Original Research

Fenbendazole Residue in Marketed Pork of North-East India

D. C. Roy*, A. Kafle, S. K. Laskar, H. Baruah and R. K. Roy

Department of Pharmacology and Toxicology, College of Veterinary Science, A.A.U., Khanapara, Guwahati-781022, Assam, INDIA

***Corresponding author:** roydula@gmail.com

Rec. Date:	Dec 11, 2018 12:39
Accept Date:	Mar 09, 2019 19:57
DOI	10.5455/ijlr.20181211123914

Abstract

The present study was conducted to analyze the residue of anthelmentic, Fenbendazole in marketed pork samples of four different north-eastern states namely Assam, Nagaland, Manipur and Mizoram. 720 samples were collected from the local vendors of the respective states in a clean sterile container and transported to the laboratory in ice packs. The sample comprises of liver, kidney and muscle. The collected samples were transported to the laboratory in ice cold packs and kept at – 20 ℃ till analysis. Ultra High Performance Liquid Chromatography was used to analyze the samples. Out of 720 samples 37 (5.2%) samples were found to contained the residue of fenbendazole. The level of fenbendazole residue was found to be maximum in liver followed by kidney and muscle (wherever detected). However, none of the detected values were above the permissible limit. This study clearly showed that there is no public health risk from pork in the states tested.

Key words: Fenbendazole, MRL, Residue, UHPLC, Public Health

How to cite: Roy, D., Kafle, A., Laskar, S., Baruah, H., & Roy, R. (2019). Fenbendazole Residue in Marketed Pork of North-East India. International Journal of Livestock Research, 9(4), 154-159. doi: 10.5455/ijlr.20181211123914

Introduction

Environment and quality of foods are the two main pillars of human health, EPA (2015) and Ames (1983). Anthelmintic drugs are widely used for treatment of parasitic worms in livestock, however little is known about the presence of their residues in food obtained from such animals. Residues, as defined by the European Union as "pharmacologically active substances (whether active principles, recipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the drug in question has been administered. Under the normal physiological conditions, following administration of a drug to an animal, most drugs are metabolized in order to facilitate elimination, and to a large extent detoxification as well. In general, most of the parent product and its metabolites are excreted

in urine and a lesser extent via faeces, Short *et al*. (1988). However, these substances may also be found in milk and eggs, and in the meat. Fendbendazole is the most commonly used anthelmentic in India followed by ivermectin and closental owning to its cheap and broad spectrum of activity against helminthes. Keeping this in view the present study was undertaken to study the fate of residue in pork of north-east India.

Materials and Methods

Chemical and Reagents

Fenbendazole standard (sigma aldrich), HPLC grade Acetonitrile (Fisher), Methanol (Fisher) and HPLC grade water (Fisher) were used for the study.

Sample Collection

A total of 720 samples of pork were collected from local vendors of different districts of north-eastern states for residue analysis. The slaughter pigs were of 6 months to 1 year of age of either sex and were of Hampshire (cross) and local origin. The samples were collected aseptically in a clean sterile container and were transported to the laboratory in ice cold packs and kept at – 20^0 C (to prevent autolysis and subsequent loss of drug residue) till analysis in laboratory.

Standard Calibration Curve of Fenbendazole

10 g pure fenbendazole standard was dissolved in a mixture of 40% di-methylsulphoxide and 60% acetonitrile to obtain a concentration of 100µg/ml. Further dilutions were made from this solution in the descending concentration of 4.0, 3.0, 2.0, 1.0 and 0.5 µg/ml respectively. An aliquot of 20 µl each of these solutions were injected into the UHPLC system. Peak areas were recorded. A standard calibration curve with coefficient of determination of 99.56 % was obtained by plotting concentration of standard solutions against peak areas obtained (Fig. 1).

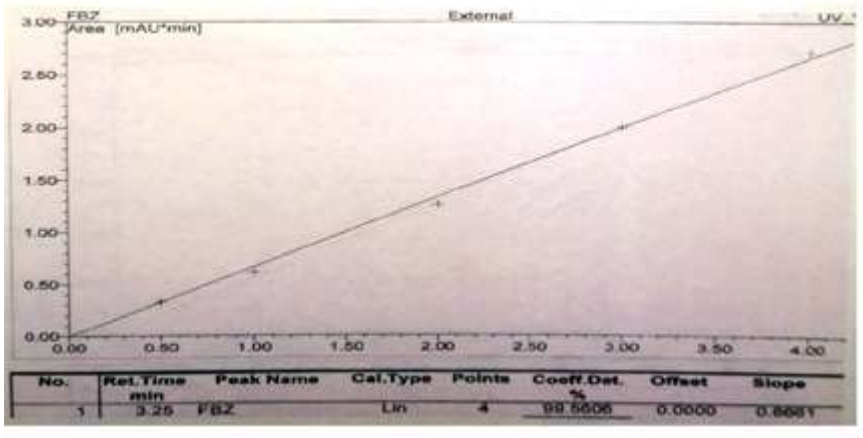

Fig. 1: Standard curve of Fenbendazole (FBZ)

DOI 10.5455/ijlr.20181211123914

Chromatographic Conditions

The levels of fenbendazole were determined using a UHPLC system of Make: Dionex equipped with an autosampler and Diode Array Detector (DAD) operated at 290 nm as per method described by Cooper *et al.* (2011 & 2012). The samples were separated on an RP-C 18 column and were eluted with a mobile phase of a mixture of water and acetonitrile in the ration of 67:33 v/v. The isocratic mode was run at a flow rate of 1.0 ml/min with coefficient of determination of STD curve 99.0%. Further, the Limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.008 µg/g and 0.010 µg/g respectively. The repeatability percentage was 83.0-95.0.

Preparation of Samples

The fascia and fat of pork samples were removed and then cut into small pieces. 10 g of each sample was taken in a blender and to it added an equal volume of distilled water. 10 g of each blended sample was transferred to a centrifuge tube. After 15 minutes 10 ml of acetonitrile was added. The sample was ultrasonicated at 100% amplitude for 10 minutes and left undisturbed for 10 minutes. The samples were centrifuged and the collected supernatant was filtered. The filtrate was then passed through C18 polymeric cartridge after which it was further filtered using 0.22 um filter paper. The filtered sample was then ready for injection.

Result and Discussion

Table 1: Fenbendazole residue analysis in the state of Assam

Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL (ug/g) As per Codex Alimnetarius
Fenbendazole	Kidney	60	6	0.016-0.070	ND	ND	0.1
	Liver	60	5	0.090-0.234	ND	ND	0.5
	Muscle	60	4	0.030-0.070	ND	ND	0.1

Table 2: Fenbendazole residue analysis in the state of Nagaland

Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL (ug/g) As per Codex Alimnetarius
Fenbendazole	Kidney	60	4	0.025-0.056	ND	ND	0.1
	Liver	60	5	0.010-0.427	ND	ND	0.5
	Muscle	60	2	0.042-0.075	ND	ND	0.1

DOI 10.5455/ijlr.20181211123914

Table 3: Fenbendazole residue analysis in the state of Manipur

Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL (ug/g) As per Codex Alimnetarius
Fenbendazole	Kidney	60	4	0.023-0.087	ND	ND	0.1
	Liver	60	2	0.137-0.285	ND	ND	0.5
	Muscle	60	ND	ND	ND	ND	0.1

Table 4: Fenbendazole residue analysis in the state of Mizoram

Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL (ug/g) As per Codex Alimnetarius
	Kidney	60	3	0.021-0.080	ND	ND	0.1
Fenbendazole	Liver	60	2	0.135-0.286	ND	ND	0.5
	Muscle	60	ND	ND	ND	ND	0.1

**ND: Not Detected*

The approved maximum residue limit of fenbendazole as established by Codex Alimentarius are 0.1 µg/g for kidney and muscle and 0.5 µg/g for liver, Codex Alimentarius (2015). Fenbendazole in pigs have a half-life of 5.5 hours. Three groups of pigs 5 in each groups were treated orally with fenbendazole 1.5% pellets at a dose rate of 5 mg/kg BW. Combined residues, expressed as oxfendazole sulfone were determined in liver, kidney, muscle, fat and skin 12, 24, 72, 120 and 168 hours after oral administration. The detectable level of fenbendazole and its metabolites were found up to 24 hours after dosing in muscle, kidney, skin and fat and up to 72 hours in liver. The peak residue concentration of fenbendazole and its metabolites were peaked at 24 hours in liver, kidney and muscle: 6.3, 1.1 and 9.2 µg/g, respectively. The peak level in skin and fat were observed at 12 hours after treatment: 9.6 and 1.2 µg/g, respectively FAO (1991).

In the present study, out of 720 samples tested, 37 (5.2%) samples were found to contained the residue of fenbendazole. The level of fenbendazole residue was found to be maximum in liver followed by kidney and muscle (wherever detected). Liver being the major organ for detoxification of xenobiotics is the major organ to be effected, Alvarez *et al*., (2000). Amongst the four states the level of fenbendazole residue, although below the permissible limit, was found to be maximum in the states of Assam which is similar to the findings of other authors, Zhou *et al.* (2010). The metabolites so obtained from the parent compound (fenbendazole) is basically sulfoxide derivative which is excreted via urine and to a lesser extend via bile, Boothe and Reevers (2012). In kidney the deposited metabolites of fenbendazole has been found to exert Renal tubular hyperemia and glomerular capsule dilation, increased serum creatinine and hepatocellular granular degeneration when fed to rats at dose rate of 500-3000 mg/kg/day, ICHTRRVMP (2011). In the present study the level of fenbendazole was not detected in muscle in the states of Mizoram and Manipur

DOI 10.5455/ijlr.20181211123914

while the residue in muscle was detected in Assam and Nagaland. Strict implementation of the withdrawal period or rather the level of residue in the muscle were below that could be detected by the machine in our study (LOD=0.008 µg/g) in the states of Mizoram and Manipur could be the result of not detection despite being used in larger extent.

Similar findings were recorder by the present author while studying the residue status of common anthelmentics and antibiotics in the pork samples of North-Eastern region, Roy *et al*. (2014). The present research is in line with the results shown by authors who studied fenbendazole residues in pig tissue after treatment with medicated feed, Bettencourt *et al*.(1999). Similar pattern of results were also been reported earlier, Cooper *et al*. (2012).

Conclusion

Upon administration of fenbendazole in pigs, most of the parent product and its metabolites are excreted in faeces and urine. However, these substances may also be found in milk and eggs, and in the meat. Two major concerns of fenbendazole residue in foods of animal origin (meat, milk and egg) are allergic reaction to the consumer and development of resistance to the parasites (causing harm to the animal and humans) both are detrimental to the mankind. Apart, the irrational, overdose of fenbendazole in animals leads to deposition or rather contamination of ecosystem as the fenbendazole metabolites are often excreted via dung and urine into the environment. This in turn disturbs the soil microbial flora. Although Fenbendazole being the most commonly used anthelmentic the residue detected in the states of Assam, Nagaland, Manipur and Mizoram were below the permissible limits. Hence, it can be concluded from the present study that there is no toxicity risk of fenbendazole in the above mentioned states. Routine public awareness and proper withdrawal period as stated by the regulatory authorities must be maintained all over the country to safeguard the health of the consumers as well as to minimize environmental contamination.

References

1. Alvarez L, Imperiale F, Sanchez S, Lanusse C. 2000. Uptake of albendazole and albendazole sulphoxide by Haemonchus contortus and Fasciola hepatica in sheep. *Veterinary Parasitology*. 94(1-2): 75-89.
2. Ames BN. 1983. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science*. 221: 1256-1264.
3. Bettencourt PS, Capece and Perez. 1999. Liquid chromatographic determination of fenbendazole residues in pig tissues after treatment with medicated feed. Drugs, Cosmetics, Forensic Science. 82(5):1007
4. Boothe DM, Reevers PT. 2012. Pharmacology introduction In: Aiello SE, Moses MA, Steigerwald MA (Eds). The Merck Veterinary Manual. Merck Publishing Group.
5. Codex Alimentarius. 2015. Maximum residue limits and risk management recommendations (RMRS) for residues of veterinary drugs in foods. Available online at: http://www.fao.org/fao-who-codexalimentarius.

 DOI 10.5455/ijlr.20181211123914

6. Cooper KM, Whelan M, Danaher M, Kennedy DG. (2011). Stability during cooking of anthelmintic veterinary drug residues in beef. Food Additives and Contaminants 28(2): 155.
7. Cooper KM, Whelan M, Kennedy DG, Trigueros G, Cannavan A. (2012). Anthelmintic drug residues in beef: UPLC-MS/MS method validation, European retail beef survey, and associated exposure and risk assessments. Food Additives and Contaminants 29(5): 746-760.
8. Environment and human health. 2015. Environmental Protection Agency. FAO.1991. First draft on Fabental, Fenbendazole and Oxfendazole. available online at- http://www.fao.org/fileadmin/user_upload/vetdrug/docs/41-11-febantel_fenbendazole_oxfendazole.pdf
9. International Cooperation on Harmonization of technical Requirements for registration of veterinary medicinal products (ICHTRRVMP). 2011. Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: metabolism study to determine the quantity and identify the nature of residues. VICH GL 46 (MRK)–Metabolism and Residue Kinetics.
10. Roy DC, Gogoi R and Laskar SK. 2014. HPLC and Spectrophotometric analysis of tetracycline residues in marketed pork of Assam. *Indian Journal of Veterinary and Animal Sciences Research*. 43(2):98-103.
11. Short CR, Flory W, Hsieh LC, Barker SA. 1988. The oxidative metabolism of fenbendazole: a comparative study. *Journal of Veterinary Pharmacology and Therapeutics.*11 (1):50-5.
12. Xi-Zhou H, Jian-Xing W, Yu-Qi F. (2010). *Journal Agricultural Food Chemistry*, 58(1): 112-119.

DOI 10.5455/ijlr.20181211123914