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Pathology and prevalence of hepatic lesions of Azeri buffaloes (bubalus bubalis) slaughtered at Urmia Abattoir, Northwest Iran

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ABSTRACT

This study was carried out for the evaluation of the prevalence rate and the pathology of various lesions in the confiscated livers of slaughtered Azeri buffaloes at Urmia Abattoir, Northwest Iran. Livers of 306 Azeri buffaloes were inspected grossly during five months according to their color and consistency changes in Urmia Abattoir. Among the inspected carcasses, livers of 28 buffaloes had various lesions. The collected tissue samples of confiscated livers were placed in 10% buffered formalin as a fixative solution in order to prepare pathologic sections with a thickness of 6 µm. The sections were stained by haematoxylin and eosin (H&E) and periodic acid schiff (PAS) methods. The most common pathologic changes in the confiscated buffalo livers were fatty change (46.4%), hepatitis and cholangitis (42.9%), and bile duct hyperplasia (39.3%) respectively. The results of this study have shown that there was a significant difference ($p<0.05$) between hepatic congestion and sex. In addition, there was no significant difference ($p>0.05$) between hepatic lesions and age. The results of this study have revealed that fascioliasis has an important role in the creation of some hepatic lesions such as bile duct hyperplasia, fatty change, and hepatitis in buffaloes. Since various hepatic lesions can reduce buffalo productions and has negative influence on animal reproduction, it is necessary to pay more attention to accurate performing of anti-parasitic program and the evaluation of possible role of poisonous plants, heavy metals, and mycotoxins in the generation of hepatic lesions of buffaloes.

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1. Introduction

Vegetable Buffalo fostering has been increased recently due to the fact that it is an important and economical animal. The superior quality of buffaloes meat and milk is still unknown in public (1). Buffaloes are used to supply a part of human need for red meat. The global production of buffalo meat is 12.4% of the total production of beef annually.

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Urmia city is the center of West Azerbaijan province located in the northwest of Iran. Iran has about 523 thousand buffaloes that 27.7% of them that are called Azeri buffaloes live in West Azerbaijan province (2). Liver, which forms about 1% of body weight, is the biggest organ in the body of herbivores. This organ has very important functions including the metabolism of bilirubin, bile acid, carbohydrate, fat and xenobiotic, protein production, and immune function (3). In addition to vital importance of liver, many researches on the ability of commercial use of buffalo liver in comminuted meat products show that

commercial utilization of buffalo liver for preparing acceptable comminuted meat products in food industries is possible (4). Pathological changes of liver may have various causes such as bacteria, virus, and fungus. Consequently, they may have direct economic losses such as the confiscation of liver or may have indirect impacts such as a decrease of productions and negative influence on animal reproduction (5). Liver products could be considered as high-risk. In this respect that these products are rich of nutritional elements and provide very suitable medium for bacterial growth. The livers or their other byproducts have contamination ability due to poor hygiene during animal slaughtering (workers or instruments). Salmonella typhimurium, Salmonella enteritidies, and Escherichia coli O157:H7 are potential pathogens for human health and they are considered as food-borne agents that are seriously infective. In case of high consumption of affected livers, antibiotic residues and aflatoxin B1 (AFB1) can cause hygienic threat to human health (6). Furthermore, transmission possibility of some cattle diseases to human known as zoonotic diseases by the consumption of raw or under-cooked liver have been reported. These diseases include hepatitis B and E, (7), toxocariasis (T.canis)(8), campylobacteriosis (Campylobacter fetus)(9), Crimean- Congo hemorrhagic fever (CCHF)(10) and Nasopharyngeal linguatulosis (L.serrata nymph)(11). There is no similar research for pathological evaluations of hepatic lesions of buffaloes slaughtered at Urmia Abattoir yet. However, some researches have been conducted in specific fields of hepatic diseases of buffaloes such as cystic parasitic disease (hydatidosis), fascioliasis, or abscess in the different areas of the world. In an abattoir study of buffalo livers affected by Fasciola gigantica, pathological findings such as inflammation, atrophy, and necrosis in livers have been reported (12). Fascioliasis is considered as an emerging food-borne disease that accidentally infects human by the consumption of raw or undercooked liver products. Hence, this parasitic disease not only causes economic loss but also can make food unsafe and hazardous (13). In another study that was performed on 510 buffaloes in Malwa district of India, only 6 animals were affected by hepatic hydatidosis, which were about 1.19% of inspected buffaloes totally (14). Also, pathology of hepatic abscess (15) and histopathologic changes of hepatic hydatidosis (16) in buffaloes were evaluated previously. Petridou et al. introduced Stenotrophomonas maltophilia as an infective agent that caused necrotizing granulomatous hepatitis, thrombosis, hemorrhage, edema, and fibrosis in the liver in a female 7-year-old buffalo (17). Furthermore, lymphoid infiltration engendered by MCF (Malignant Cattharal Fever) disease was reported in livers of swamp buffaloes in Nakhonpathom province of Thailand (18). Borelli et al. reported toxicity caused by onion (Allium cepa) in five water buffaloes at Cacador district of Santa Catarina in Brazil. In this report, due to ingestion of large quantities of onion, toxicity caused hepatic lesions including centrilobular coagulative necrosis with hemorrhage and macrophages containing brown cytoplasmic pigment (19). Results of another study performed on pathomorphology of spontaneous hepatic lesions of buffaloes in North Gujarat of India showed the most frequent lesions were hydatidosis and fatty liver (20). The aims of current study are the macroscopic (gross) evaluation of prevalence rate and differential pathologic diagnosis of the lesions of the confiscated buffalo livers slaughtered at Urmia Abattoir.

2. Materials and methods

This research was conducted in two steps in cluding post-mortem inspection at Urmia Abattoir and the preparation of the microscopic tissue sections in pathology laboratory. Within 5 months from May 22 to October 23, 2017, livers of the carcasses of buffaloes were inspected on 4 days of every week by supervising at Urmia Abattoir. The inspected animals were divided in 4 age groups of 1.5, 2.5, 3.5 and equal or more than 4.5 years. The inspection days were constantly replaced with other days rotationally to cover all the weekdays. Hepatic lesions such as congestion, necrosis, fascioliasis, hydatidosis and abscess on dorsal and ventral surfaces of confiscated livers were recorded according to their color and consistency changes by macroscopic (gross) inspection. The sampling of the abnormal liver tissues was done for the preparation of pathologic sections and they are placed then in 10% buffered formalin for fixation. The steps of tissue processing include clearing, impregnation, embedding, and blocking were performed after fixation process. Subsequently, the tissue sections were prepared in 6 µm of thickness by a rotary microtome. Finally, the sections were stained by hematoxyline and eosin (H&E) and periodic acid schiff (PAS) staining methods according to recommended standard protocol (21). H&E staining method was used as a general method for the diagnosis of all the hepatic lesions primarily and PAS staining method was used for differential diagnosis of

clear vacuoles (fat, glycogen, and water droplet) in the affected hepatic cells (21).

Statistical analysis

We used IBM SPSS Statistics V22.0 (SPSS22, Chicago, IL, USA) software to analyze the acquired data. Chi-square test and Fisher's exact test were used for the evaluation of the independency of the demographic variables (sex and age) with abundance rate of hepatic lesions. If there is a significant difference between them, the Bonferroni post hoc test will be used to examine the differences between the groups. Meanwhile, p≤0.05 was considered significant.

3. Results

Out of 306 Azeri buffaloes slaughtered and inspected grossly, 138 were male (45.09%) and 168 were female (54.9%) that 28 of them (10 male and 18 female) had hepatic lesions. The abundance of hepatic lesions in 4 age groups including 1.5, 2.5, 3.5 and more than 4.5 years was 2, 5, 8 and 13 respectively. The results of hepatic lesion abundance according to animal sex and age provided in table 1 and table 2 respectively. Table 1 explains the frequency of various hepatic lesions in 28 confiscated and abnormal buffalo livers according to animal sex. Considering table 1, the distribution of the found hepatic lesions in female buffaloes was more than males. In table 2, the distribution of hepatic lesions was expressed based on buffaloes age. Accordingly, all of the lesions are almost related to age 3.5 and ≥4.5 while frequency of the hepatic lesions in different age group statistically was not significant (p>0.05).

Chi-square test and Fisher's exact test were used for the evaluation of dependency between buffaloes age and observed hepatic lesions as two variables. P-value was considered significant when it was equal or smaller than 0.05. The results showed that there was no significant dependency between buffaloes age and the abundance of various hepatic lesions, which is provided in Table 2. Only hepatic congestion and buffaloes sex had significant dependency (p=0.023) while the prevalence of this lesion in male buffaloes was more than females (Table 3). Some of the macroscopic and microscopic hepatic lesions were observed in slaughtered Azeri buffaloes presented in figures 1, 2, and 3.

Table 1: Absolute and relative frequency of hepatic lesions in buffaloes according to gender

	Gender			
Hepatic lesion	Male N (%)	Female N (%)	Total (livers with lesion)	Total (livers without lesion)
Bile duct hyperplasia	4(40%)	7(38.9%)	11(39.3%)	17(60.7%)
Congestion	5(50%)	2(11.1%)	7(25%)	21(75%)
Hemorrhage	4(40%)	4(22.2%)	8(28.6%)	20(71.4%)
Fatty change	5(50%)	8(44.4%)	13(46.4%)	15(53.6%)
Focal necrosis	1(10%)	2(11.1%)	3(10.7%)	25(89.3%)
Parenchymal or capsular fibrosis	3(30%)	6(33.3%)	9(32.1%)	19(67.8%)
Hepatitis and cholangitis	4(40%)	8(44.4%)	12(42.9%)	16(57.1%)
Abscess	1(10%)	1(5.6%)	2(7.1%)	26(92.8%)
Fascioliasis	4(40%)	5(27.8%)	9(32.1%)	19(67.8%)
Hydatid cyst	2(20%)	2(11.1%)	4(14.3%)	24(85.7%)
Total	33(42.3%)	45(57.7%)	-	-

Table 2: Absolute and relative frequency of hepatic lesions in buffaloes with lesions according to age

	Age (year)					
Hepatic lesion	1.5 (n=2)	2.5 (n=5)	3.5 (n=8)	≥ 4.5 (n=13)	X2	p-value
Bile duct hyperplasia	0(0%)	0(0%)	5(62.5%)	6(46.1%)	6.070	0.119
Congestion	1(50%)	0(0%)	3(37.5%)	3(23.1%)	4.442	0.183
Hemorrhage	0(0%)	0(0%)	3(37.5%)	5(38.5%)	3.736	0.379
Fatty change	0(0%)	2(40%)	4(50%)	7(53.8%)	2.145	0.747
Focal necrosis	0(0%)	0(0%)	1(12.5%)	2(15.4%)	1.163	0.841
Parenchymal or capsular fibrosis	0(0%)	1(20%)	3(37.5%)	5(38.5%)	1.629	0.826
Hepatitis and cholangitis	0(0%)	1(20%)	4(50%)	7(53.8%)	3.374	0.433
Abscess	0(0%)	0(0%)	1(12.5%)	1(7.7%)	0.891	1.00
Fascioliasis	0(0%)	0(0%)	3(37.5%)	6(46.2%)	4.591	0.252
Hydatid cyst	0(0%)	0(0%)	1(12.5%)	3(23.1%)	2.008	0.848
Total	1	4	28	45		

p≤0.05 is considered significant.

Table 3: Pearson's Chi-square test for the dependence of buffalo gender and hepatic congestion·

Variable	Gender	No. of Congestion		X2	df	P-Value
		(+)	(-)			
Congestion	Male	5(50%)b	5(50%)	5.185	1	0.023
	Female	2(11.1%)a	16(88.9%)			

Note: Different letters as subscript (a,b) indicate significant difference ($p<0.05$) between animal gender and congestion in confiscated buffalo livers.

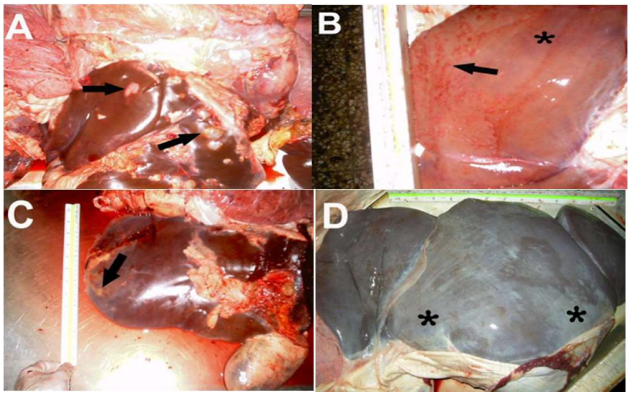

Fig. 1 (A) Hydatid cyst (arrow) in affected liver. (B) Congested area (asterisk) and capsular fibrosis (arrow). (C) Focal necrosis and calcified cyst (arrow). (D) Severe capsular fibrosis (asterisk).

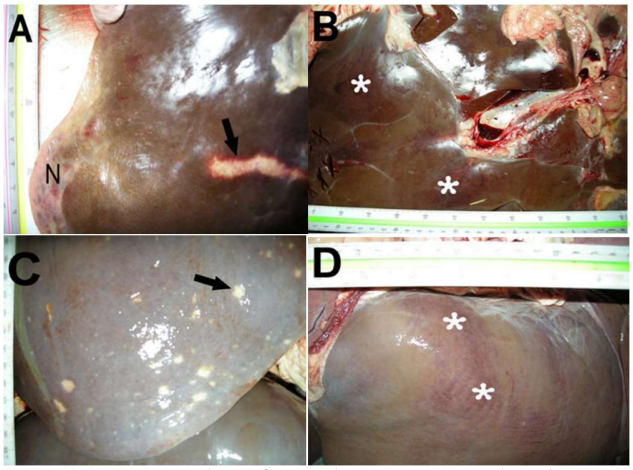

Fig. 2 (A) Migration line of Fasciola parasite (arrow) and necrotic (N) area. (B) Congested and hemorrhagic areas (asterisk). (C) necrotic white colored points (asterisk). (D) congestion and fatty change (asterisk).

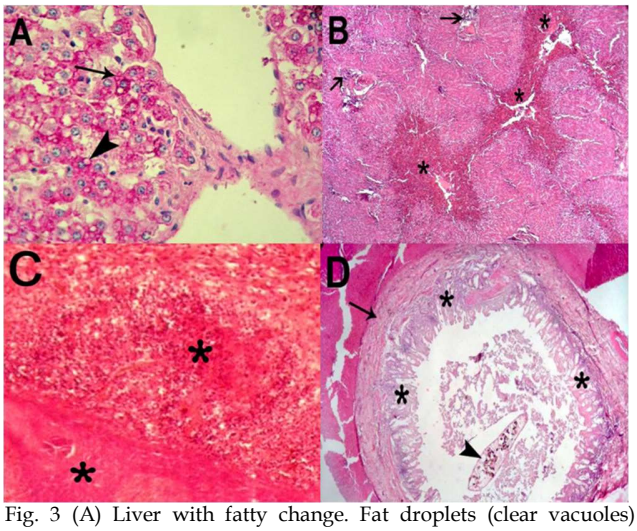

Fig. 3 (A) Liver with fatty change. Fat droplets (clear vacuoles) (arrow head) and red pink colored glycogen (arrow) (×40 objective, PAS). (B) Cholangitis in portal areas (arrow) and congestion in central veins' around (asterisk) with fatty change in peripheral hepatocytes of the veins (Nutmeg liver) (×4 objective, H&E). (C) Chronic lymphocytic hepatitis with necrotic area (asterisk) (×40 objective, H&E). (D) Bile duct hyperplasia (asterisk) caused by Fasciola parasite (arrow head) and severe peripheral fibrosis (arrow) (×4 objective, H&E)

4. Discussion

The most observed hepatic lesions in Azeri buffaloes among abnormal livers were fatty change (46.4%), hepatitis and cholangitis (42.9%), and bile duct hyperplasia (39.3%) respectively. It is notable that the cause of hepatic bile duct hyperplasia is usually fascioliasis. The most commonly observable bovine hepatic diseases in abattoirs are telangiectasia, hepatitis, perihepatitis (adhesions), fascioliasis, hydatidosis, tuberculosis, abscess, and Cirrhosis (fibrosis) (22). In a research on buffalo livers affected by Fasciola gigantica performed in Hyderabad's Abattoir in Pakistan, the results of microscopic observations of the affected livers revealed severe damages and subsequent breakdown of hepatic cords, inflammation, atrophy, and necrosis. Furthermore, there was bile duct hyperplasia with proliferation of epithelial tissue as the typical changes in affected livers (20). In the current study, the rate of contamination to fascioliasis in affected livers was 32.1% and the found parasites was of Fasciola gigantica type. Also, in the pathologic evaluation of these liver tissue sections, bile duct hyperplasia,

proliferation of connective tissue, and lymphocytic infiltration were observed. In another study focused on the prevalence of hepatic fatty change in the slaughtered cattle in Urmia abattoir, hepatic fatty change was reported in most of the livers affected by fascioliasis (23). The results of current study are consistent with the results of the latter research. It seems that in the studied buffaloes in our study, hepatic fascioliasis is the common cause of other lesions such as bile duct hyperplasia, hepatitis, and fatty change in the livers of buffaloes. Given the zoonotic nature of fascioliasis and its transmission hazard to human, it could be considered as a critical point in meat inspection and hygiene (24). Of course, according to the necessity of intermediate host snail and food-borne ability for transmission of infection, comprehensive strategies for controlling of parasite and its intermediate host are of vital importance (24). The other lesion found in our study was hydatidosis with the abundance of 14.3% in abnormal livers (1.30% in the total inspected livers). In a slaughterhouse study conducted by Jatav and Garg in Malaw area of India on 510 buffaloes, the results showed that only 6 animals (about 1.19%) had hepatic hydatidosis (14). Moreover, the results of another study revealed that hepatic hydatidosis was the most observable lesion (171 buffaloes, 13.48%) in 1269 total inspected buffalo livers (19). In comparison, the incidence rates of hepatic hydatidosis in the two last studies were more than our research. In the current study, fatty change was the most observable hepatic lesion in the abnormal livers with 46.4% and in the total inspected buffalo livers with 4.24%. The diagnosed cases of hepatic fatty change were usually in female animals. Surprisingly, there had not been clinical signs of this lesion in buffaloes before slaughtering. Moreover, there was no relation between fatty change and fatty liver syndrome in female buffaloes. The results of our research revealed that accidental hepatic fatty change may be related to agents such as mycotoxines, heavy metals, food regime, parasitic disease (fascioliasis), some drugs, and poisonous plants. The abundance of hepatic abscess in current study was 7.1% in abnormal livers and 0.65% in the total inspected animals. The results of a pathologic research of buffalo liver performed on two abattoir of Barisal district of Pakistan showed that the more commonly found lesions were Gigantocotyle explanatum infection (31.25%), Fsciola gigantica infestation (22.5%), abscess (3.75%), hemorrhage (3.5%), and hydatidosis (2.5%). Pathologically there were some changes in damaged liver tissues including cirrhosis (31.25%), nodular hepatitis (7.5%), granulomatous hepatitis (5%), and parasitic cholecystitis (15%) (15). In the current study, the abundance of hepatic congestion was 25% in abnormal buffalo livers. Additionally, its abundance in male animals was more than females and there was significant difference ($p=0.023$) between animal sex and congestion in the confiscated livers. Ozmen and Mor reported the existence of hepatic congestion in the cattle living near a battery factory and they suggested that it may be caused by acute lead toxicity (25). Moreover, Fujimoto et al. reported hepatic congestion provoked by mercury toxicity (26). Indeed, the causes of hepatic congestion in our research were not determined. However, physical handling by the workers of abattoir during or after slaughtering might be the cause of congestion in the livers of buffaloes (23). The abundance of hepatic focal necrosis in our research was 0.98 in the total inspected buffaloes. Some researchers have explained that poisonous plants and heavy metals storage could be the causes of hepatic necrosis (26,27). In another research, toxicity due to Baccharis megapotamica var. weirii was reported in water buffaloes of Brazil. The hepatic injuries that arisen from this toxicity were severe hepatocellular vacuolization with eosinophilic globules (councilman bodies) and clear lymphatic dilation in the portal areas (28). The hemorrhage abundance in the current study was 28.6% in confiscated livers (table 2). The hepatic hemorrhage might be occurred due to various causes such as diseases, traumatic lesions, internal anomalies, poisonous plants, and parasites. Bovine hepatic hemorrhage due to Thread leaf Groundsel toxicity was reported previously (29). This plant contains toxic pyrrolizidine alkaloids (PAs) that induce acute and chronic hepatotoxicity in farm animals and man. In necropsy of the poisoned cattle, severe hepatic necrosis, multiple hemorrhages, and icterus were observed (29,30).

5. Conclusion

From the current study of the Azeri buffaloes slaughtered at Urmia Abattoir, it is concluded that fatty change and fascioliasis were the most important lesions in the livers. Furthermore, fascioliasis can trigger other hepatic lesions such as fatty change, bile duct hyperplasia, necrosis, and hemorrhage. Hence, according to the economic losses, food wastes, and public health concerns, it is a necessity to consider a regular anti-parasitic program to eradicate hepatic parasitic diseases such as fascioliasis and hydatidosis.

Besides, it is suggested to study the possible role of poisonous plants, heavy metals, and mycotoxins in the generation of hepatic lesions in buffaloes.

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Conflict of interest

The authors have no conflict of interest to declare.

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