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RESEARCH ARTICLE



Assessment of aflatoxin B₁ in maize and awareness of aflatoxins in Son La, Vietnam

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ABSTRACT

Aflatoxin B₁ (AFB₁) is a fungal by-product which causes acute and chronic toxicity in humans and many other animals. This research was conducted to evaluate the prevalence of AFB₁ contamination in maize and residents' awareness of aflatoxins in Son La province, Vietnam. Maize samples were randomly collected from Son La province using multi-stage sampling. We used cut-off levels of 5 and 20 µg/kg and calculated the mean, median and range for each district. In addition, a questionnaire collected information from households about their knowledge, attitude and practice related to moldy maize. Out of 378 maize samples from Son La, 204 (54.0%) and 141 (37.3%) were contaminated with AFB₁ at more than 5 µg/kg and 20 µg/kg, respectively. Mai Son district had the highest proportion of samples (54.0%) using a cut-off level > 20 µg/kg, and Yen Chau district the lowest (4%). People from the Thai ethnic group were 30.9 times more likely to consume meat from animals fed moldy maize than people from the Kinh ethnic group ($p = 0.003$). Maize in Son La is contaminated with AFB₁ at levels which imply better control of aflatoxins in maize for human consumptions and animal feed is needed.

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KEYWORDS

Aflatoxin B₁; Son La; ELISA; prevalence; molds; awareness/perception


Introduction

Vietnam has a tropical monsoon climate with high temperature and humidity creating favorable conditions for fungal growth, especially molds which can produce toxic metabolites [1]. Aflatoxins, produced by ubiquitous fungus *Aspergillus* species have attracted attention because they are the most toxic among mycotoxins and potent carcinogens [2,3]. The International Agency for Research on Cancer (IARC) classified aflatoxin B₁ (AFB₁) as group I carcinogens; proven human carcinogens [4]. Specifically, they are involved in the development of liver cancer (hepatocellular carcinoma); individuals infected with hepatitis B virus are at higher risk [5–7]. AFB₁ is a common contaminant in several agriculture crops, mainly maize, groundnuts, pistachio nuts and cotton seeds before harvest and in storage [1,8]; Similarly, it is present in animal feeds which may affect the growth and production of livestock negatively, and it may also be present in animal-source foods as the result of animals consuming contaminated feed (Atherstone 2016). Therefore, from a food safety point of view, it is important to assess and control the levels of aflatoxins in human food and animal feed.

In Vietnam, maize is the second most important staple crop for human consumption, after rice. It is a substitute for rice during times of shortage, especially for people in the rural and mountainous areas [9]. It is also the major component of feed for Vietnam's livestock industry [9]. Unfortunately, maize is also a good substrate for mycotoxins producing fungi, especially those producing aflatoxins [10,11]. Previous studies on AFB₁ in maize in Vietnam have provided some information on contamination; however, these were based on small sample sizes or relied on the data from one point in the maize value chain [12–15], making it difficult to generalize. The northern province of Son La is the largest maize producing area in Vietnam; it is also one of the poorest areas and has limited development opportunities over the past years [16]. Only one screening AFB₁ investigation has been carried out to evaluate the prevalence of AFB₁ contamination in maize and perception and knowledge of aflatoxins in Son La province [17]. Therefore, the main objective of this research was to assess the levels of AFB₁ in maize during the rainy season and in-depth knowledge/behavior of aflatoxins among people in Son La province.

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Materials and methods

Study location

Son La province comprises Son La city and 11 rural districts (Figure 1). There are two distinct seasons: the dry winter from October to March, and the hot rainy summer from April to September. Most precipitation occurs in June, July, August and September. The average annual temperature and humidity are 23°C and 79%, respectively [16]. Similar to other Northwest provinces, Son La's population consists of many ethnic minorities, predominately Thai (54%), Kinh (18%: the major ethnic group nationwide) and Mong (13%) [18]. Other ethnicities are Ma, Dao and Muong.

Maize sampling

The field work was conducted from 10 September to 15 September 2016 at the end of the rainy season. A targeted sample size of 385 was determined based on an estimated 50% prevalence, a precision level of 5% and 95% confidence interval (CI). For each of five districts (Mai Son, Moc Chau, Son La city, Thuan Chau and Yen Chau), a total of five communes were selected conveniently selected using multi-stage sampling [districts (5)-communes (25)]. Sampling was implemented at different points of the maize value chain depending on the availability, including all local retail traders, households, commercial storages and maize fields encountered in the communes (Figure 2). Maize collection followed the method recommended by the Food and Agriculture Organization (FAO) [19]. A representative sample

of at least one kg sample was collected from different parts of the supplies. Samples were then stored in paper bags to control moisture content. In the lab, representative maize samples were ground and kept at -180C in polyethylene bags whilst waiting for analysis. Selected districts were marked on the map using ArcGIS version 10.4.1 ArcMap (ESRI, Redlands, CA, USA) (Figure 1).

Laboratory analysis

All samples were analyzed at the Plant Protection Research Institute (PPRI) in Hanoi, Vietnam, using the Aflatoxin B1 Low Matrix ELISA kit (Enzyme Linked Immunosorbent Assay) (Helica Biosystems INC; Cat. No: 941BAFL01B1). We followed the manufacturer's instructions for analysis. Briefly, AFB1 was extracted from a ground sample with 70% methanol (1/5 dilution) and followed with 1/10 dilution in Phosphate-buffered saline-Tween (PBS) prior to running the assay (a total of 1:50 dilution), giving a final limit of detection (LOD) of 1 µg/kg in samples. Optical densities (OD) of the reactions for AFB1 were quantified using a microplate reader with an absorbance filter of 450 nm. Test values were calculated with reference to standards that were included in each micro plate. Samples were not diluted and retested if exceeding the maximum standard of the kit, but the value was calculated as an approximation

Awareness assessment

Face-to-face interviews were conducted with adults (18 years old and above) in a randomly selected

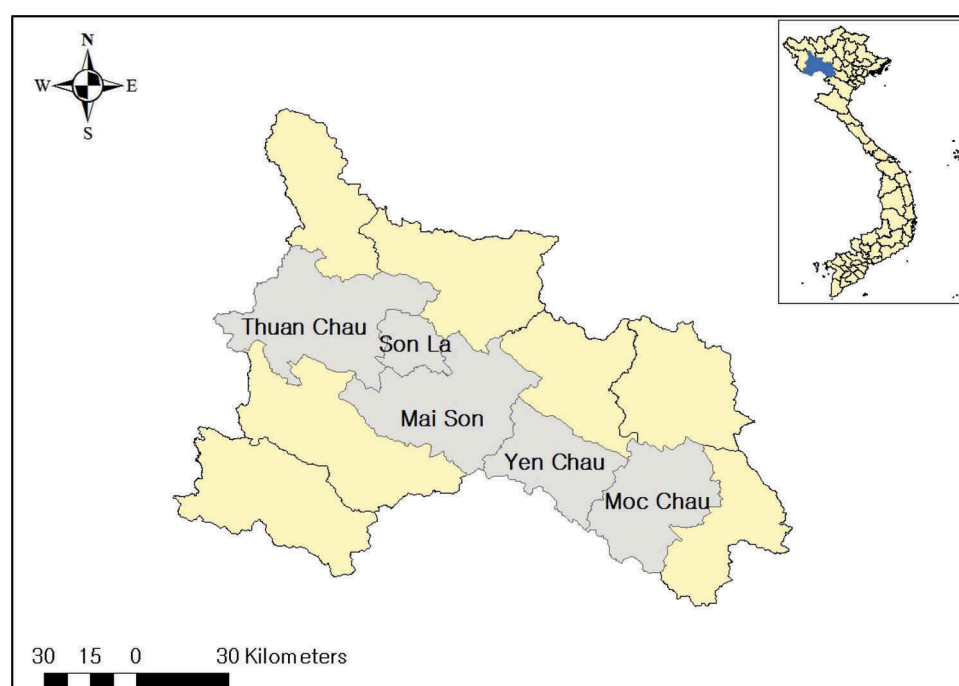


Figure 1. Sampling sites from five districts in Son La.



Figure 2. Proportion of sampling areas from five districts in Son La.

subset of the households at the same time as maize collection. Questionnaires covered socio-demographic information and knowledge, attitudes and practices about moldy maize. We investigated awareness of moldy maize rather than aflatoxins because previous research revealed that the word ‘aflatoxins’ and the concept of ‘toxic fungal metabolic by product’ were not known in the area [17]. The questionnaire was initially developed in English and later translated into Vietnamese. The final questionnaires were used in the fields after conducting the pre-testing.

Data analysis

According to the Vietnamese standards in 2011, a maximum level of 5 µg/kg AFB1 is allowed in maize for human consumption, but there are no regulations for animal feed [20]. For comparison, most European countries specify limits of 2 and 5 µg/kg for human consumptions and animal feeds, respectively [21]. The United States Food and Drug Administration (FDA) sets limits for total aflatoxins between 20 to 300 µg/kg depending on the use of foodstuff or feedstuff [22]. In Africa, Asia/Oceania and Latin America the most common limit for AFB1 in food is 5 µg/kg [21]. For our study, we calculate the proportions of samples above 5 and 20 µg/kg, respectively. Moreover, mean, and median were investigated for each district, with only samples exceeding limit of detection (LOD) (1µg/kg) included for calculation.

To assess awareness around moldy maize, demographic information were collected in the survey, including age (<29 years, 30–39 years, 40–49 years and ≥50 years), sex (male and female), education

level (none, primary/middle school, high school and college/university or more), ethnic group (Kinh, Thai and H’Mong) and occupation (retailers, farmers, feed manufactures and others). We have mainly 4 outcomes of interest as follows: (1) ‘Are molds harmful to human & animal health?’, (2) ‘Do you think that moldy maize consumption can cause disease?’, (3) ‘Is it safe to eat meat from animals which were fed with moldy feed?’ and (4) ‘Do you consume animal meat which were fed with moldy maize/cereal?’). Cronbach’s alpha was used to evaluate the consistency of answers to the main 4 outcomes if there is a logical way to combine the 4 variables. However, the test scale is 0.34 which was not high enough to combine the variables so that we decided to develop separate models. For the screening, variables with $p < 0.20$ were included in the building of a multivariable regression model, using manual backward elimination for optimization. Variables with P -values < 0.05 were considered to be significant in the final models. Clustering was taken into account by having districts and communes as random effects. Goodness-of-fit was conducted using the Hosmer-Lemeshow test [23]. All data were entered into Microsoft Excel 2010 and analyzed using STATA (version 14.2, StataCorp, College Station, TX, USA).

Results

A total of 378 (98%) maize samples were analyzed from five districts (Mai Son = 76, Moc Chau = 75, Son La city = 76, Thuan Chau = 76 and Yen Chau = 75). In total, 204 samples (54.0%, 95% CI: 48.8–59.1%) and 141 samples (37.3%, 95% CI: 32.4–42.4%) had levels above 5 and 20 µg/kg, respectively (ranged from below LOD to 417.0 µg/kg, median: 19.4 µg/kg and mean: 37.3 µg/kg)

(Table 1). Specifically, the results showed that Thuan Chau district had the highest proportion (76.3%, 95% CI: 65.2–85.3) of samples above 5 µg/kg whereas Yen Chau district had the lowest proportion (17.3%, 95% CI: 9.6–27.8). Using the cut-off level > 20 µg/kg, the highest proportion of positive samples was found in Mai Son district with 54.0% (95% CI: 42.1–65.5) while Yen Chau district had the lowest proportion with 4% (95% CI: 0.8–11.3). The highest level of AFB₁ in a sample was found in Mai Son (417.0 µg/kg) (Table 1).

In the awareness survey, a total of 107 people were interviewed (Mai Son = 25, Moc Chau = 7, Son La city = 25, Thuan Chau = 25 and Yen Chau = 25). For Moc Chau, due to lack of human resources and time, we were not able to reach the target participants (25 people per district) as the other districts. Respondents ranged from 18 to 71 years old. Male (59%), Thai ethnic group (90%) and farmers (87%) were the most common in each category. Overall, 70.1% of respondents were aware that molds are harmful to humans and animals while 55.1% of participants believed that consumption of moldy maize can cause disease. Overall, 62.6% thought that eating meat from animals which were fed with moldy feed was not safe; however, 84.1% of respondents consumed meat of animals so fed (Table 2, only data shown for question 4). Women were more aware of risks but the differences were not significant. For multivariable regression analysis, four separate models were developed based on our outcomes of interest. Apart from question 4 (Do you consume the meat of the animals which were fed with moldy maize/cereal?), none of the variables were significant in the models. In the final model, random effects were not included as variations were negligible in each level. Our final model showed that the odds ratio (OR) for consumption of meat fed by moldy maize/cereal in H'Mong group was 8 times higher than in Kinh group, but this difference was not significant, whereas the Thai ethnic group was 30.9 times higher than the Kinh ethnic group which was significantly different (Table 3). The Hosmer-Lemeshow goodness of fit test showed that there was evidence of poor fit.

Furthermore, 58% of households used maize mainly for animal feed, only 0.9% of households used it mainly for human consumption, and the

Table 2. Demographic characteristics of survey respondents from Do you consume the meat of the animals which are fed by mould maize/cereal?

Category	Characteristic (n)	Do you consume the meat of the animals which are fed by mould maize/cereal (Yes/percentage)?
Overall	n = 107	90 (84.11%)
Age (years)	<29 (n = 15)	15 (93.33%)
	30–39 (n = 30)	26 (86.67%)
	40–49 (n = 34)	25 (73.53%)
	≥50 (n = 28)	25 (89.29%)
Gender	Male (n = 63)	52 (82.5%)
	Female (n = 44)	38 (86.4%)
Education	None (n = 10)	10 (100.0%)
	Primary & Middle school (n = 52)	44 (84.6%)
	High school (n = 38)	31 (81.58%)
	College/University or more (n = 7)	5 (71.43%)
Ethnic	Kinh (n = 5)	1 (20.0%)
	Thai (n = 96)	85 (88.54%)
	H'Mong (n = 6)	90 (84.11%)
Occupation	Retailer (n = 6)	6 (100.0%)
	Farmer (n = 93)	79 (84.95%)
	Feed manufacture (n = 1)	0 (0%)
	Others (n = 7)	5 (71.43%)

Table 3. The final ordinal logistic regression model of awareness of aflatoxins in Son La.

Question: 'Do you consume the meat of the animals which were fed by mold maize/cereal?'				
Variable	Category	Odds ratio	95% CI	P-value
Ethnic group	Kinh (n = 5)	Reference	Null	Null
	Thai (n = 96)	30.91	3.16–302.05	0.003*
	H'Mong (n = 6)	8.00	0.5–127.90	0.141

* Statistically significant at P < 0.05

rest mainly sold it (Table 4). Most people (70.3%) rarely consumed maize, reporting they (23.57%) ate it only 2 or 3 times per year (with an average reported amount consumed each time being 316 g). Most participants (53.3%) did not remove visibly spoilt moldy maize before milling and 75.7% of participants did not de-husk maize kernels before cooking.

Discussion

This study found AFB₁ contamination in maize samples from all sampled districts, with more than half of the samples exceeding the level allowed. We found

Table 1. Prevalence of AFB₁ contamination in maize in Son La.

Districts (No. sample)	AFB ₁ level > 5 µg/kg		AFB ₁ level > 20 µg/kg		Mean*	Median*	Range (<LOD)
	Prevalence (%) (No. sample)	95% CI	Prevalence (%) (No. sample)	95% CI			
Mai Son (76)	65.8 (50)	54.0–75.3	54.0 (41)	42.1–65.5	73.7	41.0	<LOD–417.0
Moc Chau (75)	45.3 (34)	33.8–57.3	25.3 (19)	16.0–36.7	16.0	4.5	<LOD–66.8
Son La city (76)	64.5 (49)	52.7–75.1	50.0 (38)	38.3–61.7	45.8	34.3	<LOD–141.8
Thuan Chau (76)	76.3 (58)	65.2–85.3	52.6 (40)	40.8–64.2	26.6	26.7	<LOD–55.6
Yen Chau (75)	17.3 (13)	9.6–27.8	4.0 (3)	0.8–11.3	11.3	4.6	<LOD–69.4
Total (378)	54.0 (204)	48.8–59.1	37.3 (141)	32.4–42.4	37.3	19.4	<LOD–417.0

*Mean and median (µg/kg) were calculated from the sample above limit of detection (LOD = 1 µg/kg)
CI Confidence Interval.

Table 4. Practices associated with consumption of maize in Son La.

Characteristic	Definition	Proportion (%)
After harvesting maize in the field, what do you use maize for?	Human food	0.9
	Animal feed	58.0
	Sale	41.1
Do you remove visibly spoilt maize before milling?	Yes	46.7
	No	53.3
Do you de-husk maize kernel before cooking?	Yes	24.3
	No	75.7
If maize is used for human, how often do you consume the maize?	Rarely (2–3 times/year)	23.57
	Sometimes (1x/month)	6.08
	Never	70.34
If maize is used for animals, how often do you feed to animals?	Rarely (2–3x/year)	0.0
	Sometimes (1x/month)	0.0
	Usually (2–3x/week)	0.0
	Everyday	100.0

a variation between districts and the prevalence of AFB1 showed the lowest in Yen Chau. One possible explanation is that all maize samples in Yen Chau district were directly collected from the fields which may have less opportunities for contamination with aflatoxins (Figure 2) while in the other districts stored maize was sampled. In comparison, Mai Son, Son La city and Thuan Chau districts had a relatively higher level compared to the other two districts, possibly because of maize sampling from households.

The prevalence of AFB1 contamination in Son La is likely to vary from season to season. Our study conducted during the end of the rainy season showed relatively higher levels of AFB1 compared to the data from Lee et al. (2017) conducted during the dry season. Therefore, further study is necessary to evaluate the association between AFB1 contamination and climate risk factors in Vietnam.

Most people in Son La were aware of molds and their impacts on human and animal health. A recent study found that awareness of the specific problem of 'aflatoxins' in Son La was extremely low (only 1.09%) [17]. Our study shows that even though people are not familiar with the names of fungal toxins, they are aware that moldy food may have health impacts on animals and people which was consistent with a study conducted in Malawi [24]. In addition, we found that the Kinh ethnic group (accounting for 86% of the population across the Vietnam) had relatively higher awareness of mold health risks than other ethnic minority groups, perhaps related to better education [25]. One study showed that people with higher education had better knowledge/perception of food safety than those with low education [26]. Another study in Kenya showed a relatively high level of awareness of 'aflatoxins' (>90%) in areas subject to repeated outbreaks [27]. However, no outbreaks have been

officially reported in Vietnam, therefore the population is likely to be less aware. Our study suggests that ethnic minorities need to be more educated for enhancing public awareness of aflatoxins.

There was also a discrepancy between the high level of awareness of health effects of molds and the common consumption of animals fed moldy maize. Studies elsewhere also found that people consume moldy food even though aware of health risks, because of hunger and poverty [24,28].

Overall, in spite of the low number of people interviewed, our study indicates that maize is not commonly consumed as a staple food in Son La. but H'Mong people who are living in very remote areas had higher consumption compared to Thai and Kinh, but this may be biased by the low number of H'Mong (n = 6) that were interviewed. Because of the low consumption of maize by people in this study, aflatoxins are not likely to pose a significant health risk in Vietnam compared to African people who consume maize as a food staple with ranges from 52 to 328 g/person/day [29]. However, further investigation may be warranted to have better understanding of behavior and health impact of aflatoxins and other mycotoxins among H'Mong people.

Aflatoxins can be transferred to livestock products; however, relatively small amounts may be transferred to meat products. Further studies are needed to assess whether this exposure route poses any non-negligible health risk to people. Maize constitutes 70% of livestock feed in Vietnam [30]. Prolonged consumption of AFB1 can cause immunosuppression, acute gastrointestinal effects, lesion in liver and even death within 12 hours in pigs [31–34]. However, a national standard has not yet set the regulatory acceptable levels of AFB1 for animal consumption. Therefore, there is a need for further investigations into how the aflatoxins fed are affecting the livestock production, and the potential health impacts of aflatoxins on human and animal health through risk assessments, which is useful for the establishment of regulations.

The main limitation of this survey was that subgroups (for ethnic, age and occupation groups) had small sample size so the estimates had wide confidence intervals. In addition, due to the uneven distribution of samples from different steps in the value chain in the different districts, statistical comparisons were not done. It should also be noted that the questions around health risks associated with moldy maize may have been leading, and hence may have over-estimated concern.

In Son La, where the average annual temperature and humidity are 23°C and 79% [16], offering a favorable condition for development of molds, particularly for thermo-tolerant species such as *Aspergillus* spp [14,35,36]. mold contamination is difficult to avoid, and it is important with proper drying and storage of crops. In

Son La, moldy contamination producing AFB1 in maize is unavoidable in which the average annual temperature and humidity are 23°C and 79% [16], offering a favorable condition for development of molds, particularly for thermo-tolerant species such as *Aspergillus* spp [14,35,36]. Based on our observations, farmers in Son La were insufficiently aware of handling and storage of maize. Maize was often dried, spread or stored unshelled in heaps on the ground/floor; in some cases, under the verandah after harvesting.

We confirmed that they did not follow safe practices for the processing of maize samples. Another study found that farmers left maize on the soil after harvesting, and they did not understand that maize can be contaminated by molds through this contact [37].

Practical guidelines for proper handling of maize pre- and post-harvest should be widely disseminated to farmers, including information on separation between good maize and bad maize, and cleaning maize. In addition, improved storage facilities may reduce the risk of AFB1 contamination, and pest and insect control measures in maize need to be implemented widely. However, attention should also be paid to the gap between awareness and practice and this may require interventions aimed at behavior change through providing incentives or nudges. This study can conclude that maize in Son La is contaminated with AFB1 to varying degrees with relatively high levels during the rainy season. Control strategies are recommended as well as awareness raising and appropriate regulation for aflatoxins in animal feed in Vietnam.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Availability of data and materials

All datasets supporting our findings are available from the corresponding author on reasonable request.

Supporting information

Supplementary table 1 Awareness of molds among 107 respondents in each model corresponding questions with answers

Authors' contributions

Conceived and designed the experiments: XNTT, HNV and HSL. Performed the data collection and laboratory analysis: XNTT, TNTT, KTN and THM. Analyzed the data: XNTT and HSL. Wrote the paper: XNTT, HNV, JL, DG and HSL gave inputs and revised the paper. All co-authors approved the content of the paper.

Ethics approval and consent to participate

The study was approved by the Hanoi Medical University Institutional Review Board (HMU IRB: no. 00003121), Vietnam.

Competing interests

The authors declare that they have no competing interests.

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Viet Hung Nguyen's research focuses on the link between health and agriculture, food safety, infectious and zoonotic diseases with an emphasis on the use of integrative approaches (One Health and Ecohealth). He co-founded and led the Center for Public Health and Ecosystem Research (CENPHER) at the Hanoi University of Public Health (HUPH) in Vietnam until 2013. He is currently the regional representative for East and Southeast Asia and senior scientist in food safety and Ecohealth at the

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which are to contribute to towards reducing the economic losses by preventing or better dealing with climate sensitive disease outbreaks, antimicrobial resistance (AMR) use and food safety.

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