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Analyses of Selected Heavy Metals and Aflatoxin M1 in Milk for Human Consumption in Jhang City, Pakistan

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ABSTRACT

Chemical contaminants in milk affect public health and levels above permissible limits can constrain exports under sanitary and phyto-sanitary agreements. A screening survey was conducted during 2012-2013 to determine concentrations of Copper (Cu), Lead (Pb), Cadmium (Cd), Chromium (Cr), and Aflatoxin M1 (AFM1) in unprocessed, non-branded liquid milk available at conventional milk shops in Jhang city of Punjab. For the heavy metals mentioned above, samples were also collected directly from dairy herds near a wastewater drain in suburbs of the city. Concentrations of the studied contaminants were compared between winter and summer samples. Median concentrations of Cu, Pb, and Cd were significantly higher than the standards of the International Dairy Federation and levels in a very high percentage of the samples exceeded these standards, however, there was no permissible level available for Cr to make a comparable analysis with. Within each season, levels of Cu, Pb, and Cr differed significantly in milk collected from shops and dairy farms. For each type of milk source, there was a statistically significant difference in mean concentrations of Cu, Pb, and Cr between summer and winter. The AFM1 levels in 17% of samples were higher than the maximum tolerance limit accepted by the United States (0.50 µg/L). AFM1 levels were significantly high in the samples with median concentrations of 0.333 and 0.416 in summer and winter, respectively. The findings, i.e. detection of heavy metal contaminant levels above permissible limits in most samples, warrant continuous monitoring of those contaminants and a policy for their control. The levels of AFM1 in samples indicate that feed for animals was contaminated with aflatoxin B1- the precursor of AFM1. The sampling methodology adopted in this study can be a template for executing similar surveys is urban areas of developing countries (and informal markets) where a sampling frame of shops is generally not available. We recommend surveillance of aflatoxin B1 in commercial concentrate feeds and industrial waste management.

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INTRODUCTION

Livestock plays an important role in the national economy of Pakistan. It contributes 11.2% to GDP, and its share in the value of all agricultural commodities is 52.10%. Livestock is one of the major sources of livelihood for small marginalized and landless stockowners. Over 8 million families are directly involved with production and marketing of livestock. Several products from livestock are part of the food basket and a source of high quality nutrients. However, among all livestock products, milk is the most important, and in many rural areas perhaps the only source of nutrition, especially for children (Intercooperation, 2009).

Dairy farming in Pakistan is practiced mainly by the private sector on various scales in both urban and rural settings. However, the sector is generally characterized as fragmented and subsistence. With the exception of some peri-urban units, most dairy farming is practiced in mixed crop-livestock systems. The share of rural milk production in the total national production of 33 billion liters is 71%. The remaining comes from peri-urban and urban producers. Of the total milk produced, 97% is in the informal sector (i.e. milk consumed in the villages and/or sold in the cities through "doodhis" in unhygienic conditions). Only 3% of milk is processed and marketed through formal channels. The UHT milk market is growing. There are 24 units processing fresh as well as dry milk in the private and corporate sector (Intercooperation, 2009; Zia et al., 2011). The detailed description of the milk marketing set up is given in the Annex.

Milk consumers in Pakistan are often faced with low-quality, adulterated milk. Lack of hygiene, adulteration with various agents, and absence of cold chains are primary contributors to this low quality. The various chemical contaminants in milk include heavy metals, mycotoxins, pesticides, and antibiotics. Intake of heavy metals through the food chain by human populations has been widely reported throughout the world. Due to their non-biodegradable and persistent nature, heavy metals are accumulated in vital organs in the human body such as the kidneys, bones and liver and are associated with many serious health disorders (Singh et al., 2010). Animal feed, drinking water, and environmental exposure (e. g. irrigation of agricultural land with sewage and industrial wastewater) might be the source of heavy metals in animal products including milk (Awasthi et al., 2012). Milk and dairy products also become contaminated during manufacturing and packaging processes (Abdulkhaliq et al., 2012). Studies have been published reporting levels of heavy metals in milk sampled from urban shops (Gashu, 2010; Navarro-Blasco and Alvarez-Galindo, 2005; Qin et al., 2011), farms, and near wastewater irrigation sites (Aslam et al., 2011; Gonzalez-Montana et al., 2012; Singh et al., 2010). Aslam (2010) measured concentration of heavy metals in milk from cows and goats along a sewage water drain passing through Faisalabad city of Punjab province in Pakistan. The same drain receives effluents along its route across other districts before it falls into a river on the outskirts of Jhang city (Figure 1). Khan et al., (2013) investigated concentration of lead (Pb) in soil, forage, and milk in a district of the central Punjab. The researchers found abnormal level of Pb and pointed out temporal variation. At present, there are no reports on heavy metal contamination from milk being informally sold in urban areas and random sampling using geographic information system.

Aflatoxins are secondary metabolites of Aspergillus flavus and Aspergillus parasiticus. These two moulds occur in cereals and oil seeds - the major concentrates in ruminant diet. Their growth is influenced by various factors like temperature, relative humidity, oxygen availability, and damaged or broken grain kernels (Awasthi et al., 2012). Aflatoxin B1 (AFB1) present in feed of lactating animals gets transformed to 4-hydroxylated metabolite and is excreted in milk as aflatoxin M1 (AFM1). The fungal toxin is resistant to high temperature treatments including pasteurization and sterilization. The presence of AFM1 in milk poses a major risk for humans, especially children, as it can have immunosuppressive, mutagenic, teratogenic, and carcinogenic effects (Sefidgar et al., 2011). Although AFM1 is less toxic than AFB1, it has been classified as a possible human carcinogen, Group 2B agent by the International Agency for Research on Cancer. There have been several studies reporting AFM1 concentrations above permissible levels in milk samples from different countries of the world including Pakistan (Hussain et al., 2008; Iqbal et al., 2011; Muhammad et al., 2010; Salas et al., 2003).

Figure 1: Industrial and urban wastes being used for agriculture in and around Jhang

Keeping in view the public health impact, and considering it a constraint to exports, the Nestlé Corporation in Pakistan has launched a project to monitor AFM1 in milk at its collection centers, but results are not published. Despite studies in the past, there is no up-to-date information about AFM1 levels in milk after an increase in the number of small unregistered companies preparing formulated compound cattle feed locally called "wanda" - a potential source of mycotoxin in milk (Figure 2).

Figure 2: Unregulated trade of commercial formulated concentrate feed (wanda) is a potential cause of AFM1 in milk of dairy animals

Problem statement

Pakistan is the fourth largest milk producing country located in a milk deficient region. It has rapidly increasing demand and competition in national and international markets. Milk consumers in Pakistan are often faced with low-quality, adulterated milk. Malicious chemicals in various farm inputs, agriculture and drinking water, environmental pollution, and use of veterinary drugs lead to excretion of residues in milk. However, there is little data on the content of these chemicals in milk nor is there a system for their effective surveillance. There are a few studies on levels of important chemical contaminants in unprocessed, non-branded liquid milk available at conventional milk shops in various cities. The objective of this project is to quantify residues of selected heavy metals and AFM1 in milk sold on street shops in Jhang city (Figure 3) using an innovative sampling technique and in light of recent developments in the dairy sector.

Figure 3: Traditional milk shops in urban areas selling milk and milk products informally

Hypotheses maintained and to be tested

- The median concentration of residues of any selected[1] chemical contaminant in unprocessed, non-branded liquid milk available at conventional retail shops of Jhang city is more than maximum permissible level set by international standards.
- For any selected chemical contaminant, there is an association between proportion positive samples[2] and season.

MATERIALS AND METHODS

Description of the study area

Jhang (Urdu: جھنگجھنگ :Punjabi ,), is the capital city of district Jhang located in central Punjab; the milk belt of Pakistan. It is situated where the rivers Jhelum and Chenab join each other. It is a neglected area, however it is rich in livestock and focused on improvements in the dairy sector; particularly the Sahiwal breed of cattle. The city has

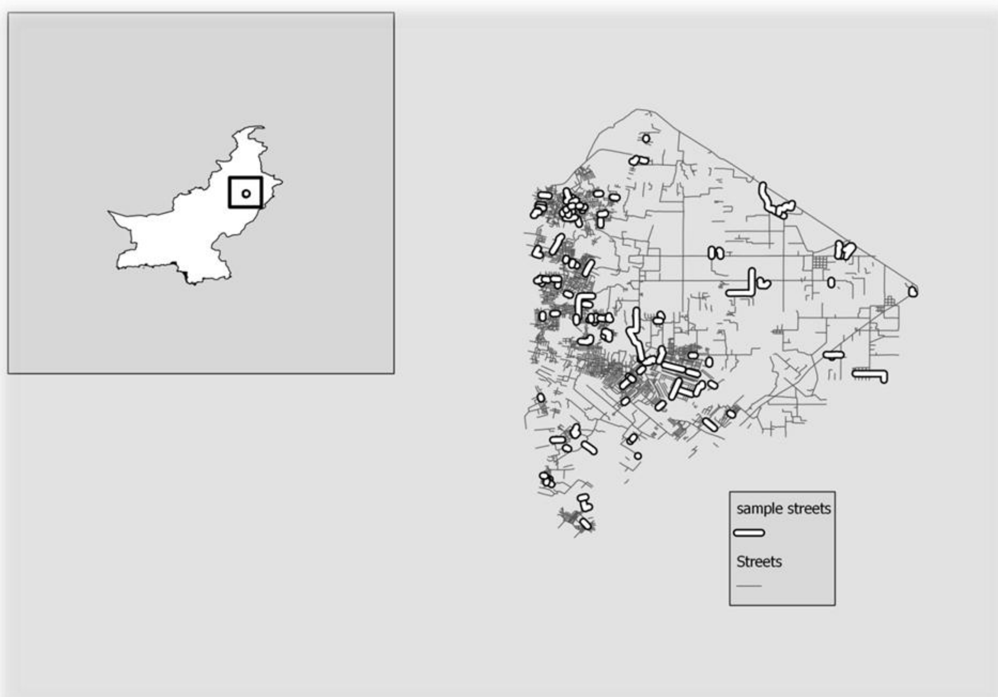
[1] Selected for inclusion in the survey
[2] Samples with content more than maximum residue level

been purposively selected because its dairy production and marketing is representative of the whole country. In 2006, The College of Veterinary and Animal Sciences was established. The college intends to make Jhang a model district and central for livestock related research.

Sampling design

In developing countries with informal markets, a sampling frame of shops in urban areas is generally not available and is difficult to construct. GIS based selection of administrative areas of a city (even use of random geographic coordinates) are relatively better options for sampling in surveys for monitoring quality of animal products in such markets. From the shapefile of Jhang city, one hundred streets were randomly selected and geo referenced in Quantum GIS Lisboa version 1.8.0 (Figure 4). Within each street, one milk shop was selected. Systematic sampling was also performed to recruit one hundred households (HH) of non-commercial livestock farms located within about a 3 km buffer around wastewater drains in peri-urban areas of the city. The sampling of shops and farms was done during winter (November, 2012 to January, 2013) and summer months (May, 2013 to July, 2013). Each shop/HH was sampled once in each season. Each month of the winter and summer sampling seasons, we made two visits. During each visit 15-20 shops and 10-20 HH were sampled. At each location, a total of 250ml of unprocessed liquid bulk milk was purchased, out of which 25ml was sampled in clean sterilized screw capped glass tubes. Immediately after collection, milk samples were transported to the laboratory by placing the tubes in ice packs and stored at -20 °C until further analysis.

Figure 4: Map of streets included in sampling



Analytical methodology

After wet digestion with nitric acid, the concentration of metals in samples was determined by atomic absorption spectrometer with Zeeman-effect background correction (AAS-Perkin Model: AA analyst 300). Standard calibration solutions were prepared by diluting commercially available stock solution (Applichem®) using purified de-ionized water. All the glass apparatus was immersed in 8N HNO3 overnight and washed with de-ionized water prior to use throughout the process of analysis. Three different quality assurance standards other than working calibration standard solutions were prepared and analyzed at the start of assay on daily basis. All the quality assurance parameters were observed according to the AOAC (2000). The AFM1 in milk samples with assayed through competitive colorimetric ELISA (Aflatoxin M1 ELISA Test Kit by IDDEX). Five standards were run and R2 was more than 90%.

Data analyses

The data was analyzed in SPSS version 21. The data was not normal and could not be transformed, therefore non-parametric tests were used. Wilcoxon signed rank and Chi square tests were performed to test hypotheses.

RESULTS AND DISCUSSION

Main findings of the survey are summarized in Table 1, Figure 5 and Table 2. Median concentrations of Cu, Pb, and Cd were significantly higher than standards of the International Dairy Federation (IDF).[3] However, there was no permissible level available for Cr with which to make a comparable analysis. Within each season, levels of Cu, Pb, and Cr differed significantly in milk collected from shops and dairy farms near a wastewater drain canal. For each type of milk source, there was a statistically significant difference in mean concentration of Cu, Pb, and Cr between summer and winter samples. Aslam et al. (2011) studied uptake of heavy metal residues from sewerage sludge in the milk of goat and cattle during summer season. They concluded that the levels of Cd, Cr, Ni, Pb, as and Hg in the milk of goat and cattle were higher than reported values in the literature. Similar findings were obtained from studies from India (Singh et al., 2010), Palestine (Abdul khaliq et al., 2012), and Egypt (Malhat et al., 2012). Heavy metals are added into the environment in large quantities through atmospheric deposition, solid waste disposal, sludge application, and wastewater irrigation. Food animals reared on contaminated fodder become a continuous source of heavy metal residues in edible tissues and milk. Heavy metal contamination in meat and other edible tissues is a matter of great concern for food safety and human health.

Concentration of Cu was significantly more in milk from urban shops and during the summer. In winter, the proportion of abnormal (exceeding the international standard) samples was more in milk from the market. All samples collected near drains during summer contained Cu above the permissible level. A possible source of Cu may be the feed contamination, water contamination, or copper alloys used in equipment.

Statistically speaking, concentrations of Cr were higher during summer, importantly in milk from dairy farms located near a wastewater drainage canal. There was no international standard available to declare proportion of samples containing “unsafe” level of Cr. Cr deposits have been found in much higher concentrations in shoots, leaves, nodes, and the tender parts of plants during summer. Higher levels of chromium were found in the milk of dairy animals fed sewerage grown fodders.

Abnormal levels of Cd were found in milk from shops in both seasons. Interestingly, no sample from farms containing detectable limit of Cd could be found in winter despite a high level measured during summer. The proportion of samples containing abnormal level of Cd was more in summer, both in milk from shops and farms. Concentration of Pb was higher in winter and comparatively more in milk from the urban shops. The proportion of samples with Pb above safe limits was more in winter irrespective of source of milk. All samples collected from urban shops in winter had a Pb level above safe limit.

There are seasonal variations in heavy metal content of soil, wastewater, fodder, and particulate matter (Khan et al., 2013). Moreover, the uptake of these metals by plants is influenced by soil pH and agriculture practices such as use of fertilizers. Level of soil ingestion by the animals and vegetation types in different seasons are the other factors which may lead to varying degree of heavy metals exposure to animals and hence apparent differences in proportion of abnormal samples between summer and winter. The sources of heavy metals are multiple and their entry into dairy chain also depends on biological variables (e.g. rate of absorption into the animal body). Aslam

[3] The IDF standards for permissible maximum are Cu = 0.01 parts per million (ppm), Cd = 0.0026 ppm, Pb = 0.02 ppm.

(2010) determined contents of heavy metals in cattle milk collected from two areas of Faisalabad city of Pakistan. The author estimated the mean which is sensitive to outliers and skewed nature of data in such studies. This constrains us to compare the findings quantitatively. Apparently, the findings of our study are in agreement with those of Aslam (2010). In addition, we observed significantly higher concentration of Pb in winter samples which may be attributed to regional differences.

Overall, the AFM1 levels in 17% of samples were higher than the maximum tolerance limit of 0.50 parts per billion (0.50 µg/L) accepted by the Food and Drug Administration (FDA) of the United States and by food safety authorities in China, Japan and Mexico (the European Union sets a stricter limit of 0.05 µg/L). AFM1 levels were significantly high in samples with median concentrations of 0.333 and 0.416 in summer and winter, respectively and with interquartile ranges of 0.253 and 0.322, respectively. There was a statistically significant difference in median concentration, and the proportion of samples containing levels of AFM1 exceeding the FDA standard differed significantly when compared across the two seasons (summer: 12%, winter: 24%). For comparison with other studies, the mean value of AFM1 in our samples (0.38 µg/L) was higher than that reported by Iqbal *et al.* (2011) [0.046 µg/L] and Hussain *et al.* (2008) [0.027 µg/L and 0.044 µg/L in buffalo and cow milk respectively] but lower than Muhammad *et al.* (2010) [17.38 µg/L]. The proportion of samples containing abnormal level of AFM1 in our survey (17%) was similar with Iqbal *et al.* (2011) while differed from Hussain *et al.* (2008) and Muhammad *et al.* (2010). It appears that there is variation in exposure across different regions of country possibly due to variations in feeding regimes, climate and husbandry practices.

Table 1: Median and interquartile range of concentration of studied metals with milk samples stratified by season and source

Source of Milk	Contaminant	Season		
Urban milk shops	Copper	Summer	Median	1.354
			Interquartile Range	3.229
		Winter	Median	0.500
			Interquartile Range	0.500
	Cadmium	Summer	Median	0.092
			Interquartile Range	0.079
		Winter	Median	0.250
			Interquartile Range	0.250
	Lead	Summer	Median	0.455
			Interquartile Range	1.136
		Winter	Median	2.000
			Interquartile Range	1.750
	Chromium	Summer	Median	8.400
			Interquartile Range	10.000
		Winter	Median	3.750
			Interquartile Range	3.000
	AFM1	Summer	Median	0.333
			Interquartile Range	0.253
		Winter	Median	0.416
			Interquartile Range	0.322

Note: For heavy metals levels are reported in parts per million; for AFM1 in parts per billion.

Table 1: (Continued)

Source of Milk	Contaminant	Season		
Dairy farm near drain	Copper	Summer	Median	0.938
			Interquartile Range	0.729
		Winter	Median	0.000
			Interquartile Range	0.250
	Cadmium	Summer	Median	0.092
			Interquartile Range	0.092
	Lead	Summer	Median	0.227
			Interquartile Range	0.909
		Winter	Median	1.250
			Interquartile Range	1.500
	Chromium	Summer	Median	12.400
			Interquartile Range	5.600
		Winter	Median	4.250
			Interquartile Range	1.000

Figure 5: Median concentration of the studied contaminants with bars showing 95% confidence intervals

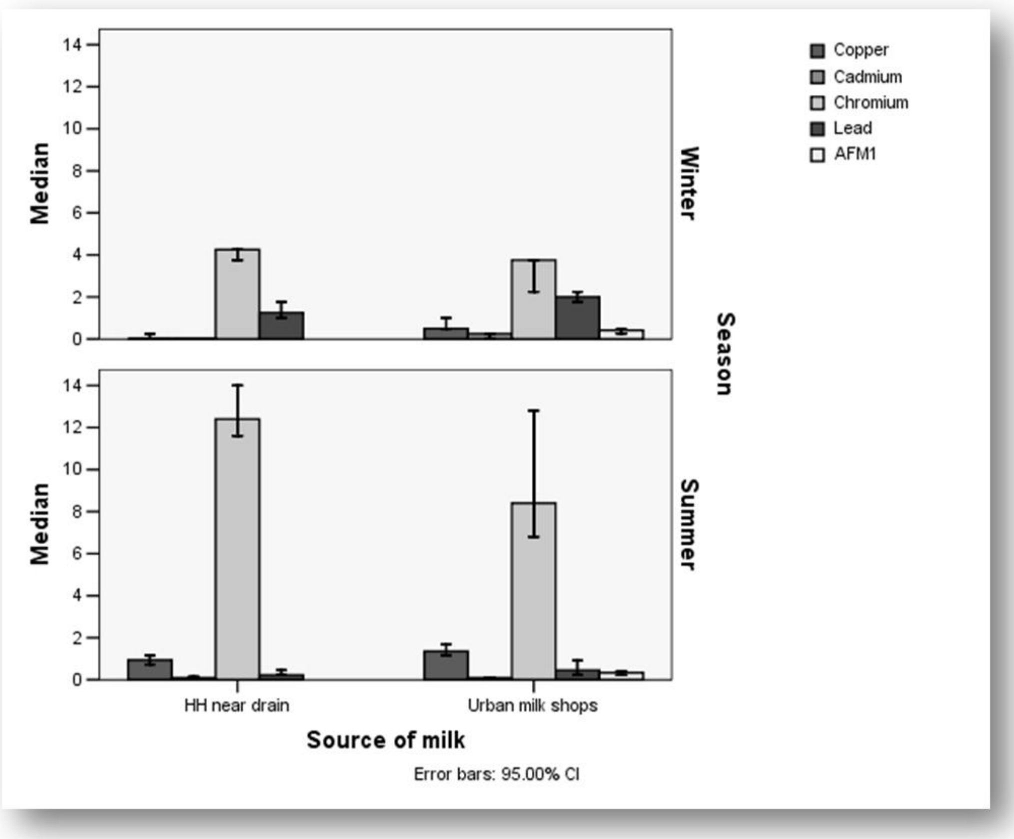


Table 2: Percentage of samples with concentration more than permissible level

	Summer		Winter	
	HH near drain	Urban milk shops	HH near drain	Urban milk shops
Copper	100	94	44	97
Cadmium	86	94	N/A*	54
Lead	70	67	97	100
Aflotoxin M1	N/A*	12	N/A*	24

*All samples below detection limit of the assay for Cd w inter HH near drain; measurement of AFM1 for HH near drain not included in the study design

CONCLUSIONS AND RECOMMENDATIONS

There is a need to set a legal limit for some heavy metals in milk and for surveillance in markets. It would provide a base from which processing companies and producers of bio-products can promote their business for the wellbeing of the consumers and to avail export opportunities. Appropriate risk communication and mitigation strategies are required for farms near the wastewater drain canal crossing several districts of Punjab.

This survey also generated data on the occurrence of AFM1 in informally marketed milk from one city of Pakistan. The levels of AFM1 in samples indicate that the feed of dairy animals are contaminated with AFB1. It was concluded that the occurrence of AFM1 in milk samples were considered to be possible hazards for public health, especially children. Therefore, there is a need to limit exposure to aflatoxins by imposing regulatory limits. Production of safer and healthier milk and other dairy products with minimum AFM1 levels can be achieved by adopting prophylactic measures including control of humidity and water content of feedstuff (which favors mould production). Monitoring of the AFB1 level in feed is important to prevent the risk of AFM1 contamination in milk. For the future, we suggest active surveillance of AFB1 in commercial feed.

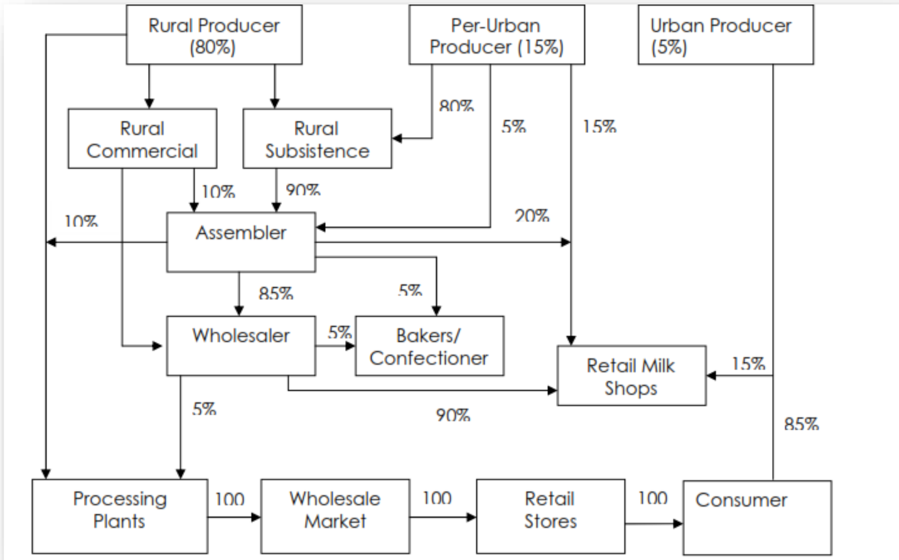
Residues of chemical contaminants in animal products and by products may have public health implications in addition to being a trade barrier under sanitary and phyto-sanitary agreements. This research has provided data about concentrations of some heavy metals and AFM1 in milk being marketed informally in one selected city of Pakistan. The findings, i.e. detection of level above internationally recommended permissible limits in most samples, warrant continuous monitoring of those contaminants and a policy for their control. At the moment, the focus of national veterinary services is mainly on control of infectious diseases, which is not enough to sustain public health or opportunities in international markets. Unregulated trade and injudicious use of antibiotics and pesticides is also quite common. It will also be interesting to assess their levels in milk and devise a strategy for an integrated testing and control at least in the formal dairy sector.

ANNEX

Milk marketing channels in Pakistan

Milk production in rural areas constitutes about 80% of the total milk production in the country. Of the remaining 20%, peri-urban production accounts for about 15% and the urban about 5% (Figure 6). About 90% of the milk marketed is collected from the subsistence farmers and the remaining 10% is contributed by the commercial dairy farms. The situation in Jhang is more or less the same.

Figure 6: Milk marketing channels in Punjab - Pakistan



Source: Value Chain Analysis of Livestock Sector (Dairy) in District Vehari of Punjab, prepared by Intercooperation, Pakistan 2009

Owing to consumer preferences and lack of technology, almost 95% of the milk in Pakistan is marketed raw through informal marketing chains; the remaining 5% is processed by the formal processing industry and marketed through the formal chain. The major difference between the two types of marketing chain is the sophistication of their storage and handling infrastructure and practices (Zia et al., 2011).

A generalized description of the milk supply/value chain in one district in Punjab has been provided by Intercooperation et al. (2009). As that text explains and is summarized herein the usual concept of organized markets is not prevalent in the case of milk. The marketing system for milk is predominantly informal and is characterized by the presence of a number of small sized farmers, milkmen (doodhis), milk processors, milk/dairy shops, vendors or halwais operating at different stages of milk collection and distribution. The milkmen or doodhis are the main intermediaries linking milk farmers in rural areas with consumers in urban centers. The milk is handled in crude way. A typical dhodhi owns a few metallic containers/cans or plastic drums. The dodhis transport milk in these containers to shop keepers or khoya makers who maintain a set of boiling pans, buckets and earthen pot for making yogurt.

The milk processors have introduced an organized system of milk collection. They have introduced chillers and refrigerated carriers. Milk processors, especially Nestle and Halla, have set up collection centers in milk production areas where they have created basic infrastructure in the form of chillers. The milk collected at these chillers (Nestle calls them as Village Milk Collection Centers) is transported to sub-centers and processing plants in refrigerated carriers. This has provided competition to the traditional milk collection system dominated by dodhis and has thus enabled rural farmers to obtain better prices. A variety of modes of transportation are used for milk collection from the farmers in rural areas to its haulage to processing plants, shop keepers and consumers in urban centers. Bicycles, motorbikes, three wheelers, animal carts, etc are used for collection and haulage of milk. The risk of milk spoilage during transportation is higher in summer than in winter. The dodhis add ice, water and chemicals to minimize the risk of spoilage. In summary, the milk marketing supply/value chain is characterized by the presence of a number of participants operating at different stages along the distribution chain as follows:

Producers: Dairy farmers are the sole point of milk production. They can be classified into two basic categories determined by the farm's geographical location and size of operations:

i. Subsistent Farmers: Constitute the majority of dairy farmers in the country and are responsible for 90% of the milk produced. They normally keep 2-5 milk producing animals on the farm.

ii. Market Oriented or Commercial Farmers: They are responsible for the remaining 10% of the milk production and keep anywhere from 10 to 500 milk producing animals on the farm.

Milk Collectors: For a majority of dairy farmers in the country, milk collectors usually provide the only market linkages. Based on the scale of their operations, milk collectors can be classified into five main groups:

i. Doodhis: They are primary and traditional milk collectors. Small (katcha) dodhis collect milk from farmers' doorstep. The collected milk is usually sold to urban consumers at their door step, retailers, mini-milk collectors and milk collection centres of the processing plants.

ii. Mini Milk Collectors: The medium-sized (pucca) dodhis collect milk at their established points mainly from small dodhis, aggregate and supply to processing plant.

iii. Contractors: Contractors (large dodhis) are large scale milk collectors and after purchasing milk in the bulk sell them directly to milk retail shops or dairy processors.

iv. Village Milk Collection Centers (VMCC): The VMCCs are set up by processing plants. Milk is collected from farmers, held in chillers (cooling tanks) and later transported to sub-centres.

v. Milk Collection Sub Centres (SCs): The SCs are also set up by the processing plants. The milk collected at VMCCS further is bulked at SCs and then shifted to processing plant in refrigerated carriers. The SCs have comparatively bigger chilling set up.

Milk Processors: The processors of can again be categorized into two types based on their scope and the products manufactured by them:

i) Traditional Processors: These are shops that process traditional dairy products to be sold locally. Products produced by them include ghee, lassi, yogurt, khoya, etc.

ii) Corporate Processors: These are national or multi-national companies that have well defined procurement and distribution mechanisms in place. They own processing plants and primarily produce UHT, Pasteurized, and Powder milk.

Milk Retailers: They run retail shops mostly in urban centers. They buy fresh milk at wholesale prices primarily from dodhis and sell to consumers. They also sell milk products like yogurt, khoya, confectionery, etc.

Consumers: Consumers are located in urban and rural areas. However, most milk marketed is consumed in urban centre. In addition, there are institutional consumers as well, e.g. hotels, cafeterias, hospitals, etc.

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