

Are Aflatoxins a Cause of Parenchymal Liver Disease in Humans?

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Abstract

In 2014 a hepatitis research team noticed that an unusually high amount of people in eastern Ethiopia had chronic liver disease (CLD) and cirrhosis. Aflatoxins, a group of mycotoxins produced by *Aspergillus* fungi, were suspected as the culprit. Aflatoxins attack certain agricultural products grown or stored under certain conditions and are known hepatocarcinogens. This literature review looks at the evidence for and against aflatoxins as a cause of parenchymal liver disease in humans. In January 2015 the most common medical databases were searched using search terms including aflatoxin, liver disease and cirrhosis, with 68 articles identified as relevant. Further reading led to 17 articles being selected, with two of these identified via the reference lists of the other articles. Three articles were about previous incidents of acute liver failure, where aflatoxins were proven to be the culprit. Seven looked into CLD in humans, where about half of the articles supported that aflatoxins could cause CLD, while a few showed some evidence against it. The last seven articles were various animal studies, mostly using birds, where a few showed cirrhotic changes and several showed various histopathological parenchymal liver changes, though different animals seemed to react differently to aflatoxin exposure. From the articles identified in this literature review, aflatoxins have been shown to cause acute parenchymal liver disease in humans and may cause chronic parenchymal liver disease in humans. However, little research has been done in this field, and further studies are required to draw a conclusion.

Contents

1	Introduction	1
1.1	Objective.....	1
1.2	Background.....	1
1.2.1	What are aflatoxins?.....	1
1.2.2	Historical note	2
1.2.3	Aflatoxin metabolism.....	2
1.2.4	Carcinogenicity	3
1.2.5	Parenchymal liver disease	4
2	Method	5
3	Results	6
3.1	Aflatoxins and parenchymal liver disease in humans.....	6
3.1.1	Acute parenchymal liver disease.....	6
3.1.2	Chronic parenchymal liver disease.....	8
3.2	Aflatoxins and parenchymal liver disease in animals.....	11
4	Discussion	16
4.1	Aflatoxins and acute parenchymal liver disease in humans	16
4.2	Aflatoxins and chronic parenchymal liver disease in humans	17
4.3	Aflatoxins and parenchymal liver disease in animals.....	18
5	Conclusion.....	20
	References	21

1 Introduction

1.1 Objective

A group of Norwegian researchers working on a hepatitis project in Ethiopia in 2014, observed unusually high levels of chronic liver disease (CLD) and cirrhosis in the eastern parts of the country, with no known cause. Aflatoxins were hypothesised as a possible cause, as this is a known cause of hepatocellular carcinoma as well as acute hepatitis. Several other substances that can cause acute hepatitis are known to cause CLD in lower doses. Aflatoxins are well controlled in most parts of the world, but in rural Africa it is both common to produce your own food, thus avoiding the control mechanisms regulating aflatoxins in commercially sold foods, and store it in non-ideal conditions.

The objective of this literature review was therefore to review existing research for evidence regarding aflatoxins as a cause of parenchymal liver disease in humans.

1.2 Background

1.2.1 What are aflatoxins?

Aflatoxins are a group of mycotoxins produced mainly by the common fungi *Aspergillus flavus* and *Aspergillus parasiticus*, but also by other *Aspergillus* species. *A. flavus* only produces B aflatoxins, while *A. parasiticus* produces both B and G aflatoxins. Several other aflatoxins derive from these, including M aflatoxins and aflatoxicol. Aflatoxin B₁ is known to be the most toxic and Aflatoxin G₁ the second most toxic (4-6).

The *Aspergillus* fungi attack certain agricultural products such as rice, soybeans, peanuts, almonds, pistachios, hazelnuts, cereals and maize (7). The fungi are found worldwide, but as their growth and toxin production are determined by humidity and moisture content of plants, they are mainly a problem in resource-poor tropical and subtropical countries (8). They are particularly problematic in drought-stressed maize and groundnuts (1). Maize, peanuts and cottonseed may have high levels of aflatoxins as a result of being invaded by the fungi before harvest. In other crops, the aflatoxins develop during storage in high temperatures and humidity, which can be avoided by good storage practices and rapid drying (5).

In Sub-Saharan Africa food is stored in various ways, including delaying of harvest and storage in underground pits. Maize and other grains are commonly dried in the fields, and thereafter on mats outside or on floors, before being stored in granaries. Improper drying and storage is a risk factor for aflatoxins (1).

Aflatoxins are regulated in more than 75 countries, with industrialised countries generally accepting lower levels

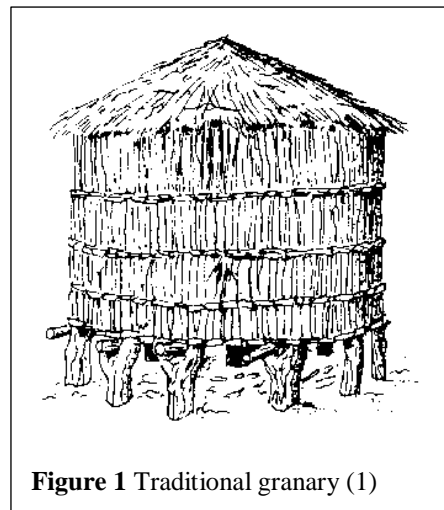


Figure 1 Traditional granary (1)

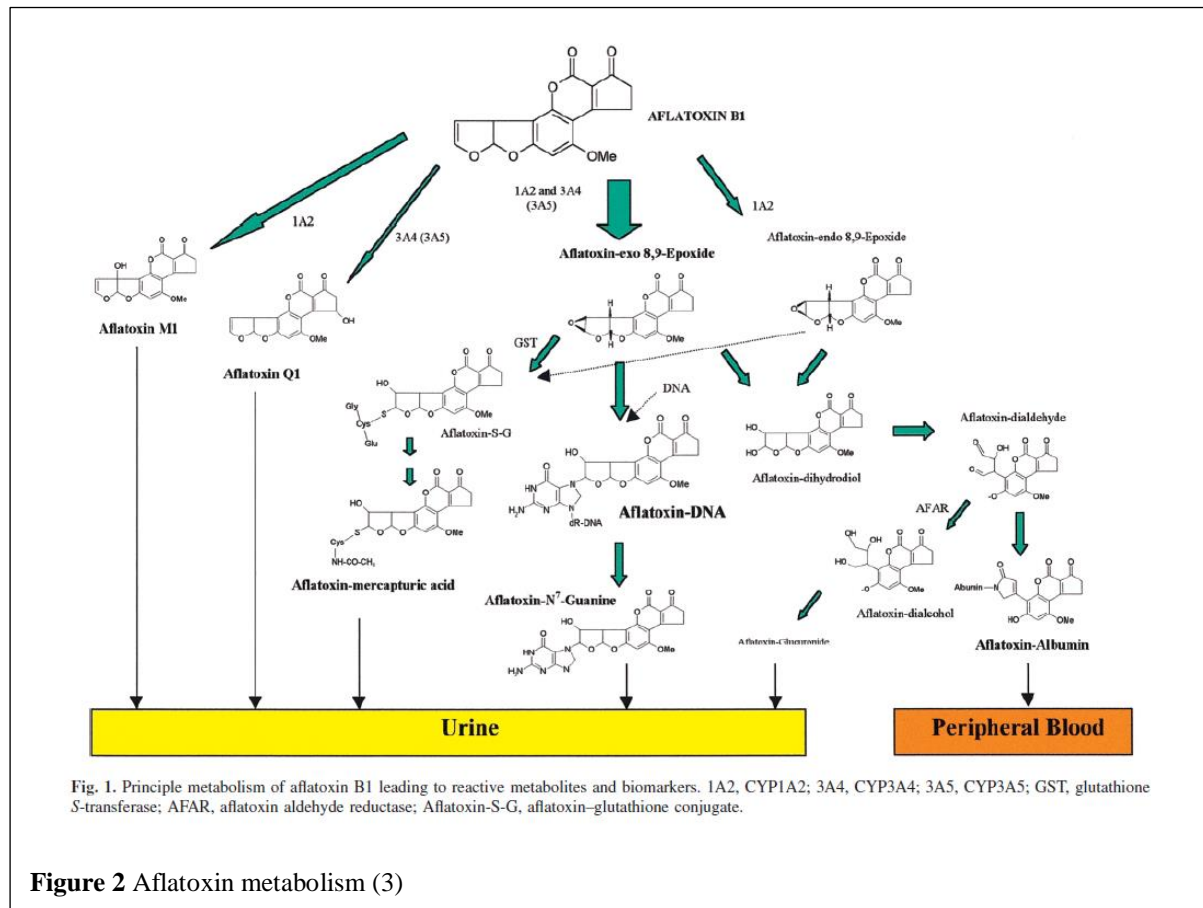
than developing countries. As examples, the acceptance level for aflatoxin in foods is 5µg/kg in Sweden, 10µg/kg in Japan and 30µg/kg in Brazil (9).

1.2.2 Historical note

In England during the spring and summer of 1960 thousands of turkeys were affected by an unknown disease, named “Turkey X” disease. Lethality was high and the terminal stages were characterised by neurologic symptoms and coma. The most significant pathological changes were found in the liver and several causative agents were proposed. It was discovered that they had all been fed diets containing peanut meal from South America, and research showed that the toxicity was related to the amount of *Aspergillus flavus* present. The toxic agent was thus named “aflatoxin”, from *A. flavus* toxin.

These findings stimulated further research, and it was soon clear that the contaminated ground nuts could induce acute liver disease in ducklings and liver cancer in rats. Methods from these investigations could then help to detect and quantify aflatoxins in foodstuffs. Observational epidemiologic studies from the 1960s through the 1980s also used these methods to find an association between aflatoxin ingestion and the incidence of hepatocellular carcinoma (10, 11).

1.2.3 Aflatoxin metabolism



When they first are ingested, the aflatoxins themselves are thought to be harmless. They only become toxic when metabolised, which happens for the most part by members of the cytochrome P450 superfamily in the liver.

In phase I of the metabolism, the parent molecule (here aflatoxin B₁, known as AFB₁) gets oxidised mainly by CYP3A4 and mainly into the highly reactive AFB₁-8,9-exo-epoxide. This is the toxic stage of the aflatoxin.

Phase II of the metabolism sees the epoxide detoxified through a number of pathways. The main route is glutathione S-transferase-mediated conjugation with reduced glutathione, to form epoxide-glutathione S-transferase conjugates.

It is thereafter converted to an aflatoxin-mercapturic acid conjugate and excreted in the urine.

Accumulation of the aflatoxin metabolites from the phase I metabolism will happen if either the ingested quantity of aflatoxin exceeds the capacity of the phase II pathways, or if the activity of the phase II pathway is decreased. The AFB₁-8,9-exo-epoxide will then bind with high affinity to guanine bases in cellular DNA and form AFB₁-N⁷-guanine adducts, which in turn can give rise to guanine-to-thymine transversions in DNA. The epoxide may also form products that react with serum albumin to form long-lived lysine adducts (8, 10).

1.2.4 Carcinogenicity

Cancer of the liver was the second most common cause of cancer death worldwide in 2014, responsible for 745,000 deaths (12). Hepatocellular carcinoma (HCC) makes up 75-90% of the primary liver cancers and is responsible for most of these deaths. The highest prevalence of HCC is found in China, South-East Asia, sub-Saharan Africa and North Africa (13). Common etiologic factors are chronic hepatitis B virus infection (HBV), chronic hepatitis C virus infection (HCV), heavy alcohol consumption and high dietary exposure to aflatoxins. The difference in prevalence throughout the world reflects the different prevalences of these etiologic factors (14).

Aflatoxins themselves are regarded by the International Agency for Research on Cancer (IARC) as a Group-1 agent, meaning they are proven to be carcinogenic to humans. This conclusion was made after two independent cohort studies in Shanghai and Taiwan showed statistically significant effects of exposure to aflatoxin on the development of HCC (5). Furthermore, the previously mentioned transversion of guanine to thymine in DNA caused by the AFB₁-N⁷-guanine adduct have been shown in clinical studies to selectively target the third base position of codon 249, of the human tumour suppressor gene TP53, and cause arginine to be exchanged to serine. This is a known mutational hotspot for HCC and is detected in over 50% of HCC in high-incidence areas of China and Africa, as well as being rare in HCC in areas with low or no dietary intake of aflatoxins (15, 16).

Aflatoxins are also shown to have a synergistic effect with HBV in causing HCC. Cohort studies have shown a 5-10 fold increase in risk of HCC when both factors are present, compared to HBV or AFB₁ alone (16). Several mechanisms have been thought to cause this synergism. Chronic HBV infection may induce cytochrome P450s and thereby increase the amount of the mutagenic AFB₁-8,9-epoxide. HBV encodes an HBVx protein that inhibits nuclear excision repair, which is normally responsible for removing aflatoxin-DNA adducts. This will increase the frequency of TP53 mutations and lead to uncontrolled cell cycling. The

generation of oxygen and nitrogen reactive species seen with chronic HBV infections also increases the likelihood of the TP53 mutations (4).

1.2.5 Parenchymal liver disease

Hepatitis is hepatocyte injury due to inflammation, and will result in scarring if it becomes chronic. Causes include toxins, viral infection and obesity. It can involve the entire liver or patches, and if the causal agent is a toxin, the periportal areas are often affected first. The hepatocyte injury in acute hepatitis can either result in swelling followed by necrosis, or apoptosis of the hepatocytes. There can also be a degree of cholestasis. If severe enough, acute hepatitis can lead to massive hepatic necrosis, liver failure and death (17, 18).

Fibrosis is a response to any hepatocellular injury, where chronic wound healing activates hepatic stellate cells, which transform into myofibroblasts. This leads to increased secretion, decreased degradation and accumulation of extracellular matrix (ECM). In addition, the sinusoidal endothelial cells lose their fenestrations and the hepatocytes their microvilli, leading to hepatocyte dysfunction and tissue hypoxia (13). Fibrosis is a dynamic process, and if the cause of the injury is eliminated, restoration of liver function is possible (17).

Cirrhosis is a diffuse process involving most or all of the liver and is characterised by fibrosis and the conversion of normal liver architecture into structurally abnormal regenerative nodules (17). The amount of extra connective tissue created by fibrosis will eventually leave little or no space for hepatocyte regeneration, and the damaged hepatocytes are permanently replaced by connective tissue. This leads to chronic liver failure (18).

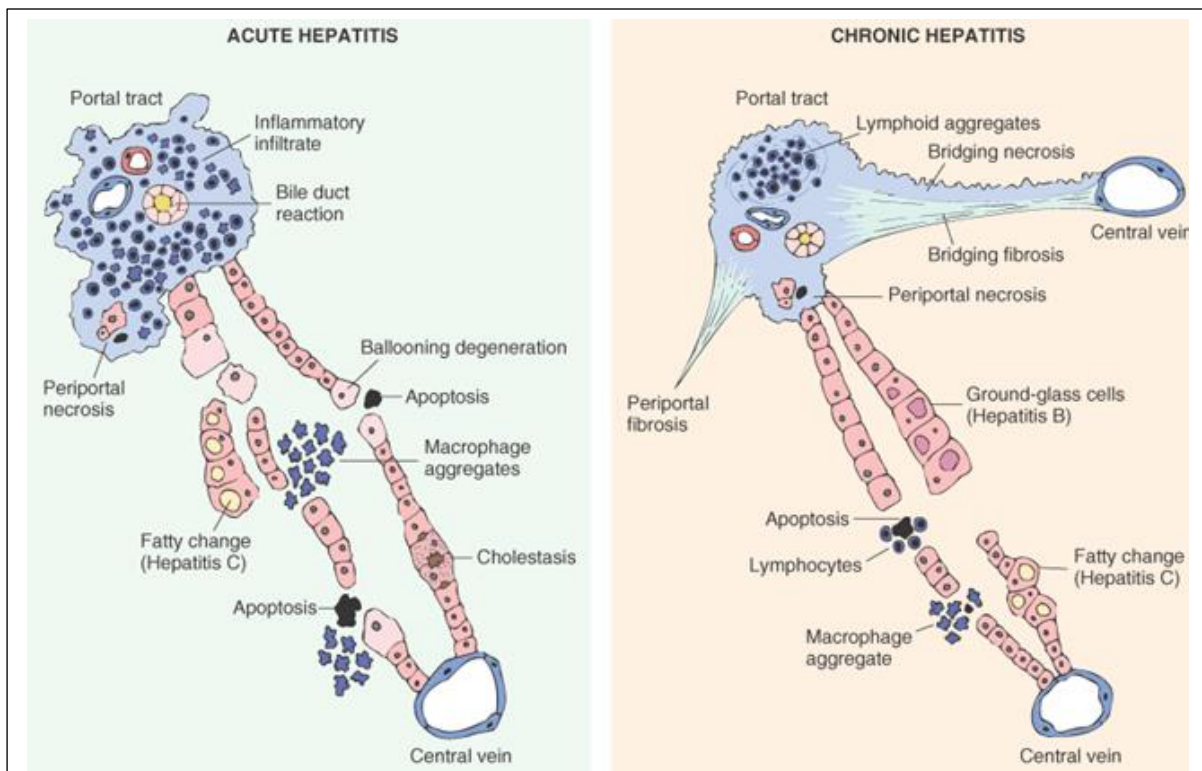


Figure 3 Morphologic features of acute and chronic hepatitis (2)

2 Method

In January 2015 the following databases were searched for literature: PubMed, McMaster Plus, Cochrane, Ovid and Embase. In PubMed the following MeSH terms were used “(aflatoxin OR aspergillus flavus OR aspergillus parasiticus) AND (liver cirrhosis OR parenchymal liver disease)” which gave 266 articles. In McMaster Plus the search words “aflatoxin(s) and liver cirrhosis” and “aflatoxin and liver disease” were used and gave non-relevant UpToDate hits as well as the same hits on PubMed found in the previous search. Cochrane Library was searched for “aflatoxin” in title, abstract and keywords, giving 40 articles. In Ovid the search words “aflatoxin and liver disease” gave 75 articles, while in Embase both “aflatoxin and liver disease” and the standardised terms “aflatoxin/b/b1/b2/g1/g2/m1 AND liver disease” were used, amounting to 292 and 147 articles. Most of the different databases showed the same articles. The chosen articles were limited to those written in English. This only excluded two articles, which were in Romanian.

Based on titles, the 68 articles most relevant to the objective were chosen. All abstracts were then read, or the full article where an abstract was not available, which led to 25 articles being shortlisted and carefully read through. The references of these articles were checked, and two extra articles added (Ortatatli and Coulter). A total of 17 articles were chosen for analysis based on their relevance to the objective.

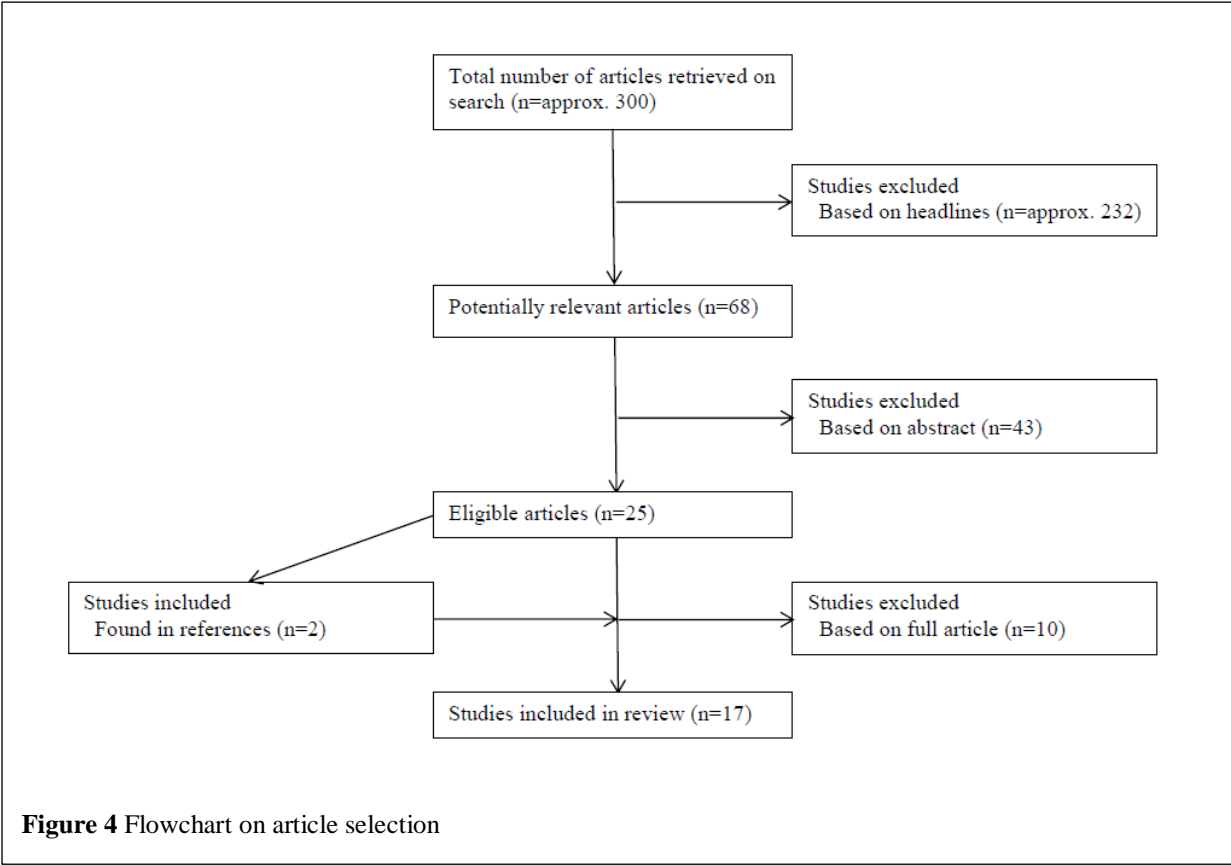


Figure 4 Flowchart on article selection

3 Results

3.1 Aflatoxins and parenchymal liver disease in humans

3.1.1 Acute parenchymal liver disease

In 1975, Krishnamachari et al published an article in The Lancet reporting an outbreak of hepatitis in Western India. The outbreak lasted for two months, starting in October 1974, and affected 397 patients from 200 villages in two adjacent states, leaving 106 dead. The areas of the outbreak were normally drought-stricken, with a poor nutrition level in the general population, and had a few weeks prior to the outbreak been affected by unseasonal rains. The syndrome seen was characterised by jaundice, rapid onset of signs of portal hypertension and a high lethality rate. Death was usually sudden and often preceded by massive gastrointestinal bleeding, while hepatic coma was rare. Males were affected twice as often as women, and no infants, nor contacts of the deceased, were affected. A large number of dogs who shared the household food developed ascites, icterus and died during the outbreak, while no animals who did not share the household food were affected. Five maize samples were collected from affected households and all showed *Aspergillus flavus* and aflatoxin levels between 6.25 and 15.6 ppm, meaning people living there would have consumed 2-6 mg of aflatoxin daily for several weeks. One liver was obtained from necroscopy and showed extensive bile duct proliferation and periductal fibrosis. No aflatoxin B₁ was detected in extracts from the liver. Blood samples were obtained from 7 cases and urine samples from 7 cases, with only two of the blood samples and none of the urine samples showing detectable levels of aflatoxin B₁. They concluded that aflatoxins were very likely to be the causal agent behind the outbreak. (19)

Key points:

- India, 1974
- First known aflatoxicosis outbreak among humans.
- 397 affected, 106 died
- Syndrome of jaundice, portal hypertension and GI-bleeding.
- Aflatoxin levels in consumed maize up to 700 x today's legal limit.
- Aflatoxin concluded to be likely cause.

Another article in The Lancet was published by Ngindu et al in 1982, titled "Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya". Between March and early June of 1981, 20 patients with hepatitis were admitted to three hospitals in the Machakos district of Kenya, 12 of whom died. Initially they had mild symptoms of abdominal discomfort, anorexia, malaise and low grade fever, and sought medical attention after developing jaundice and dark urine, with a mean time from onset to hospital admission of 10.2 days. They were all managed conservatively and those who died developed hepatic failure and passed within 12 days of admission. 80% of the cases occurred in family groups, only sparing wholly breastfed infants, and the lethality in family groups was significantly higher than in the isolated cases (p=0.02). Two homesteads accounted for 12 of the admitted patients, including eight of the dead. The first homestead had dogs which were fed the same diet as the family, and died immediately before the humans got ill. Food samples

Key points:

- Kenya, 1981
- 20 admitted to hospital, 12 died
- Syndrome of jaundice, malaise and dark urine.
- Aflatoxin levels in consumed maize up to 600 x today's legal limit.
- Aflatoxin concluded to be the likely cause.

were collected from these two homesteads and compared with samples from outside the immediate epidemic area. Maize grains from the affected homesteads had 12,000 and 3200 ppb of aflatoxin B₁, while the control samples had a maximum of 500 ppb. Blood samples were collected from 10 patients and 19 contacts or neighbours, and no viral antibodies against regional diseases were detected, though three subjects were HBsAg positive. Liver samples from two children of the same family contained 39 and 89 ppb of aflatoxin B₁, and both showed marked centrilobular necrosis, slight fatty infiltration and no bile duct proliferation. It was noted that 1980 had been a very dry year in the region, which caused food shortages and most likely storage of grains which would normally have been discarded, while the rains of 1981 were heavier than normal. Aflatoxin poisoning was concluded to be the cause of the outbreak. (20)

Azziz-Baumgartner et al produced a case-control study of an acute aflatoxicosis outbreak in Kenya in 2004. The outbreak that lasted from January until June had 317 cases of acute hepatic failure and 125 deaths. Seven patients had their serum analysed for viruses known to cause hepatic disease in Kenya, with no significant findings. In 2004, the rainy season normally lasting from March through May was early and short, leading to drought and food shortage, with people storing maize in their homes rather than granaries to be sure it was not stolen. Maize from affected areas was sampled and found to have aflatoxin B₁ concentrations up to 4400 ppb. The case definition was acute jaundice of unknown origin leading to hospitalisation, during the peak of the epidemic, in the areas most affected by the outbreak. 40 cases were chosen, and matched with two controls from each case patient's village, excluding infants, amounting to 80 controls. They all received questionnaires about food intake, storage and cooking as well as illness or death of family members or pets during the exposure period. Maize samples were collected, if possible from the maize consumed during the exposure period, and blood samples were taken and analysed for aflatoxin B₁-lysine adducts and HBsAg. 11 case patients were dead at the beginning of the study, did not have blood samples taken and their questionnaires were completed by family members.

Key points:

- Kenya, 2004
- 317 cases, 125 died
- Aflatoxin levels in consumed maize up to 200 x today's legal limit.
- Case-control study, 40 cases, 80 controls.
- Strong association between acute hepatic failure and aflatoxin concentration in maize, serum B₁-albumin adducts and HBsAg titers.

Case patients were more likely than controls to be male (62.5% vs 33.8%, p=0.003). Home-grown maize kernels from case households had a significantly higher aflatoxin concentration than control households (mean 354.4 ppb vs 44.1 ppb, p=0.04). Eating home-grown maize was significantly associated with case status (adjusted OR=3.0, 95%CI 1.01-8.8), and owning "bad" home-grown maize (discoloration, unusual odour, signs of mould) was found to be a risk factor for aflatoxicosis (adjusted OR=5.9, 95%CI 1.9-18.2). Case patients who fed their dogs household food reported dog deaths more often than controls (43% vs 15%, adjusted OR=15.2, 95%CI 1.8-127.4). Having a concentration of aflatoxin B₁-lysine adducts at or above the median was a risk factor for aflatoxicosis (adjusted OR=14.8, 95%CI 3.0-72.2). Case patients had higher aflatoxin B₁-lysine adduct concentrations in serum than did controls (median 1.2 ng/mg of albumin vs 0.15 ng/mg of albumin, p<0.001). There was a positive association between concentrations of aflatoxins in home-grown maize and aflatoxin B₁-lysine adduct concentrations in serum (p<0.05). Positive HBsAg titres were found in 44% of cases and 7% of controls, and this was found to be a risk factor for acute hepatic failure (adjusted OR=9.8, 95%CI 1.5-63.1). Looking at only HBV-negative cases and controls, having aflatoxin B₁-lysine adduct concentrations at or above the median was also a risk factor for developing aflatoxicosis (95%CI 2.1-∞, p=0.004). They concluded that aflatoxin

concentrations in maize, serum aflatoxin B₁-lysine adduct concentrations and positive HBsAg titres were associated with case status. (21)

3.1.2 Chronic parenchymal liver disease

In 2008, Kuniholm, Lesi et al published an article about aflatoxin exposure and viral hepatitis in the aetiology of liver cirrhosis in the Gambia. They included 97 cirrhotic patients and 397 controls. Both groups underwent a standardised clinical examination, provided blood samples and completed an interviewer administered questionnaire. In addition, the cirrhotic group had an ultrasound examination to exclude those with space occupying hepatic lesions. The questionnaire looked into demographics, medical history, lifestyle characteristics and food consumption including groundnut consumption over the last two months, and compared this to their probable lifetime exposure.

The blood tests assessed hepatitis B and C status as well as 249^{ser} TP53 mutation. They chose the latter as a measurement of aflatoxin exposure over aflatoxin-albumin adducts or urinary adducts, as these are more likely to be affected by the severity of liver disease.

They found that aflatoxin exposure alone, both assessed by high lifetime groundnut consumption (OR = 2.8; 95% CI, 1.1-7.7) and presence of 249^{ser} TP53 mutation in plasma (OR=3.8; 95% CI, 1.5-9.6), was found to significantly increase the risk of cirrhosis. Aflatoxin and HBV exposure combined appeared to have a synergistic effect on cirrhosis, but the results did not give statistical significance (p=0.37 and 0.35). (22)

In 1971, Amla et al published an article about cirrhosis in Indian children due to aflatoxin-contaminated peanut meal. Three hospitalised children with kwashiorkor had accidentally been given peanut protein meal with aflatoxin content of 300/μg per kg. After clinically improving for 10 days, their condition deteriorated a week later, with pale stools, anorexia, abdominal distention, visible abdominal veins and hepatomegaly. All other children known to have consumed the same contaminated peanut meal were included in the study, comprising 20 children aged 1.5 – 5 years who had consumed the meal for periods varying from 5 days to 1 month. 18 had protein-calorie malnutrition, one had nephrosis and one was a normal child. They had no history of consumption of common aflatoxin contaminated foodstuffs prior to this. Most of the children attended clinic weekly and had liver biopsies taken every 2-3 months. They had no control group, but had been giving 3500 children with protein-calorie malnutrition the same treatment for a decade prior to this, and 70% of the children had between 1 and 3 years of follow up with examinations and biopsies. In these children, they had seen livers recover within 6 weeks, and in no cases had they seen cirrhosis of the liver clinically, nor on the biopsy material.

After 2 months, 16 of the children who had consumed the aflatoxin-contaminated peanut meal had a firm hepatomegaly. After 1 year, the number was reduced to 12 and after 2 years only six had firm enlarged livers. However, three children died of hepatic coma after 1.5 years. Over a 1 year period, the liver biopsies in general showed a gradual transition from an increased central and periportal fatty infiltration to formation of fatty cysts, fibrosis and

Key points:

- The Gambia, 1997 – 2001.
- 97 cases, 397 controls
- 249^{ser} TP53 mutation used to assess aflatoxin exposure.
- Both high lifetime groundnut consumption and 249^{ser} TP53 mutation significantly increased the risk of cirrhosis.

Key points:

- India, 1969
- 20 children affected, most already suffered from kwashiorkor, 3 died.
- Aflatoxin-contaminated peanut protein meal.
- Six still had hepatomegaly after 2 years. Biopsies showed fibrosis and cirrhosis.

cirrhosis. Two of the children, one being the nephrotic child, who had only been exposed to the contaminated meal for a week, had only mild histopathological changes that regressed over a 1 year period. Children who had consumed the contaminated meal for a month showed moderately severe histopathological changes that progressed throughout the observation period. (23)

A study looking into aflatoxin exposure and hepatitis C virus in advanced liver disease was conducted in hepatitis C virus-endemic areas in Taiwan in 2002 by Chen et al. It included 314 thrombocytopenic adults ≥ 40 years of age who had their sera tested for HBsAg, anti-HCV, α -feto-protein (AFP) and aflatoxin-albumin adducts and underwent an upper abdominal ultrasound. The ultrasound rated severity of liver parenchyma disease on a scale from 0 to 6, where 5 or 6 were defined as liver cirrhosis. A modified simple scoring system was used, assessing change of angle and edge of the liver, coarseness of liver parenchyma and splenomegaly. The aflatoxin exposure was expressed as nanograms of aflatoxin-albumin per milligram of albumin.

Key points:

- Taiwan, 2002
- HCV-endemic area.
- 314 thrombocytopenic adults underwent blood tests and ultrasound.
- High levels of aflatoxin-albumin adducts were a statistically significant risk factor for advanced liver disease in the whole group, but not in the anti-HCV-negative group.

In the entire group, high levels of aflatoxin-albumin/albumin was found to be an independent risk factor for advanced liver disease, where >8 versus ≤ 8 AFB-albumin/albumin had an OR of 2.29, 95% CI = 1.23-4.27 and $p = 0.009$. A high level of aflatoxin-albumin/albumin was found to be a determinant of advanced liver disease in HCV patients, where >8 versus ≤ 8 AFB-albumin/albumin had an OR of 2.09, 95% CI = 1.09-4.0 and $p = 0.026$. However, aflatoxin levels were not associated with advanced liver disease in those without HCV infection. Aflatoxin-albumin/albumin levels increased significantly with ultrasonographic parenchyma scores, with $p < 0.001$. (15)

Coulter et al conducted clinical studies on aflatoxins and kwashiorkor in Sudanese children from '81 to '83, and published the results in 1986. The study was conducted at two hospitals in Khartoum, one of which was a major children's referral hospital and the other a university hospital. Children with kwashiorkor were index cases and were compared with children of similar age with marasmus, marasmic kwashiorkor and controls. A full medical and nutritional history was taken, and they went through clinical examination, anthropometric measurements, blood tests and urine collection by a clean catch method. The sera and urines were analysed for aflatoxins B₁, B₂, G₁, G₂, M₁, M₂ and aflatoxicol.

Key points:

- Sudan, 1981 – 1983
- 404 malnourished children, 180 controls
- Children with kwashiorkor had a significantly higher positivity rate of aflatoxins in sera compared to controls.
- Aflatoxicol was more often found in children with kwashiorkor than the other groups.

Of all the children, 141 had kwashiorkor, 111 marasmic kwashiorkor, 152 marasmus and 180 were controls. Their median age was 18.1 months, 17.9 months, 18.1 months and 17.5 months respectively. Regarding aflatoxins in sera, there was a significant difference in positivity rates between the groups ($p < 0.05$), with the largest difference being between the kwashiorkor group (37.7%) and the controls (21.3%). When looking at the mean concentration of aflatoxins in the aflatoxin positive sera, they found values of 154 pg/ml in the kwashiorkor group and values ranging from 77 to 82 pg/ml in the other groups, but the difference between the groups was not significant ($p = 0.43$). As for urine aflatoxin levels, no significant difference was found between the positivity rates of different groups, nor in mean concentration in positive samples. A major difference was found when looking at the individual aflatoxins and

metabolites, where aflatoxicol was found in 11.6% of the kwashiorkor group, 6.1% of the marasmic kwashiorkor group, 0.7% of the marasmic group and in no control cases. This difference in prevalence between the groups was highly significant, with $p < 0.0001$. They also found that the ratio of AFB₁ to AFM₁ was higher in the kwashiorkor group than in the control group, in both serum and urine. They concluded that this provided evidence of differences in the aflatoxin metabolism of children with kwashiorkor compared to children with other types of malnutrition and normally nourished children. (24)

As a follow up to Coulter's article, De Vries and Lamplugh published an article about aflatoxins in liver biopsies from Kenya in 1989. They had 15 needle liver biopsies, nine of them from living subjects and six post-mortem. From living patients, the biopsies were taken for diagnostic purposes, and they had consent to obtain two samples from each procedure. One sample was for histology while the other was stored and shipped to Liverpool for aflatoxin analysis. They also took blood and urine samples from each patient which were also sent for aflatoxin analysis. The post-mortem patients had liver biopsies taken the day they died and the blood was taken from the heart. Histology was not available for this group of patients. Aflatoxin concentrations were all determined by high performance liquid chromatography using fluorescence detection.

Key points:

- Kenya, published 1989
- Nine live patients with different liver disorders, six post-mortem patients dead from various disorders.
- Aflatoxins detected in blood and urine of all three cases of cirrhosis, but only in one liver specimen.
- Possible role for aflatoxins in pathogenesis of liver cirrhosis, but too few patients to conclude.

Of the nine living subjects, five had HCC, two cirrhosis and two had hepatitis. Of the five HCC patients, aflatoxin was found in the liver specimens of three and in no blood samples. Only four urine samples were taken, and one showed aflatoxins. Of the patients with cirrhosis and hepatitis, aflatoxin was found in none of the liver samples, but in the two cirrhotic patients, aflatoxins were found in both blood and urine. One of the hepatitis patients had aflatoxin in the blood, but none in the urine.

In the post-mortem group, the patients had died from stomach cancer, cirrhosis, cerebral malaria, marasmic kwashiorkor, peritonitis and pregnancy complications. Aflatoxins were found in liver biopsies from four of the patients, including those with stomach cancer, cirrhosis, marasmic kwashiorkor and peritonitis. Three had blood samples positive for aflatoxins, more specifically the patients with stomach cancer, cirrhosis and marasmic kwashiorkor. Their findings supported a possible role for aflatoxins in the pathogenesis of cirrhosis, but they could not draw any conclusions due to their small number of patients. (6)

In an article from 2008, Hosny et al performed a study on 249^{ser} TP53 mutations in circulating free DNA of Egyptian patients with HCC versus CLD. 249^{ser} was chosen due to it being described as a hallmark of mutagenesis by aflatoxin. 255 serum samples were collected and included 76 cases of HCC, 110 cases of CLD, sero-positive for either HCV (74 cases) or HBV (36 cases), and 69 cases without liver symptoms, sero-negative for both HCV and HBV. Circulating free DNA (CFDNA) was both quantified and used to detect 249^{ser} TP53 mutations by PCR.

Key points:

- Egypt, published 2008
- 255 subjects: 76 HCC, 74 CLD+HCV, 36 CLD+HBV, 69 controls
- 249^{ser} TP53 mutation was most often found in the CLD+HBV group, and was rare in the HCC group.
- They did not calculate any statistical significance.

The HCC group had one 249^{ser} TP53 mutation (1.3%), the CLD

+ HCV group had four (5.4%), the CLD + HBV group had six (16.7%) and the healthy sero-negative group had one (1.4%). No calculation of statistical significance was performed in the article. (14)

Sayed et al conducted a cross-sectional study to assess different factors that may be associated with some common liver diseases in a rural population of Egypt, published in 2005. The different factors were some trace elements (copper, iron, selenium and zinc), heavy metals (lead, mercury, arsenic, aluminium, manganese, nickel, chromium and cadmium), aflatoxin B₁ and *Schistosoma mansoni* infection. The common liver diseases were hepatitis B, C, bilharzial or fatty liver. The village was close to the Nile, as well as several industrial areas, with the population mainly involved in agriculture. 84 subjects from 15 households from different parts of the area were studied. They all had blood taken and their liver assessed by abdominal ultrasound. The blood was analysed for serum aflatoxin B₁, the heavy metals and trace elements previously mentioned, HBsAg, anti-HCV antibodies and circulating schistosomal antigen (schist. ag).

Key points:

- Egypt, published 2005
- Cross-sectional study
- 84 subjects
- Several statistically insignificant results where AFB₁ was found in higher values and more often in the serum of those with viral hepatitis than sero-negatives.
- Statistically significant lower levels of AFB₁ in the serum of those with fatty livers than normal subject.
- Concluded with an association between AFB₁ and liver disease in the area

The reference value of aflatoxin B₁ was 13.6-36.2 ng/kg, and the sero-negative, HBV, HCV and combined infection groups all had a median value above the reference (45.0, 65.0, 37.5 and 52.5). Aflatoxin B₁ was found in the serum of 76.5% of the sero-negatives, 95.2 % of HBsAg positive (OR 6.2, 95% CI 0.7 – 53.3), 88.9 % of the anti-HCV Ab positive (OR 2.5, 95% CI 0.3 – 22.8) and in 100% of those with a combined infection. These findings were statistically insignificant. When grouped after ultrasound findings, the normal livers had a median value above the reference (50.0), while both the bilharzial and fatty liver were within reference range (25.0, 22.5). Aflatoxin B₁ was found in the serum of 90.7% of the livers which appeared normal on ultrasound, 77.8% of the bilharzial livers (OR 0.4, 95% CI 0.1-2.3) and 63.6% of the fatty livers (OR 0.2, 95% CI 0.04-0.9). The difference between normal livers and bilharzial livers was found to be statistically insignificant, however a statistically significant lower incidence of aflatoxin B₁ was found with fatty livers compared to normal livers. It was concluded that aflatoxin B₁ had a definitive association with liver disease in the area that was studied. (25)

3.2 Aflatoxins and parenchymal liver disease in animals

In 2013 Siloto et al published an experiment looking into the lipid metabolism of layers fed diets containing aflatoxin (AF), fumonisin (FU) and a binder. Fumonisin is a mycotoxin contaminant of corn based foods produced by *Fusarium verticillioides*. They took 168 37-week-old Hisex Brown layers and randomised them into seven treatments with six replications of four birds each, with an experimentation period of 56 days or two 28 day cycles. There were three diets with no binder, consisting of AF, FU and AF+FU, three diets with binders and the same arrangement of mycotoxins, and one control diet with

Key points:

- South America, published 2013
- Layers (type of chicken)
- Their objective was to see if a binder could reduce the effects of AF and FU
- Layers fed aflatoxin had significantly higher relative liver weight and percent of liver fat compared to controls.

neither mycotoxins nor binders. The aflatoxins were AFB₁ and AFB₂ in concentrations of 1 mg/kg, and the fumonisins FUB₁ and FUB₂ in concentrations of 25 mg/kg. After 56 days two layers from each replicate were randomly selected for blood collection, and very low-density lipoproteins (VLDL), high-density lipoproteins (HDL) and triglyceride were measured. Six layers from each treatment were weighed and killed, their livers removed, weighed and analysed for fat content.

The layers fed aflatoxin, both with and without binders, had a significantly higher relative weight of the liver compared to the control group. The layers fed aflatoxin and no binder showed a yellowish liver, and they had a significantly higher percent of liver fat compared with the layers on the control diet. The layers on aflatoxin diets had a lower value of plasma VLDL and triglycerides. (26)

An article about the effects of feeding corn naturally contaminated with aflatoxin B₁ and B₂ on the hepatic function of broilers was published by Yang et al in 2012. They took 1200 one-day-old Cobb male broilers and randomised them into five treatments with eight replications of 30 birds each, with an experimentation period of 42 days. The five treatments consisted of one control diet without aflatoxins, and then four diets with 25%, 50%, 75% and 100% aflatoxin B₁ and B₂ contamination.

The 100% contaminated diet contained 82.4 and 14.2 µg/kg AFB₁ and AFB₂ for the first 21 days, and 134.0 and 23.6 µg/kg for the last 21 days. On day 21 and 42, one bird per replicate was selected for blood sampling for serum biochemistry and another for liver samples, where the latter was used both for measurement of hepatic antioxidant enzyme activities and histopathological examinations.

Key points:

- China, published 2012
- Broilers (type of chicken)
- Several changes that were seen on day 21 had improved on day 42.
- Diets containing ≥ 50% aflatoxin-contaminated corn could induce pathological liver lesions.

The body weight of the 100% contaminated diet group was significantly decreased compared to the control group on day 21 ($p < 0.001$), while there was no significant difference on day 42. The relative weight of the liver was significantly lower in the 25% contaminated group compared to the more contaminated groups on day 42 ($p = 0.006$), but also lower than the control group. Regarding the biochemical parameters on day 21, there was a significant increase in serum aspartate aminotransferase (AST) in the 75% and 100% contaminated groups compared with the others ($p = 0.004$), and in the serum γ -GT in the 100% contaminated group compared to the 25% and 50% group, as well as the 75% contaminated group compared to the 25% contaminated group ($p = 0.02$), though no significant difference in γ -GT between any of the groups and the control group. All other biochemical parameters on day 21, as well as day 42, were found to have no significant difference. As for the hepatic antioxidant enzymes there were several significant effects, the most important being the significant reduction in hepatic protein on day 42 in all the contaminated groups compared to the control ($p < 0.001$), and the increase in glutathione reductase (GR) and glutathione-S-transferase (GST) in the 75% and 100% groups compared to the control on day 42 ($p = 0.008$ and $p = 0.006$). Regarding the histopathology of day 21, 83.3% of the 75% contaminated group had light hyperplasia of bile duct epithelium, while the rest had severe lesions, and the entire 100% contaminated group had severe hyperplasia. On day 42, the lesions had improved overall. Only 1/3 of the 75% group and half of the 100% group showed very light lesions and the rest had normal histopathology. Comparing the 100% group with the control group on day 42, there were several ultrastructural changes, including fatty degeneration, lipid droplets in the cytoplasm and swollen endoplasmic reticulum. Compared to the control group there was significantly more apoptosis in the 50%, 75% and 100% groups on day 21 ($p < 0.05$) and in the 75% and

100% group on day 42 ($p < 0.05$). They concluded that diets containing $\geq 50\%$ aflatoxin-contaminated corn could induce pathological liver lesions, slightly change serum biochemical parameters and damage hepatic antioxidant functions. (27)

Ortatatli et al published an article in 2005 in which they evaluated pathological changes in broilers during chronic, low level aflatoxin exposure. They had 567 one day old Ross-308 type broiler chicks of both sexes, who were weighed and randomised into six groups. The groups consisted of one group with 50 ppb ($\mu\text{g/L}$) aflatoxin, one with 100 ppb ($\mu\text{g/L}$) aflatoxin, one with 50 ppb aflatoxin + a binder (clinoptilolite = CLI), one with 100 ppb aflatoxin + a binder, one with just a binder, and one control group. The experiment lasted for 42 days, after which 10 broilers from each group were randomly selected, weighed and killed. Liver, spleen, kidneys, thymus and the bursa of Fabricius were removed and weighed, relative organ weights calculated, and tissue samples collected for histopathological examination. The hepatocellular degeneration was graded as follows: Slight – mild hepatocellular swelling and fatty changes in centrilobular areas. Moderate – clear hepatocellular swelling in centrilobular and midzonal areas. Severe – diffuse and severe hepatocellular swelling, cytoplasmic paleness and rupture.

Key points:

- Turkey, published 2005
- Broilers
- Low level and chronic aflatoxin exposure.
- Significant microscopic changes in the livers of the 100 ppb AF-group compared to controls (hydropic degeneration, fatty change, bile duct-hyperplasia, periportal fibrosis).

There was no statistical difference in the relative weights of the different organs, nor any gross pathological changes. The 100 ppb aflatoxin diet compared to the control had significant ($p < 0.05$) slight to moderate changes, with hydropic degeneration and small fatty vacuoles in hepatocytes in centrilobular and midzonal areas. Some also had bile-duct proliferations in portal areas and periportal fibrosis. In the 50 ppb aflatoxin diet only very mild and statistically insignificant changes were seen. (28)

In 1989, Rajan et al published a paper on aflatoxin induced hepatopathy in pigs and ducks. They did three different studies: Two received carcasses from pigs and ducks and performed detailed post-mortem examinations and analysis of feed samples for aflatoxin, while the third was an experimental study on pigs. Twenty healthy male piglets were randomised into three groups, with seven in each group, who were given 0.1 and 0.2 mg aflatoxin per kilogram bodyweight daily, and one control group of six piglets. The experiment lasted for 3 months, and the piglets were weighed before the experiment started and every two weeks after this. At the end of the experimental period, all piglets were killed and subjected to post-mortem macroscopic and histopathological examination.

Key points:

- India, 1986 – 1989
- Ducks and pigs
- Three different studies.
- In the post-mortem pig group they saw both acute and chronic liver disease.
- In the post-mortem duck group moderate fibrosis was common.
- In the experimental pig group there was a significant increase in liver weight compared to controls, and several histopathological changes.

1034 ducks were examined, while it was not stated how many pigs were examined. They examined 127 feed samples for pigs, where 26.8% contained AFB_1 between 20 and 100 ppb, and 9.4% contained AFB_1 between 101 and 1600 ppb. For the ducks they did 20 feed samples, where 40% contained AFB_1 between 20 and 100 ppb and 20% between 101 and 600 ppb. They did the post-mortem examinations of pigs for three consecutive years, finding 25%, 18% and 33% with hepatosis for each of the years ('86-'88). Hepatosis was seen as acute, sub-acute and chronic. The acute hepatosis was characterised by congestion, sinusoidal engorgement, haemorrhage, diffuse fatty changes and necrosis. The sub-acute and chronic had diffusely enlarged livers with necrotic spots, and some also had

nodular and atrophic livers. They saw periportal necrosis, interstitial fibrosis, pseudolobulation and regenerating nodules, among many things. In the experimental study the pigs became depressed, didn't eat much and the animals in both experimental groups developed mange infection. There was a dose dependent increase in liver weight ($p < 0.05$), and histologically there was diffuse fatty change, hepatocyte necrosis, pseudolobulation and interstitial fibrosis. As for the ducks 40.5% had hepatosis, with a diffuse enlargement of the liver. Histologically, there was necrosis, pseudolobulation and often a moderate fibrosis, as well as regenerating nodules. 50 of the ducks also developed neoplasms. (29)

Uchida et al published an article on the influence of aflatoxin B₁ intoxication on White Peking duck livers with duck hepatitis B virus infection (DHBV), in 1988. In the short-term experiment, they had 20 one day old ducklings with DHBV, where half were given AFB and half were not, and five ducklings without DHBV who were not given AFB. The ducks in the latter group were all sacrificed in the fourth week, while the former had two to three ducklings sacrificed at the second, third and fourth week. In the long-term experiment they had 53 one day old ducklings. Group one consisted of 22 ducks with and 16 ducks without DHBV, given 0.1 mg/kg body weight of AFB twice a week for a full 54 weeks. Group two had five ducks with and three ducks without DHBV who were given the same amount of AFB but only for five weeks, and then kept off AFB for the remaining 49 weeks. Group three had five ducks with DHBV and none without, and they were given a toxin free diet for the first 16 weeks followed by 25 weeks of AFB. Group four had two ducks with DHBV who had a toxin free diet for 41 weeks. The ducks were sacrificed at the end of the experiment or when they became weak.

Key points:

- Japan, published 1988
- Ducks
- Short- and long-term experiments with combinations of AFB and duck HBV
- Several ducks died due to liver cell necrosis.
- Long-term AFB administration induced nodular changes and cirrhosis.

Two ducklings died during the short-term experiment. In the DHBV+AFB-treated group the livers were shown to have hepatocellular necrosis in periportal areas and biliary cell proliferation, while there were no signs of cirrhosis. In the long-term experiment, 22 ducklings who had received AFB-diets died within 10 weeks due to extensive liver cell necrosis. Half of all the ducks surviving for more than 10 months developed amyloidosis. The ducks who received long-term treatment with aflatoxin were found to have nodular changes, cirrhosis and, in a few, hepatocellular tumour, regardless of whether they were given aflatoxin from birth or if they first received it after 16 weeks. They concluded that aflatoxin B-intoxication provoked various liver disorders independent of DHBV infection. (30)

In 1969, Newberne and Butler wrote a review looking into the pathologic changes seen in the livers of different species after single doses and continuous administration of aflatoxin. With turkeys they found that birds dying after only a short period of exposure had severe periportal necrosis and nodular regeneration, while birds dying after 23 weeks had nodular livers with fibrosis, biliary proliferation and a variation in the size of the hepatocyte nuclei. In ducklings, a single high dose gave periportal necrosis and lakes of fat over a 48 hour period, and ducks fed low dose diets of aflatoxin for 16 months were all found to have cirrhosis, in addition to many developing liver cell tumours. An experiment with chickens fed 1.5ppm of AFB₁ showed periportal fatty infiltration after a few days, over a

Key points:

- Published 1969
- Review of acute and chronic effects of aflatoxin on different animals.
- Ducks: Low dose, long-term exposure gave cirrhosis.
- Some animals appeared not to be affected by aflatoxin.
- Different species had different distributions of hepatic lesions.

month the development of scattered liver cell necrosis, biliary proliferation and increased connective tissue, and after six weeks biliary proliferation, fibrosis and increased lymphocytic hyperplasia of the portal tracts. Calves and cattle experimentally fed on a diet containing 2.0 ppm aflatoxin for four months showed progressive biliary proliferation, increased connective tissue and some degeneration of centrilobular hepatocytes. Many species were reported to have similar findings after aflatoxin exposure, including pigs, guinea pigs, dogs, rabbits and monkeys. There were a few species which seemed to react differently. Newberne and Butler found that sheep had no field reports, and experiments showed them to be relatively unsusceptible to aflatoxin exposure. Rats had many of the same findings mentioned in the previous species, but seemed to have no increase in fibrous tissue and, in at least two studies, had no evidence of cirrhosis at the stage where carcinomas were observed. Mice have appeared to be resistant to the chronic toxicity of aflatoxin in feeding trials. Different species appeared to have a different distribution of the hepatic lesions, with rats and ducklings having periportal lesions, pigs and guinea pigs centrilobular, dogs periportal and centrilobular, and rats having midzonal. (31)

Wannop published a paper in 1961 on the histopathologic observations made on field cases from the outbreaks when Turkey X disease was first discovered. Organs were collected from turkeys immediately after death, and blood was collected from the wing vein.

The most significant changes were found in the liver. Acute cases were found to have swollen hepatocytes and nuclei, frequently associated with an enlargement of the nucleolus. The affected areas had eosinophilic parenchymatous cells and in places they contained several small vacuoles within the cytoplasm. Many areas had degrees of necrobiosis, and throughout the liver were small periportal areas of regeneration. Turkeys who survived for longer showed widespread regeneration of liver parenchyma and formation of bile ductules. A few birds survived, and developed pale, very hard, shrunken livers, with islets of parenchyma but no thick fibrous bands. (11)

Key points:

- England, published 1961
- Histopathologic examination of first known outbreak
- Turkeys
- Most significant changes were found in the liver
- Survivors developed pale, hard and shrunken livers.

4 Discussion

Finding recent articles concerning the objective was difficult, as most of the research was published in the first few decades after aflatoxins were discovered. Because of this, quite a few older articles were used, and nine out of the 17 articles in this review were published before 1990. Although aflatoxins are still a major health issue worldwide, with known carcinogenic effects, they are well controlled in more developed parts of the world and it is likely considered a less profitable research area. Many of the studies used in this review did not have the objective of this review as their main hypothesis or result, but they still had relevant findings. Since there were very few studies focusing on this one subject, it was also difficult to compare the findings to one another, as the methods used varied widely.

4.1 Aflatoxins and acute parenchymal liver disease in humans

It is worth mentioning that there have been more aflatoxicosis outbreaks among humans than the three mentioned in this review, but these seemed to be the most researched.

In the acute outbreaks, aflatoxin levels in maize have been very high, with the '74 outbreak in India finding total aflatoxin levels between 6250 and 15,600 ppb, the '81 outbreak in Kenya finding aflatoxin B₁-levels between 3200 and 12,000 ppb, and the 2004 outbreak in Kenya finding aflatoxin B₁-levels up to 4400 ppb, with a mean of 354.5 ppb in affected cases. In comparison, the Kenyan regulatory limit is 20 ppb (10).

While the aflatoxin levels in maize were all very high, the aflatoxin levels measured in the affected patients varied. In the '74 outbreak, aflatoxin B₁ was only detected in two of seven serums, in none of the seven urines, nor in the one liver extract. Liver samples from two of the children in the '81 outbreak showed aflatoxin B₁, while the case patients from the 2004 outbreak had a higher concentration of aflatoxin B₁-lysine albumin adducts than controls. The half-life of aflatoxin B₁-lysine albumin adducts is similar to that of unbound albumin, namely 20-60 days, while unbound aflatoxin remains in the blood for only 13-120 minutes after exposure (21). It was not specified what technique they used to detect aflatoxin B₁ in '74, nor was it specified how much time had passed between when aflatoxin exposure ended and the blood and urine tests were taken. Aflatoxin B₁-albumin adducts were, however, not discovered until the 1980s, and due to this it is highly likely that the aflatoxins found in the sera from '74 were unbound (32). It is therefore possible that the aflatoxin B₁ concentrations would have been detectable, had the investigations had been conducted sooner.

The presenting clinical picture in the 2004 case was not described in the study. In the two earlier outbreaks, an initial phase of low grade fever and anorexia, followed by jaundice and oedema of the lower extremities was described. Microscopic examination of the liver was done with the first two outbreaks, showing quite different findings. In the '74 outbreak they looked at the liver of one deceased patient, finding periductal fibrosis and extensive bile duct proliferation. In the '81 outbreak they looked at the livers of two young deceased siblings and saw centrilobular fibrosis and no bile duct proliferation. This was, of course, a very small selection but even though the two findings were very different, neither of them showed the pattern one would expect, with fibrosis found in the periportal area, as this is where toxins normally damage the liver first. It is worth noting that Amla's study from 1971, which mostly

looked at cirrhosis, saw an initial increase in central and periportal infiltration after the children were accidentally fed high doses of aflatoxin over a short period of time.

Hepatitis B and aflatoxins are known to have a synergistic effect when it comes to hepatocarcinogenicity, but the possibility of them having a synergistic effect on acute hepatic failure was looked into in the '81 and 2004 outbreaks. In the '81 outbreak they studied the sera of 29 people (10 who were or had been ill and 19 contacts/neighbours) and three were positive for HBsAg, of which at least two had been ill with acute hepatic failure. In the 2004 study, eight of 18 cases were HBsAg positive, and it was found that positive HBsAg titres were a risk factor for acute hepatic failure. This is too little data to conclude any synergistic action, but several other agents are proven to have synergistic actions when it comes to liver disease, for example alcohol and hepatitis C on CLD (33). Thus it is possible that a synergistic action can exist between HBsAg and aflatoxins when it comes to acute hepatic failure.

Another noteworthy finding was that wholly breastfed infants were spared in every outbreak, reflecting that only those ingesting contaminated crops would receive a high enough toxin dose to become clinically unwell.

Regarding the lethality rates in the aflatoxicosis outbreaks, the '74 outbreak in Western India left 27% dead, while the Kenyan outbreaks of '81 and 2004 had lethality rates of 60% and 39% respectively. While the '74 and 2004 outbreaks had between 300 and 400 affected, the '81 outbreak had only 20, so the significance of the high lethality rate of the latter could be uncertain due to the small number of cases.

4.2 Aflatoxins and chronic parenchymal liver disease in humans

There were several results indicating that aflatoxins may cause CLD, and more specifically cirrhosis, in humans. Kuniholm and Lesi did a recent and rather large study with almost 500 participants, concluding that aflatoxin exposure alone, measured in two different ways, significantly increased the risk of cirrhosis. Amla's study showed that several of the 20 children had increasingly firm and enlarged livers both one and two years after getting a high dose of aflatoxin, and after one year their biopsies showed a transition to fatty cyst formation, fibrosis and cirrhosis. In Coulter's study of Sudanese children they found a significant difference between kwashiorkor children and controls when it came to aflatoxins in sera, and in the presence of aflatoxicol between the groups. Aflatoxicol was most often found in kwashiorkor children and none of the controls. As a statistically insignificant observation, De Vries' and Lamplugh's article found aflatoxins almost consistently, with one exception, in blood, urine and liver samples of cirrhotic patients.

On the other hand, some results provide arguments against this. In Chen's study aflatoxin levels were only associated with advanced liver disease in combination with HCV, and not as a risk factor in itself. In Sayed's study from Egypt, he found that AFB₁ levels were lower in patients with bilharzial and fatty livers than normal livers, and AFB₁ was found in significantly fewer patients with fatty , compared to those with normal livers, which was the opposite of what would be expected looking at the other results. While Chen used aflatoxin-albumin adducts, Sayed et al checked for serum aflatoxin B₁, which has already been

mentioned to only stay in the blood for up to 120 minutes. This could be an explanation for these unexpected findings.

An important point to make is that liver damage and disease may cause increased levels of aflatoxin, either instead of, or in addition to aflatoxin causing disease, meaning the two variables may be considered correlated with an unclear causal pathway. As explained by Chen et al, liver injury has been shown in experimental animal studies to increase cytochrome P450 enzyme, which could increase the activation of AFB₁ into the more dangerous AFB₁-8,9-epoxide, which would cause more damage, but would also create adducts with albumin, which was what many of the studies used to measure aflatoxin exposure. This was cleverly avoided in the Kuniholm and Lesi study by using 249^{ser} TP53 mutations, as this was presumed to be less likely to be affected by the severity of liver disease. The 249^{ser} TP53 mutation has mostly been used as a cancer marker for hepatocellular carcinoma caused by aflatoxins, and the mutation has been proven to be less prevalent in areas with low aflatoxin exposure (34). Kuniholm and Lesi also performed ultrasonography on all cirrhotic patients included in the study, to ensure absence of liver tumours.

Based on this, some of the results may be invalidated by unclear causal pathways between liver damage and aflatoxin exposure. Others results, however, would still remain valid, with Kuniholm and Lesi using a better method for measuring aflatoxin exposure, and Amla doing a prospective study in which all the children were known to have been exposed to aflatoxins over a short amount of time.

4.3 Aflatoxins and parenchymal liver disease in animals

Most of the animal studies of chronic aflatoxin exposure looked at the changes in microscopic liver structure. While most of the studies saw pathologic lesions, only two reported cirrhosis. Both Uchida and Newberne and Butler reported that ducks fed low doses of aflatoxin for over a year developed cirrhosis, many also developing hepatocellular tumours.

There were several parenchymal changes seen throughout the animal experiments: classic steatosis changes like lipid droplets in cytoplasm, fatty change, increased liver size and yellow colour. More steatohepatitis-like changes were seen, with hepatocellular swelling and a mostly periportal necrosis. Fibrotic changes included increased connective tissue and different types of fibrosis such as interstitial, periportal, central and a more generalised fibrosis. Nodular changes were also seen, as well as the birds surviving the initial “Turkey X”-disease showing pale, hard and shrunken livers.

Most of the animals used for experiments in these articles were various birds, such as ducks, broilers and layers, and they generally seemed to be susceptible to both acute and chronic effects of aflatoxin, including fibrotic and cirrhotic changes. While Newberne and Butler found that several other animals had a similar response to aflatoxin exposure, some reacted differently. Rats were susceptible to the acute reactions and tumours, but seemed not to get fibrosis or cirrhosis. Sheep seemed to have few reactions to aflatoxins. Mice appeared resistant to the chronic toxicity. Different species also had differences in the distribution of fibrosis as mentioned above.

In Yang's article, they reported that the hepatic lesions seen in broilers on day 21 had improved on day 42. This could in part be explained by them also finding a significant increase in glutathione reductase (GR) and glutathione-S-transferase (GST). GST is, as previously mentioned, responsible for detoxifying the toxic aflatoxin epoxide, and GR converts oxidised glutathione to the reduced glutathione which is needed for GSTs detoxifying actions. When stimulated by low levels of AFB₁, the broilers seem to have secreted more GST and GR to meet the detoxification requirements (27). This would lead to a lower concentration of the toxic metabolites and less liver damage.

5 Conclusion

While it is generally accepted that aflatoxins cause acute hepatitis, called aflatoxicosis (7), it has been unclear if they can also cause CLD and cirrhosis in humans.

There have been a few studies on humans which have presented the conclusion that long term low-concentration aflatoxin exposure leads to cirrhosis, but the research is minimal.

Animal studies show that some species may develop CLD after long term aflatoxin exposure, but different animal species seem to have very different results from similar exposures. Due to these interspecies differences, it is very difficult to transfer the knowledge about liver disease and aflatoxin exposure from animal experiments to humans.

In summary, aflatoxins do cause acute parenchymal liver disease in humans and may cause chronic parenchymal liver disease in humans, though very little research has been done concerning the latter, and further studies are required to prove this connection. A suggested, albeit time-consuming, research design for this would be a prospective cohort study.

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