerging Mycotoxins: Knowledge Gaps and Actions



**What is the key goal from a One Health perspective?**

# Thank you for your attention!

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