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Prevalence of Aflatoxicosis in Broiler Chickens in Quetta, Pakistan

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Abstract.- A base line study was conducted to determine the prevalence of aflatoxicosis in broiler chickens in and around Quetta district during the period June 2009 to May 2010. The study was based on the examination of sick and dead birds (n=1105 broiler chickens). Congested and oedematous carcass, hemorrhages, yellowish brown or pale liver; swollen kidneys; atrophy of bursa and thymus were the common necropsy findings. Thin layer Chromatographic analysis of liver samples of suspected birds was carried out for the confirmation of aflatoxin. Aflatoxin contents of analyzed samples ranged between 3.0 - 11.7 µg/kg. It was recorded that 8.78% (n=97) birds were aflatoxicosis positive. The highest season wise prevalence was recorded in autumn (13.29%) and the lowest in winter (5.1%). It is concluded that existing situation of aflatoxicosis need strict surveillance to monitor the problem. There should be a campaign among public to create awareness about toxicological effects of aflatoxins and development of good laboratory facilities for confirmation of disease. Further use of aflatoxin ameliorators should be promoted to limit the problem.

Key words: Prevalence, aflatoxicosis, broiler, aflatoxins, mycotoxins, toxigenic fungi.

INTRODUCTION

Mycotoxins are structurally diverse, low molecular weight secondary metabolites produced by toxigenic fungi under specific environmental conditions (Charoenpornsook and Kavisarasai, 2006). They can be produced in various food crops before, during and after harvesting (Anjum *et al.*, 2011). Frequently affected crops include cereals, oilseeds, spices and tree nuts. It is estimated that approximately one quarter of the world's food supply is annually affected by mycotoxins (Azab *et al*., 2005). More than 480 compounds are documented as mycotxins with a greatest significance to aflatoxins (Saif, 2003).

Aflatoxins are a group of closely related frequently occurring myctoxins mostly produced by the toxigenic strains of *Aspergillus flavu*s and *Aspergillus parasiticus*. These are considered unavoidable natural contaminant of food and feed causing serious health effects on human and animals (Oguz and Kurtoglu, 2000; Anjum *et al*., 2012). Aflatoxins exhibit a wide array of biological effects and can be mutagenic, carcinogenic, embryotoxic, nephrotoxic, estrogenic and immunosuppressive even in very low concentrations (Jayabarathi and Parveen, 2010; Muhammad *et al*., 2010). Aflatoxin contaminated diet may lead to huge economic losses in poultry due to reduced weight gain, interference with reproductive performances, impaired feed conversion and increased mortality (Oguz, 2012).

Clinical signs and symptoms referred to aflatoxicosis in poultry include in-appetence, un-even and retarded growth, abnormal vocalization, feather picking, pale shanks and combs, ruffled feathers, depression, bruising, ataxia, catarrhal enteritis, passage of undigested feed particles, reduced bone strength leading to leg weakness and lameness, convulsions and opistotonous preceded death. While at necropsy, congested carcase with pale, oedematous and enlarged liver and kidney; in chronic cases hydropericardium and ascites, shrunken firm nodular liver, bile distended gall bladder and hemorrhages are also reported (Anjum, 1997; Saif, 2003; Dhanasekaran *et al*., 2009).

Pakistan has diverse (cold, hot and humid) type of climate in different areas and during different periods of the year, which is likely to enhance chances of occurrence of mycotoxins in poultry feed. In Pakistan, aflatoxins has already been reported in poultry feed and feed ingredients (Anjum *et al*., 2012; Rashid *et al.*, 2012) causing aflatoxicosis. The prevalence of aflatoxins in poultry feed is regularly monitored and reported

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from many countries including Brazil, Colombia, India, Malaysia, Nigeria, and Saudi Arabia (Cespedes and Diaz, 1997; Alkhalaf *et al*., 2010; Anjum *et al*., 2012). However, information regarding prevalence of aflatoxicosis is scanty in this part of country. Keeping in view the importance of aflatoxicosis, present study was designed to determine the prevalence of aflatoxicosis in and around Quetta district.

MATERIALS AND METHODS

Study area

A base line study was conducted from June 2009 to May 2010 in and around Quetta district to determine the prevalence of aflatoxicosis in broiler chickens. Quetta district is situated between 30°15 North latitude and 66°55 East longitudes, at an altitude of 1,675 meters in the North West part of Balochistan and at the West Central Pakistan. During the data collection period the average minimum and maximum temperatures were 9.63±8.75°C and 26.07±9.08°C respectively; the total rain rainfall was 215.6 mm and average humidity was 46.83±3.06%. The Quetta district was selected, as it is not only the capital city but also the most populated district inhabitant of almost half of urban population of Balochistan, the largest province comprising the 45% of land mass of Pakistan. There are approximately 150 broiler farms where approximately 945 flocks of size range from 1000 to 6000 are reared annually.

Prevalence of aflatoxicosis in broiler chicks

Seasonal prevalence of aflatoxicosis in broiler chicks was determined adopting the methodology suggested by (Anjum, 1990). Briefly the duration of study from June 2009 to May 2010 was divided into four seasons namely summer (June to August), autumn (September to November), winter (December to February) and spring (March to May). The data of 1105 cases submitted to Disease Investigation Laboratory, Livestock and Dairy Development Department Quetta and veterinary surgeons of private sector providing veterinary services was probed to find out the prevalence of aflatoxicosis. Diagnosis of the problem was based on history, clinical signs and postmortem examination of sick and dead birds whereas liver samples of suspected cases were analyzed chemically to confirm the presence of aflatoxin (AOAC, 2000). The data was analyzed to estimate the prevalence using Chi square test. Season wise comparison was made through Kruskal-Wallis test. Computer software SPSS 16 for windows and Excel 2010 were used for computation of data.

RESULTS

Prevalence of aflatoxicosis in broiler chickens revealed a significant association ($P<0.05$) among seasons (Table I). Over all prevalence during the year was 8.8% with frequency of 97/1105. The highest prevalence was observed in autumn 13.3% followed by summer 7.8% and spring 5.3%, whereas it was least in the winter 5.1%. The analysis of data revealed significantly higher prevalence of aflatoxicosis during autumn in comparison to summer, winter and spring ($P<0.05$). However, non-significant association was noted between summer, winter and spring ($P>0.05$).

Table I.- Prevalence of aflatoxicosis in broiler chicken in Quetta, Balochistan

Season	Cases reported	Positive cases*	Prevalence of positive cases (%)		
			Within season	Within cases reported	of total
Summer 2009	437	34	7.8	35.1	3.1
Autumn 2009	346	46	13.3	47.4	4.2
Winter 2009-10	78	4	5.1	4.1	0.4
Spring 2010	244	13	5.3	13.4	1.2
Total	1105	97	8.8	100	8.8

*Positive cases: number of birds affected with aflatoxicosis

The history and clinical signs of sick/dead birds revealed that 67% of the farmers/owners used pharmaceuticals (self-medication) without consulting the poultry expert. Further it was recorded there was ineffectiveness of antibiotic therapy and/or mineral vitamin supplementation, uneven and impaired growth, poor appearance, reduced feed intake, abnormal vocalization,

paleness of comb and Shank, ataxia and varying degree of mortality (Table II).

Table II.- History/clinical signs of broiler chickens (n=97) associated with aflatoxicosis

History/Clinical signs	Frequency*	%
No effect of antibiotic therapy and/or mineral vitamin supplementation	67	69.07
Un Even and impaired Growth	97	100
Abnormal vocalization	22	22.68
Ataxia	18	18.55
Pale shank /Comb	83	85.56
Poor Appearance	91	93.81
Mortality	97	100
Reduced feed intake	97	100
Diarrhea	68	70.10

*Birds having the history/sign out of total aflatoxicosis positive (n=97) birds.

Table III.- Post-mortem findings of broiler chickens (n=97) associated with aflatoxicosis.

Organ	Post-mortem findings	Frequency*	%
Abdominal Cavity	Ascites	12	12.37
Carcass	Congested & Oedematous	89	91.75
Sub Cutaneous Tissues & Internal Organs	Hemorrhages	92	94.84
Liver	Multiple	97	100
Kidney	Enlarged	94	96.84
Spleen	Enlarged	91	93.81
Pancreas	Enlarged	88	90.72
Gall Bladder	Distended	92	94.84
Heart	Hydroparicardium	44	45.36
Bursa	Atrophy	94	96.90
Thymus	Atrophy	87	89.69

*Birds having the lesions out of birds (n=97) positive for aflatoxicosis

Post-mortem examination of aflatoxicosis positive birds (n=97) showed pathological changes in gross lesions including congested and oedematous carcass; hemorrhages on subcutaneous tissues and internal organs; friable, fatty, enlarged, hard, yellowish brown, pale, livers; swollen kidneys; enlarged pancreas; enlarged spleen; distended gall bladder; congestion of myocardium, hydropericardium; atrophy of bursa and thymus. In some cases ascites was also present (Tables III, IV).

The laboratory report of the liver samples of aflatoxicosis suspected birds revealed aflatoxin B_1 contents ranged between 3.0-11.7 µg/kg with mean value of 5.29±0.18 µg/kg.

Table IV.- Post mortem findings of liver associated with aflatoxicosis (n=97)

Parameters	Findings	Frequency*	%
Size	Enlarged	79	81.44
Colour	Pale	82	84.53
	Yellowish brown	15	15.46
Consistency	Friable	61	62.88
	Hard	18	18.55
Appearance	Congestion	64	65.97
	Fatty	25	25.77

*Birds having hepatic lesions out of birds (n=97) positive for aflatoxicosis

DISCUSSION

Prevalence of Aflatoxicosis in broiler chicks is not an unusual phenomenon, several researchers (Giasuddin *et al*., 2002; Yunus *et al*., 2008; Ahmed *et al*., 2009) carried out surveys in different parts of the world and reported the problem 27.59, 3.6, and 5.53%, respectively. The results of present study revealed highest prevalence in autumn season. This is in agreement with Yunus *et al*. (2009) who reported highest incidence of mycotoxicosis during July to September and correlated it with monsoon (Hot season with heavy rain fall commences from July and remains up to September) season. Poultry feed production mills and feed ingredients used for the formulation of poultry feed in Pakistan are mostly located/cultivated/produced in the monsoon region where environmental conditions like topography of land, relative humidity, ambient temperature, rain fall and wind velocity are conducive for the growth of aflatoxin producing fungi especially in summer and autumn. Invasion of aflatoxigenic fungi to the substrate (feed or feed ingredient) in the presence of favourable environmental conditions results in rapid proliferation of aflatoxin producing fungi as an outcome release of aflatoxins. This aflatoxin contaminated feed or feed ingredient is a potential risk for future out-breaks of aflatoxicosis in the

exposed poultry.

History and clinical signs of aflatoxicosis affected birds in the present study are in close agreement with the findings of Agag (2004) and Shareef *et al*. (2008). The present study highlights the clinical signs based on field aflatoxicosis whereas reports on this aspect in the literature are rare. It was observed that farmers/owners initially tried to treat the bird at their own (self-medication) and when the situation became out of control or losses become severe the poultry expert was consulted to handle the problem. Situation needs extensive extension work for farmers' education.

In the present study post-mortem findings of affected birds are in line with Shareef *et al*. (2008) and Mubarak *et al*. (2009). The slight variation in the observation of researchers might be due to the difference in concentration of aflatoxins in the feed or variation in the duration of exposure, age, sex, breed, nutritional and health status of birds (Hasan *et al*., 2010).

The analysis of aflatoxin residues in liver of poultry following ingestion of dietry aflatoxins has been cited in the literature (Hussain *et al*., 2010). The liver being a principal target organ of aflatoxicosis and main site of biotransformation of aflatoxins (Denli *et al.*, 2009) is capable to accumulate aflatoxins. Confirmation of aflatoxicosis by the estimation of aflatoxin contents in the liver might be an efficient and reliable tool. Sampling of feed for analysis to confirm the presence of aflatoxins is in practice (Banday *et al*., 2006). It is known that in the natural conditions aflatoxins are not equally distributed in the whole feed. The feed analysis might present false positive or negative results. In the situations Gastrointestinal tract contents, tissue or blood sampling might be the method of choice for the confirmation of aflatoxins poisoning. In the present study liver aflatoxin residue level ranged from 3.0 to 11.7 µg/kg, whereas in the previous studies Denli *et al*. (2009) and Hussain *et al*. (2010) mentioned mean liver aflatoxin residue level of 0.166 and 3.84 µg/kg respectively. These variations in aflatoxin residue levels might be due to variation in the concentration of ingested aflatoxin, difference in the rate of absorption, biotransformation and excretion processes of bird (Dersjant-Li *et al*., 2003).

Aflatoxicosis in poultry in the field or natural conditions is an outcome of ingestion of aflatoxin polluted feed or feed stuff, produced by attack of aflatoxigenic fungi, so the risk of aflatoxicosis could be reduced by preventing proliferation of mould growth in feed and feed stuffs to limit aflatoxins production. Controlling insects, use of genetically resistant verities of crops, biological control by introducing non toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* to soil, improvement in pre harvest and post-harvest practices, physical separation of damaged and infected grains, improvement in storage and transportation conditions, addition of mould inhibitors to the feed (Bankole and Adebanjo, 2003; Bintvihok and Kositcharoenkul, 2006, Bhat *et al*., 2010) could manage aflatoxin production. At the part of farmers end detrimental effects of aflatoxin contaminated feed could be neutralized by the use of aflatoxin ameliorators. In this regard bentonites, clinoptilolite, zeolite, montmorillonite, alluminosilicates, activated charcoal (Oguz, 2011) yeast (Hashmi *et a*l., 2006), probiotics, prebiotics and synbiotics (Hashem and Mohamed, 2009) were used in different parts of world with promising results.

To develop control and eradication strategies for a disease initial epidemiological assessments are very much important and provide baseline data. In conclusion prevalence of aflatoxicosis in broiler chicken should be observed vigilantly as contamination of feed stuff with aflatoxins is unavoidable and serious problem world is facing. Aflatoxicosis control programe, including periodic surveillance to update the existing situation, extension work to create awareness regarding toxic effects associated with aflatoxin poisoning in human and poultry; as there is every possibility that the toxin will enter the human food chain and promotion of use of aflatoxin ameliorators in feed should be launched. Further legislation and implementation of laws with regard to aflatoxin contamination has to be enforced. Emphasis should be laid towards development of newer low cost and time effective aflatoxin detection instruments, which are portable, reliable, and easy to handle at field levels can lead to limit the effect and bio proliferation of aflatoxins.

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