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Research Paper

Aflatoxin Proficiency Testing and Control in Kenya

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ABSTRACT

Texas A&M AgriLife Research (hereafter AgriLife) introduced a quality systems approach to accurately measure and manage aflatoxin that resulted in improved food safety for approximately 10 million Kenyans. A quality systems approach contains elements that ensure laboratory testing competence. In this study, quality system elements included analyst training and qualification, proficiency testing, use of reference material to support analytical traceability and define analytical uncertainty, development and implementation of a food safety plan by commercial maize (*Zea mays*) millers, and verification of testing accuracy at the AgriLife laboratory accredited by the Kenya Accreditation Service under the International Organization for Standardization/International Electrotechnical Commission 17025:2005 standard. In 2014 and 2015, five proficiency rounds were performed, ranging in aflatoxin concentrations of 5 to 40 μg/kg. Five laboratories had a *z*-score of >3, and all of these were for the fifth proficiency round with an aflatoxin content of 5 μg/kg. In 2015, 31 analysts qualified to participate in the program at 15 maize mills. The analysts' qualification for seven test samples, which ranged from 3.1 to 28 μg/kg total aflatoxin, resulted in an average relative standard deviation of 19.2% across all participants and test methods. Independent testing of participating mill verification results before and after analyst implementation of the quality systems approach revealed an improvement in measure accuracy.

HIGHLIGHTS

- Use of working controls improved aflatoxin testing in Kenya milling industry.
- Aflatoxin testing and qualification workshops prepared analysts.
- Validated aflatoxin test kits produced accurate test results in Kenya.
- Laboratory quality systems improved food safety for 10 million Kenyans.

Key words: Aflatoxin; Proficiency testing; Qualification

Aflatoxin is a toxic fungal metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Occurrence of this toxin is common in Kenya and throughout Africa *(18)*. Acute aflatoxicosis may lead to jaundice, low-grade fever, depression, anorexia, diarrhea, or death *(2)*. Fatal incidents in Kenya occurred in 2005 involving 125 individuals *(16)* and in 1981 resulting in 12 fatalities *(17)*. Aflatoxin is a group 1 carcinogen *(13),* and chronic exposure may lead to liver cancer. Emerging evidence also suggests a link between aflatoxin exposure and growth impairment during infancy *(11)*.

Kenya's annual per capita consumption of maize products is 88 kg/capita/year *(5, 19)*. The Kenyan maximum level (regulatory limit) for total aflatoxin in food products is 10 μg/kg and 0.05 μg/kg aflatoxin M1 in milk. Systems to manage aflatoxin risk within the private sector have begun to emerge, and there is no paucity of government agencies with some responsibility for regulating the toxin, including the Ministry of Health, Kenya Bureau of Standards, the Agriculture Food Authority of the Ministry of Agriculture, and county government's public health agencies *(10)*. Numerous efforts to assist Kenya and other African government and private sector entities have occurred through international donor agencies *(18)*.

In 2014, A&M AgriLife Research (hereafter AgriLife) launched Aflatoxin Proficiency Testing and Control in Africa (APTECA), a quality systems approach to accurately measure and manage aflatoxin. This quality systems approach uses principles within the International Organization for Standardization (ISO) 17025 *(14)* standard for testing competency. In particular, the APTECA program emphasizes analyst competence through demonstrating capability to accurately measure aflatoxin against a traceable standard with a validated test kit with defined uncertainty, participation in a proficiency testing program, use of reference material for analytical control, and participation in a co-laboratory verification activity per-

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formed by an ISO 17025–accredited laboratory. Aflatoxin test kits used in this program all met the validation criteria developed by the U.S. Department of Agriculture, Federal Grain Inspection Service *(6)*. Through accurate aflatoxin measurement, milling firms were able to ensure product was not introduced into commerce above the maximum level of 10 μg/kg established by the Kenya Bureau of Standards. The program mobilized the large-scale private sector millers who have organized themselves through Kenya's Cereal Millers Association. The principle focus of APTECA was the introduction of a quality systems approach to manage aflatoxin risk based on a similar program in the state of Texas *(9, 20)*. Characteristics of the quality system included development of a food safety plan, analyst qualification to accurately test for aflatoxin, use of working control material to ensure testing accuracy, participation in a proficiency testing program, and verification of testing result accuracy through use of an ISO/International Electrotechnical Commission (IEC) 17025:2005–accredited reference laboratory. Because of the intensive measurement of aflatoxin in this program, it lends itself to effectiveness evaluation. Aflatoxin risk management in Texas, through the introduction of a quality systems approach, improved testing accuracy by program participants as demonstrated by test results of working control samples, analyst qualification results, and verification of participant results in the Office of the Texas State Chemist ISO/IEC 17025:2005–accredited laboratory *(20)*.

The quantification of improved risk management by the private sector achieved through APTECA will enable regulatory risk managers in Kenya to assess the suitability of coregulation, which involves private–government sector collaboration in the adoption of a government-backed code of practices. In this article, we test the hypothesis that a quality systems approach to measure and manage aflatoxin risk is universal and is suitable for adoption in Africa. Such an effort expands the testing capacity for regulatory oversight and improves food safety. Toward this end, the research represents one building block in an effort to build a self-sustaining solution to protect the African community from this foodborne group 1 carcinogen.

MATERIALS AND METHODS

The APTECA program conducted by AgriLife explores the suitability of a quality systems approach to manage aflatoxin risk in Kenya through (i) aflatoxin proficiency testing, (ii) use of working controls to ensure testing accuracy, (iii) analyst qualification, and (iv) verification of mill test results in the AgriLife ISO/IEC 17025:2005–accredited laboratory.

Proficiency testing. APTECA hosted five rounds of a proficiency program designed to evaluate aflatoxin testing accuracy in Kenya that included two rounds in 2014 involving 5 and 8 laboratories and three rounds in 2015 involving 7, 12, and 16 laboratories. The assigned value reported in micrograms per kilogram units was determined by the Office of the Texas State Chemist reference laboratory by high-performance liquid chromatography with fluorescence derivatization (HPLC-FLD) analysis for aflatoxin in maize (*Zea mays*) described in our previous study *(3),* which was found to be highly correlated to aflatoxin test kits. The HPLC-FLD method is adapted from the AOAC International official method *(1)*. The assigned standard deviation (σ) was determined using the Horwitz function *(12),* which in the food sector determines fitness for purpose. Laboratories reported duplicate results (x) that were analyzed using the Grubbs test *(8)* and the Dixon outlier test *(4)*. The z-score was calculated based on the following equation:

$$z = (x - \mu)/\sigma$$

where a z-score ≤ 2 is satisfactory, a z-score >2 and ≤ 3 is questionable, and a z-score >3 is unsatisfactory.

Working control material production. The working controls were prepared in the AgriLife ISO/IEC 17025:2005–accredited laboratory in College Station, Texas, using naturally contaminated maize that was ground using an RAS mill (Romer, Union, MO), and a second grind was performed using an SR 300 rotor beater mill (Retsch, Haan, Germany) equipped with a 1.5-mm screen. The ground maize was mixed for 1 h in a commercial mixer (item 0241568, Kobalt, Mooresville, NC) and placed in 500-g plastic bottles. Aflatoxin concentration in the working control was measured from 12 bottles using 50 ± 0.25-g samples from each bottle and the average, standard deviation, and relative standard deviation (RSD) were calculated. The working control samples were stored in –20°C until needed.

Analyst qualification. Participants in an aflatoxin testing and qualification workshop received training through a demonstration of the test procedure accompanied by two practice analyses under instructor supervision in the morning. The aflatoxin test-kit platforms used in the workshop included the Neogen Q+, Neogen Veratox, and Vicam Aflatest. These kits are approved by the Federal Grain Inspection Service of the Grain Inspection, Packers and Stockyards Administration (GIPSA) *(6, 7)* based on validation criteria established by this agency. The Neogen Veratox test kit is an enzyme-linked immunosorbent assay (ELISA) and needs refrigeration. This kit is particularly useful for labs running a large number of tests and is a technology used in this region of Africa. The other two methods, Vicam Aflatest and Neogen Q+, use antibody technology and do not require refrigeration, enabling easy transport to remote locations where training may occur. The use of these three kits for the analyst qualification exercise was to offer a diversity of testing platforms presently available in the East African market. In the afternoon, participants analyzed seven previously tested samples with known aflatoxin concentration, and their test results were evaluated using the Dixon's Q test for outlier identification.

Verification of aflatoxin testing accuracy. Samples of miller tested maize were shipped to the AgriLife ISO/IEC 17025:2005–accredited laboratory in Nairobi, Kenya, for reanalysis. AgriLife personnel followed standard operating procedures for sample receipt, identification, and analysis. Samples were analyzed using the Vicam Aflatest procedure *(7)*. The Vicam test was selected for use at the AgriLife laboratory in Nairobi because it most closely resembles the HPLC-FLD reference method. In particular, the HPLD-FLD method uses the Aflatest affinity columns to purify the sample before separation and fluorescence detection. The Vicam platform is suitable over a wide range of matrices and can measure up to 1,000 μg/kg without dilution. A performance curve compared an acceptance probability by APTECA participants across 2 years according to the AgriLife aflatoxin test results for 2014 and 2015, assessing performance before (119 samples) and after (208 samples) adoption. Acceptance probability possesses potential type 1 (false-positive) and type II (false-negative) error.

TABLE 1. *Aflatoxin proficiency test results reported as z-scores across 16 laboratories during 2014 and 2015 and a comparison of mean results and RSD between Kenya laboratories and the AgriLife ISO 17025–accredited laboratory*

	Aflatoxin proficiency test *z*-score				
	Round 1	Round 2	Round 3	Round 4	Round 5
Lab no.					
1	1.23	0.07	1.42	0.97	1.03
2	0.33	0.00	1.30	0.63	3.28
3	1.51	0.49	0.20	0.70	0.99
4	0.60	0.70	0.68	0.49	−0.03
5	2.41	0.57	1.46	0.75	1.38
6		0.61	0.97	0.28	0.00
7		1.54	1.78	1.30	3.06
8		1.51		0.83	5.16
9				0.57	3.38
10				0.88	0.69
11				1.14	4.19
12				0.38	−0.16
13					5.00
14					−0.63
15					−0.22
16					0.72
OTSC[a] assigned mean	21	12	40	29	5
OTSC assigned RSD	29	31	26	27	36
Participant mean	20	12	29	25	7
% coefficient of variation	47	29	24	19	43

[a] OTSC, Office of the Texas State Chemist.

The purpose of the performance curve was to highlight the reduction of both types of error after the adoption of a quality system.

RESULTS AND DISCUSSION

Proficiency testing. The project in Kenya began through face-to-face visits with commercial maize millers during which they were requested to analyze known samples of aflatoxin-contaminated ground maize. After mill personnel provided their test results, the known value was given to the participant. This type of informal proficiency test led to the first formal proficiency test conducted in the summer of 2014 with five Kenya mills (Table 1). The assigned value was 21 μg/kg, and an assigned relative standard using the Horwitz function was 29%. The participating mill result mean was 20.2 μg/kg (under-measured the assigned value by 0.8 μg/kg), and the RSD was 47%. Participation increased over the course of four additional rounds of proficiency tests, to 16 labs by round 5. The actual RSD values by the proficiency test provider were 12.3, 17.7, 10.7, 12.9, and 13.9% for rounds 1, 2, 3, 4, and 5, respectively. For rounds 2 to 5, the participants' RSD was approximately double that of the test provider's RSD%.

Round 5 tested the capability of mills to accurately measure aflatoxin at the lower end of most test kits' accuracy range of 5 μg/kg. The participants' average result was 2 μg/kg higher (40%) than the assigned value. Six of the 16 labs involved in round 5 reported results leading to a *z*-score >3, indicating unsatisfactory performance. Three of these poor results could be explained by use of a test kit that was not validated by GIPSA; the others appeared to be dilution errors based on conversations with laboratory analysts. These results highlight the need to provide training in root cause analysis, to encourage mills to continue participating in proficiency testing programs and results verification, and to adopt test kits that, although slightly more expensive, are more accurate.

Proficiency test RSD values were comparable to what was observed in other proficiency testing programs, such as the programs conducted by the Association of Official Oilseed Chemists and the Association of American Feed Control Officials. For example, the Association of Official Oilseed Chemists program yielded RSD% for aflatoxin levels of 6.9, 13.4, and 25.3 μg/kg that were 37.6, 33.2, and 21.5%. The Association of Official Oilseed Chemists program uses its reported RSD% to calculate the *z*-score rather than the Horwitz function used by the Office of the Texas State Chemist.

The ISO/IEC 17043:2010 standard offers a number of options for reporting proficiency test results, including using consensus values and assigned values *(15)*. APTECA used the assigned value at the request of program participants. The selection of the standard deviation, upon which the *z*-score is calculated, is less flexible. The use of the consensus standard deviation could result in overly generous *z*-scores *(21)*. In contrast, the use of the actual standard deviation by the proficiency provider would result in too narrow of a tolerance when calculating the *z*-score, assuming the proficiency providers RSD is approximately half that of the program participants. If the consensus mean

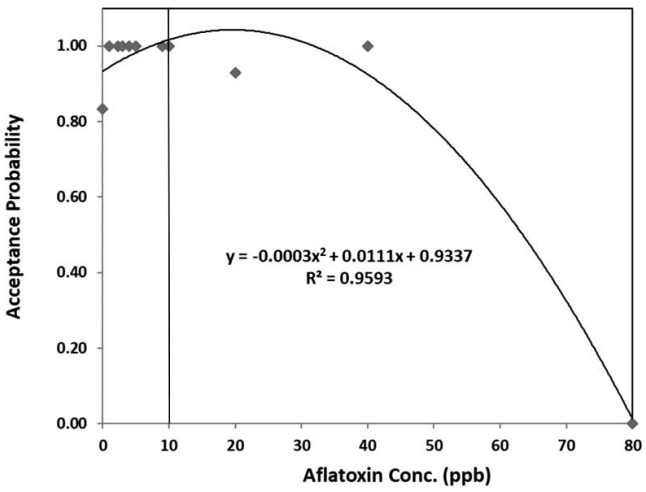

FIGURE 1. *Performance curve before APTECA implementation comparing acceptance probability by aflatoxin concentrations at Kenya flour mills compared with the AgriLife ISO 17025–accredited laboratory using retained samples in 2014 and 2015.*

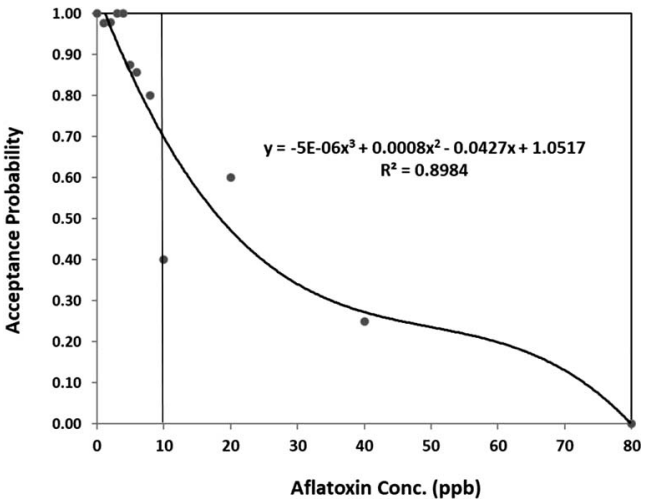

FIGURE 2. *Performance curve after APTECA implementation comparing acceptance probability by aflatoxin concentrations at Kenya flour mills compared with the AgriLife ISO 17025–accredited laboratory using retained samples in 2014 and 2015.*

and consensus standard deviation were used to calculate *z*-scores, there is a greater likelihood that none of the *z*-scores would be >3, thus compromising the purpose and benefit of a proficiency testing program.

Analyst qualification. All mills participating in the proficiency program sent laboratory analysts to the aflatoxin testing and qualification workshop (Table 2). The Dixon test results are reported by sample and test kit for the participants and instructor. Although all students passed the qualification portion of the program, the precision of the test results varied by kit. To qualify, students either needed to demonstrate no significant difference in their test results from those of the instructor based on the pair *t* test results, and/or the mean difference must be <2 times the standard deviation of the reference material results. The most variation was observed in the Neogen Veratox kit, an ELISA kit. This testing platform was offered because many of the mills were using an ELISA-based aflatoxin test kit, although the one most popular in the Kenya milling industry was not validated or approved using the GIPSA method. The advantage of the Veratox kit is its capability to analyze a large number of samples in batches, making it ideal for use in a regulatory laboratory. The Vicam Aflatest and the Neogen Q+ demonstrated comparable precision in aflatoxin testing. Because neither of these tests require refrigeration, they have been used in other workshops at remote sites, including workshops in Kigali, Rwanda; Kampala, Uganda; Dar es Salaam, Tanzania; Moshi, Tanzania; Blantyre, Malawi; and Lilongwe, Malawi.

Laboratory verification. Further verification of mills' aflatoxin test results of finished product occurs at the AgriLife laboratory. Performance curves comparing results before (119 samples; Fig. 1) and after (207 samples; Fig. 2) adoption of the APTECA protocol document an improvement in testing accuracy resulting from this program. The performance curve indicates that before adoption of the APTECA protocol, many of the labs undermeasured aflatoxin content. Although this may reduce sellers' risk, it provides companies a false impression that they are producing a low-aflatoxin product and indicates that contaminated product is entering the market even after being measured in a laboratory. After the analyst qualification, the performance curve began to resemble an equal distribution of false positives (type I error) and false

TABLE 2. *Difference in analyst qualification measured as a percent coefficient of variation (%CV) comparing test results between students and instructors at eight aflatoxin concentration levels and three test kits*

		Mean		
Kit	Sample	Instructor	Student	%CV
Veratoxin	1	32.8	35.7	14.2
	2	3.1	3.4	39.0
	3	3.5	4.1	30.0
	4	24	26.5	15.4
	5	19.6	20.9	20.8
	6	14.6	15.6	23.9
	7	5.8	6.1	26.4
	8	23.1	22.6	28.5
Vicam	1	28	27.8	6.2
	2	3.5	3.5	29.8
	3	3.2	3.4	18.3
	4	24	24.2	10.6
	5	19	19.0	10.5
	6	13	13.3	10.2
	7	5.7	6.4	7.9
	8	33	28.8	22.0
Q+	2	5	4.3	24.4
	3	5.8	5.7	14.5
	4	25	27.8	15.3
	5	15.6	16.8	19.9
	6	16.2	14.8	21.8
	7	7.7	7.6	18.9
	8	27.4	26.5	13.3

negatives (type II error). It also conformed to the type of distribution reported in Texas after the introduction of a similar program by the Office of the Texas State Chemist *(20)*.

In summary, a quality systems approach improved testing accuracy and precision to measure and manage aflatoxin in Kenya, although improvement seemed to regress during the fifth round of the proficiency testing. Mills participating in this program represent approximately 80% of the milling capacity of the formal maize-milling sector with a customer base of approximately 10 million Kenyans. The introduction of a quality systems approach to measure and manage aflatoxin, which worked in Kenya, could lead to the adoption of coregulation that is a governance option that uses government-backed standards adopted by industry, leading to shared responsibility to manage aflatoxin risk in Kenya and elsewhere in the region.

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