

Macroscopic and histopathological examinations of liver lesions in slaughtered cattle in Zabol City, Iran

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Abstract:

BACKGROUND: Liver lesions in cattle not only have negative effects on the cattle breeding industry, but also lead to loss of animal protein production, and in particular to endanger the consumers' health. **OBJECTIVES:** This study was carried out to examine the macroscopic and histopathologic lesions of liver in slaughtered cattle of Zabol City. **METHODS:** Livers of 281 cattle slaughtered at the industrial slaughterhouse of Zabol City were collected from March to September 2015 for gross and microscopic investigation. Tissue processing was conducted from specimens inspected to tissue lesions with routine protocol and histopathological changes of livers were examined under light microscopy. **RESULTS:** Macroscopic investigation of 281 cases showed 90 livers (32.02%) with gross lesions. In histopathological examination, there were 83 cases (29.53%) with hydatidosis, 3 cases (1.07%) with fasciolosis, 2 cases (0.71%) with perihepatitis and 2 cases (0.71%) with lipidosis. **CONCLUSIONS:** Based on the results of this study, the most frequent liver lesion in cattle slaughtered in Zabol City was hydatidosis indicating the active life cycle of *Echinococcus granulosus* and the outbreak of this parasitic disease in dogs and other carnivores in Sistan region.

Introduction

The key role of protein as an essential ingredient in the daily diet of humans cannot be ignored. Livestock products are one of the most important and most desirable sources of protein in the world. Considering the significant livestock population in Iran with more than 8 million cattle, and due to the fact that human health is influenced by animal health, the improvement of cattle production has attracted the cattle breeders' attention (Moshfe et al., 2003; Fallah et al.,

2010). The liver is the largest gland of the mammalian body and supports almost every organ in the body. This organ is critical for the survival of the creatures due to its many essential roles. The liver is the only internal organ of the body that can rapidly regenerate up to 70% of its lost tissues without any dysfunction. The synthesis of proteins and vitamins, participation in metabolism, drug metabolism, toxic substances' purification detoxification of body, prenatal hematopoiesis, quick provision of energy and bile production and secretion are the most

important roles of the liver in the body (So-hair and Eman, 2009; Ramin et al., 2012). Hence, liver diseases and injuries in various animal species such as cattle may lead to critical conditions like metabolic disruption, immune deficiency, reduced production and finally human exposure to infection in terms of zoonotic diseases (Hosseini et al., 2004; Ezatpour et al., 2014). Several slaughter studies have been conducted on the incidence of various liver diseases in cattle, some of which have addressed the hydatidosis (Yakhchali and Gargi, 2006; Fallah et al., 2010; Rostami Nejad et al., 2012; Taghavi et al., 2013; Ezatpour et al., 2014), and others have reported fasciolosis (Mungube et al., 2006; Kara et al., 2009; Mellau et al., 2010; Hosseini et al., 2012; Magaji et al., 2014; Mekuriaw et al., 2016) as the most important causes of these lesions. Considering the popularity of cattle products in Sistan region, the key role of liver diseases and lesions in the health and products of cattle, as well as the lack of influential researches in this area, the present study was conducted with the aim of macroscopic and histopathological examination of liver lesions in cattle slaughtered in Zabol City, in order to determine the major liver lesions in addition to the amount of infection.

Materials and Methods

This study was carried out on 281 slaughtered cattle (in order to collect 90 specimens suspected of liver lesions) over eight visits (field and random) to the industrial slaughterhouse of Mohammad Abad, Zabol City, during a six-month period beginning from March 2015. Post mortem liver examination was performed by observation and palpation of various liver lobes and incision

of suspected gross liver lesions in terms of color, consolidation, absence of necrotic points, adhesion, and so on. In addition, the specimens were examined in terms of the presence of the parasite by cutting in the liver hilum area of the biliary duct. Specimens of 1×1×5 cm were collected from 90 cattle suspected of liver lesions macroscopically and were fixed in 10% buffered formalin and transferred to the pathology laboratory of Zabol veterinary faculty. The macroscopic characteristics of each specimen were recorded separately and the formalin of the specimens was replaced after 24h for effective fixation. Dehydration, clearing and paraffin impregnation of the specimens were performed by using an automatic tissue processor and the paraffin blocks were prepared. Then, tissue sections about 5 microns in thickness were provided by microtome device and stained with hematoxylin and eosin (H&E) common staining method. Finally, the prepared glass slides were investigated microscopically with different magnifications of light microscope. Then, the frequency and percentage of different liver lesions' patterns were recorded and described.

Results

All specimens were collected over eight visits to Mohammad Abad slaughterhouse of Zabol City as follow (Table 1). The results of the macroscopic and histopathological examination of the livers in this study are summarized in Table 2. Eighty-three specimens (29.53%) were diagnosed hydatidosis in a microscopic examination. In terms of macroscopic examination, all livers with this lesion had one or more small cysts on the liver surface, which penetrated

Table 1. The characteristics of the slaughtered cattle studied in the present study in Zabol slaughterhouse.

Number of visits	Date of visit	Number of slaughtered cattle	Number of liver with lesion	Race	Sex	Number of liver lost
1	2015/03/27	48	7	Holstein	Male	-
2	2015/04/11	12	4	Holstein	Male	-
3	2015/05/08	20	9	Holstein	Male	-
4	2015/05/27	44	17	Holstein	Male	6
5	2015/06/11	49	16	Holstein	Male	1
6	2015/06/30	32	13	Holstein	Male	1
7	2015/08/18	26	5	Holstein	Male	-
8	2015/09/16	50	19	Holstein	Male	7
Total	6 months	281	90	Holstein	Male	15

Table 2. Results of the macroscopic and microscopic study on the liver of cattle slaughtered at Zabol slaughterhouse.

Type of lesion	Macroscopic		Microscopic	
	Number	Percent	Number	Percent
Hydatidosis	83	29.53	83	92.22
Fasciolosis	3	1.07	3	3.34
Liver adhesion / perihepatitis	2	0.71	2	2.22
Pale liver / Lipidosis	2	0.71	2	2.22
Total number of lesions	90	32.02	90	100
Specimens with no lesions	191	67.98
Total liver examined	281	100

into the parenchyma of the tissue in some cases. Some cysts contained dilute, clear and colorless liquid which was as plasma in some cases because of the bleeding. However, in other cases, the small cysts contain concentrated contents and sometimes mineralization due to the chronic lesion. The accumulation of mononuclear inflammatory cells with the presence of connective tissue around the cyst (Fig. 1), portal hepatitis and bile ducts hyperplasia (Fig. 2), calcification, irregular hepatic plates and pressure atrophy (Fig. 3) were observed in microscopic examination of hydatidosis. Fasciolosis was observed in three specimens (1.07%). In macroscopic examination, infested livers had rigid consolidation, swollen biliary ducts with focal calcified cysts and in one case, Fasciola parasite was observed in hepatic bile duct. In terms of microscopic

investigation, hepatic necrosis, bile ducts hyperplasia with accumulation of mononuclear inflammatory cells, as well as formation of connective tissue were the most common findings in all three cases with fasciolosis (Figure 4). Two cases (0.71%) were diagnosed perihepatitis. In macroscopic examination, thickening of Glisson's capsule and its adhesion to the liver surface was evident. In microscopic observation, increasing thickness of this capsule due to formation of large amount of collagen fibers and inflammatory cells (perihepatitis) (Fig. 5), hepatic parenchymal hemorrhage and superficial vessels fibrinous thrombosis (Fig. 6) were the common findings of both specimens. Also, the bacillus shape bacteria in the vessels were notable (Fig. 7). Two specimens (0.71%) were diagnosed as hepatic lipidosis. In macroscopic examination, the

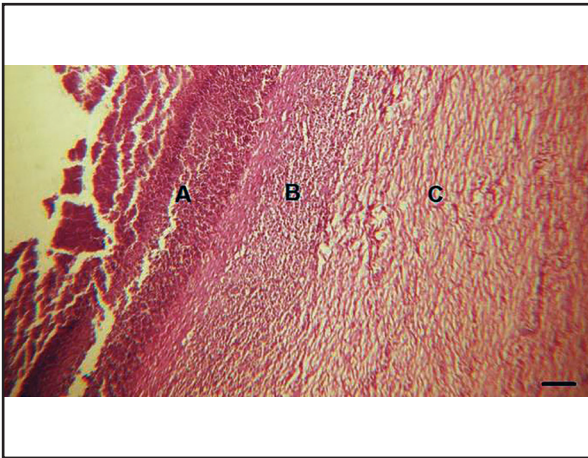


Figure 1. Hydatidosis in the cattle liver. The hydatid cyst wall (A) surrounded by mononuclear inflammatory cells (B) and high amounts of connective tissues (C) (H&E, Scale bar= 250 μ m).

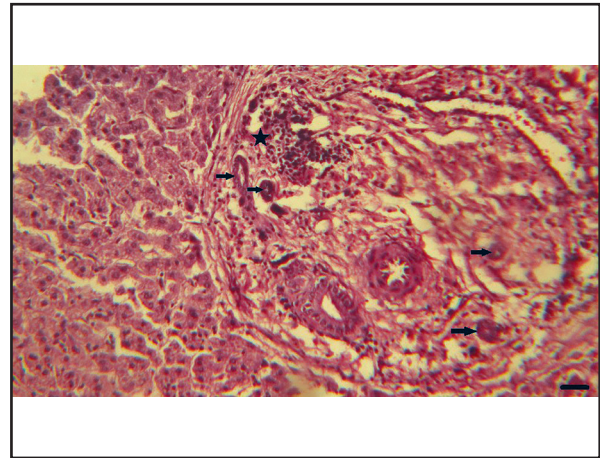


Figure 2. Portal hepatitis in the liver of cattle with hydatidosis. Bile ducts hyperplasia (arrows) with the accumulation of mononuclear inflammatory cells and connective tissue (star) around the portal area (H&E, Scale bar= 50 μ m).

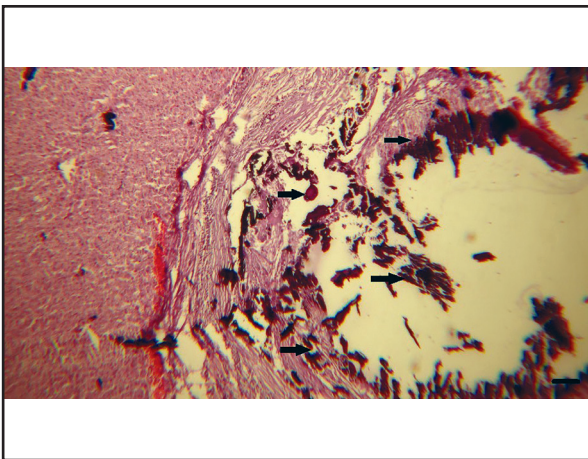


Figure 3. Hydatidosis in the cattle liver. Extreme calcification (arrows) and pressure atrophy of the liver parenchyma around hydatid cyst (H&E, Scale bar = 75 μ m).

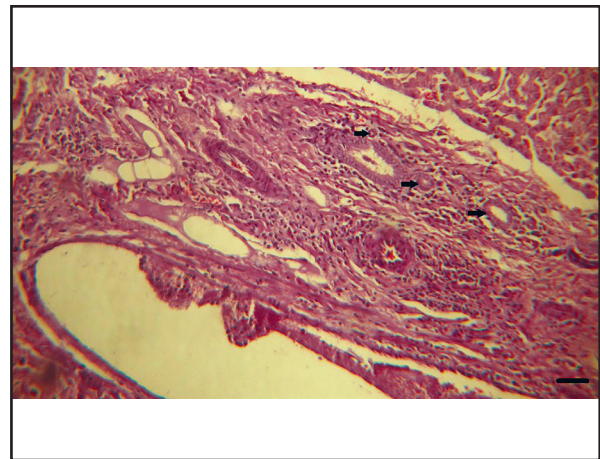


Figure 4. Bile ducts hyperplasia (arrows) with the accumulation of mononuclear inflammatory cells and connective tissue in portal area (portal hepatitis) of cattle liver with Fasciolosis (H&E, Scale bar= 50 μ m).

lobes of livers with notable lipidosis were observed enlarged, pale yellow, soft and friable with round edges. In microscopic investigation, vacuolar degeneration of hepatocytes with nuclear pyknosis was the significant finding of this lesion. Moreover, in hepatocytes with large lipid vacuole, displacement of nucleuses to the periphery was evident (Fig. 8).

Discussion

Hydatidosis was the most common he-

patic lesion (29.53%) in the present study. Various studies were reported associated with cattle hydatidosis in several Provinces. In Baneh City (Kurdistan Province) 5.69% (1.35% of livers) (Yakhchali and Gargi, 2006), in Hamadan Province 16.2% (Fallah et al., 2009), in Mashhad City 5.5% (Borji et al., 2012), in Fars Province 11.5% (Oryan et al., 2010), in Urmia City 14.93% (53.21% of livers) (Taghavi et al., 2012) and in Lorestan Province 9.4% (Ezatpour et al., 2014) of cattle were investigated for hydatid cyst.

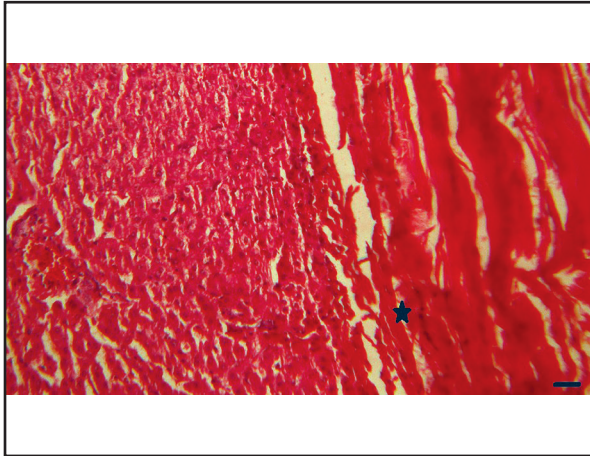


Figure 5. Existence of a large amount of collagen fibers that covered superficial inflammatory cells (star) in Glisson's capsule of cattle liver with perihepatitis (H&E, Scale bar= 50 μ m).

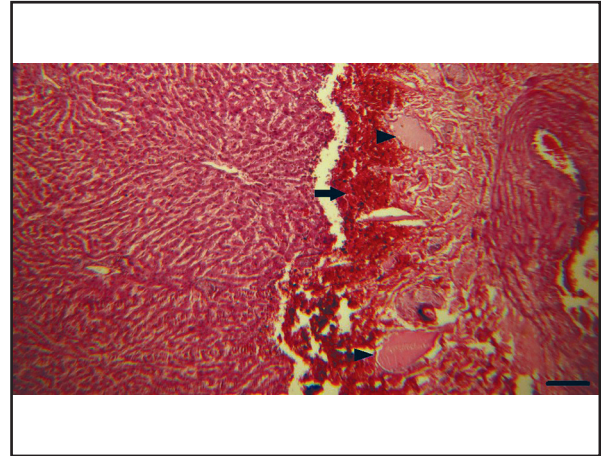


Figure 6. Hepatic parenchymal hemorrhage (arrow) and superficial vessels fibrinous thrombosis (arrow head) in the cattle with perihepatitis (H&E, Scale bar= 150 μ m).

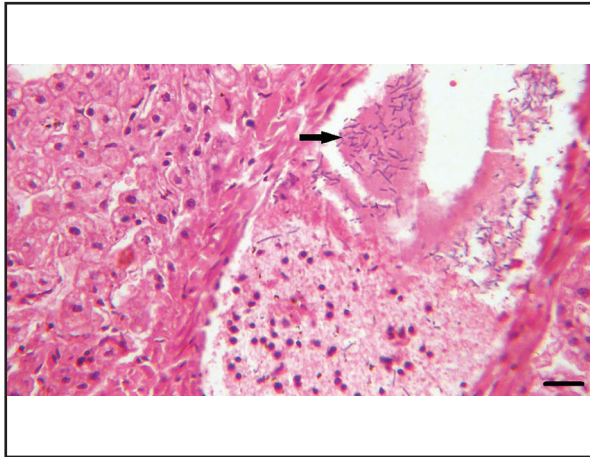


Figure 7. Bacillus shape bacteria in hepatic veins of cattle associated with perihepatitis (arrow) (H&E, Scale bar= 50 μ m).

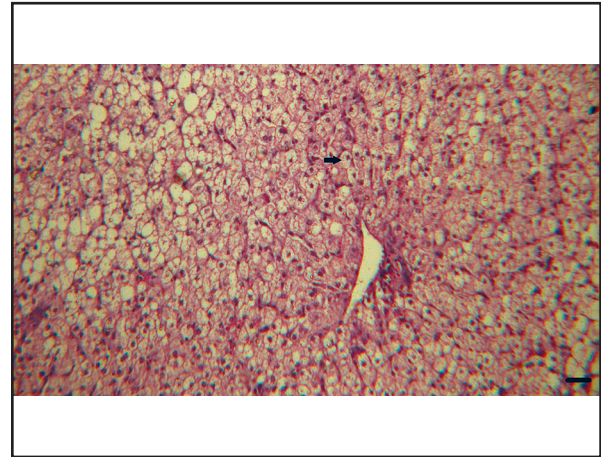


Figure 8. Hepatic lipidosis in cattle. Vacuolation of the hepatocytes, nuclear pyknosis displaced to the periphery of cells (arrow) (H&E, Scale bar = 40 μ m).

In all of these researches except Oryan et al. the percentage of hydatidosis contamination in cattle was higher than sheep and goats.

In the present study similar to the aforementioned studies the incidence of hydatidosis in cattle was higher than other diseases and lesions but the percentage of hydatid cyst infestation in cattle of Sistan region was much higher than other areas of Iran. This matter is indicative of the active life cycle of *Echinococcus granulosus* in this region. The most important causes of the significant outbreak of hydatidosis infesta-

tion in cattle in Sistan region are the favorable weather conditions for the survival of this parasite eggs, the extent of traditional livestock farming, non-sanitary slaughters in rural areas, the presence of herding dogs with no health control and the abundance of stray dogs which triggers the parasite's life cycle and also is a potential risk to human community health. Among the liver lesions observed in this study, three cases (1.07%) were diagnosed with fasciolosis. In the 2001-2002 research conducted in the industrial slaughterhouse in Yasuj City, the

prevalence of *Fasciola hepatica* in cattle was 12.5%, which was higher than sheep (11.75%) and goat (7.16%) (Moshfe et al., 2003). In a survey carried out in slaughterhouses in different cities of Kermanshah Province in 2004, the prevalence of *Fasciola* in cattle was reported 62-75% (Hosseini et al., 2004). In another study, in order to investigate the prevalence of infection with the common parasites in humans and livestock in cattle slaughtered in the industrial slaughterhouse of Hamadan in 2009, the rate of cattle infection with *Fasciola* was 9.5%, which accounted for a lower prevalence of hydatid cyst (16.2%) (Fallah et al., 2010). During 2006-2007, the rate of feces infection of cattle in Caspian Sea Provinces was investigated by Hosseini et al. in terms of fasciolosis, demonstrating 32.5%, 12.5% and 3.1% infection in Guilan, Mazandaran and Golestan Provinces, respectively. The higher prevalence of this disease in Guilan Province was attributed to the higher rainfall and humidity in comparison with Mazandaran and Golestan Provinces (Hosseini et al., 2012). In 2014, the rate of parasitic infections of the slaughtered cattle in the slaughterhouse of Lorestan Province was investigated by Ezatpour et al. and the *Fasciola* infection was reported 7.6%, which was lower than hydatid cyst (9.4%) (Ezatpour et al. 2014). The results of this study, compared with the above studies, indicate a lower percentage of infected cattle with fasciolosis in Sistan area. It seems that one of the main reasons for the low prevalence of this infection in the present study can result from the reduction of natural water resources due to successive droughts in the Sistan region, which has destroyed the habitat of the intermediate host snail and has affected their reproduction adversely. In this study,

two cases (0.71%) were diagnosed with perihepatitis. A study on foreign bodies and the occurrence of adhesions in the abdominal cavity of 200 buffaloes slaughtered in Ahvaz slaughterhouse from 2002 to 2003, 43 (21.5%) found adhesion. In addition, the adhesion rate in male buffaloes (13.5%) was reported less than female buffaloes (30.2%). This difference was because those female buffaloes were older than males, and consequently, they were exposed to external metal objects more frequently (Ghadrdan Mashhadi et al., 2007). In a California study of liver abscesses in 18 Holstein dairy cattle using ultrasound and laparotomy, six abscesses were reported due to peritonitis, which was higher than other factors, and liver adhesion was also recorded in three cases (Dore et al., 2007). In another clinical study on the type of strike retinoid peritonitis and their clinical symptoms in the cattle of Lorestan Province, the incidence of this lesion in female animals was higher due to their higher average age and direct relation between peritonitis incidence and age (Hajighahremani et al., 2010). In this study, pregnancy and associated contractions in the abdominal cavity and fore stomach pressure, especially during the last months of pregnancy, were among the important and complicating factors of peritonitis in the female livestock, which increased the risk and severity of these lesion complications in cattle (Hajighahremani et al., 2010). In the present study, the percentage and severity of adhesion in the abdominal cavity of the livestock were very low, and were observed only in two male cattle, especially into the capsule covering the surface of the liver, which in the microscopic examination caused an increase in the thickness and inflammation of the liver capsule and a

brief inflammation with hemorrhage in the infected liver parenchyma and was called perihepatitis. There was also no perforation and rupture caused by foreign bodies in the abdominal cavity as well as at the liver surface of the studied livestock, but the presence of bacilli in the surface vessels of the infected liver parenchyma increases the probability of bacterial infection of the lesion. The reason for the low incidence of this lesion in this study is the male gender of all slaughtered cattle. In the present study, two specimens (0.71%) showed liver lipidosis. During the histopathologic examination, it was found that the causes for this lesion were liver parenchymal rarefaction, swelling and vacuolation of hepatocytes, darkening and compression of their nuclei (pyknosis), and in some cases, pushing nuclei to the border of the cell. In a study conducted in Urmia industrial slaughterhouse from 2008 to 2010, 388 livestock including 114 cattle, 100 buffaloes, 147 sheep and 27 goats were investigated and the fat content was measured in dry and wet liver mass. Small ruminants (2.89%) showed the lowest amount of liver fat compared to cattle (3.61%) and buffaloes (5.29%). The data from this study revealed a difference in species, regardless of gender and age. Also, it was found that the incidence of feed related liver lipidosis in the big ruminant is greater than that of small ruminants (Ramin et al., 2012). In a study conducted in 2009 in order to investigate the levels of liver cells' triglyceride and total bilirubin and serum glucose in dairy hybrid cattle in Ahvaz slaughterhouses, fatty liver was the most frequent finding in female cattle about one month after their delivery. In this group, amount of non-esterified fatty acids was reported higher than 1100 $\mu\text{Eq/L}$, indicating that delivery in-

creases the risk of this lesion (Rezaei Saber and Nouri, 2009). In a study by Raoofi et al. on 106 Holstein dairy cattle in slaughterhouses around Tehran, 29 cattle (27%) were diagnosed with fatty liver syndrome by measuring the amount of fat in the liver by a Soxhlet extraction apparatus. It was mentioned as a significant percentage (Raoofi et al., 2001). It has been proven that fatty liver disease in female ruminants is more likely to occur, especially in late pregnancy and early lactation, due to a negative energy balance (Bobe et al., 2004). In the present study, all slaughtered livestock were males, so the incidence of liver lipidosis was not significant.

In conclusion, the results of this study indicate that the most common liver lesion in the cattle slaughtered in Zabol City is hydatidosis. This poses a potential danger to the human community health, in addition to imposing economic losses resulting from livestock losses, condemnation of the carcasses or infected organs, as well as reducing livestock production. Therefore, it requires more comprehensive health control measures.

Acknowledgements

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بررسی ماکروسکوپیک و هیستوپاتولوژیک ضایعات کبدی در گاوهای کشتار شده در شهرستان زابل - ایران

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چکیده

زمینه مطالعه: ضایعات کبدی در گاو نه تنها باعث اثرات منفی بر صنعت پرورش گاو می‌گردد، بلکه سبب افت تولید پروتئین حیوانی و خصوصاً به خطر افتادن سلامت مصرف کنندگان می‌شود. **هدف:** این مطالعه با هدف بررسی ماکروسکوپیک و هیستوپاتولوژیک ضایعات کبدی در گاوهای کشتار شده در شهرستان زابل انجام گرفت. **روش کار:** کبد ۲۸۱ رأس گاو کشتار شده در کشتارگاه صنعتی شهرستان زابل از فروردین تا شهریور سال ۱۳۹۴ جهت ارزیابی ظاهری و میکروسکوپی جمع‌آوری شد. مراحل آماده‌سازی بافتی از نمونه‌های مشکوک به ضایعات بافتی با روش متداول انجام گرفت و تغییرات هیستوپاتولوژیک کبدها توسط میکروسکوپ نوری بررسی شد. **نتایج:** ارزیابی ماکروسکوپیک ۲۸۱ نمونه، نشان دهنده وجود ۹۰ کبد (۳۲/۰۲٪) دچار ضایعات ظاهری بود. در بررسی هیستوپاتولوژیک، ۸۳ مورد (۲۹/۵۳٪) مبتلا به هیداتیدوزیس، ۳ مورد (۱/۰۷٪) مبتلا به فاسیولوزیس، ۲ مورد (۰/۷۱٪) مبتلا به پری هپاتیت و ۲ مورد (۰/۷۱٪) مبتلا به لیپیدوزیس بودند. **نتیجه گیری نهایی:** بر اساس نتایج این مطالعه شایع‌ترین ضایعه کبدی در گاوهای کشتار شده در شهرستان زابل هیداتیدوزیس بود که نشان دهنده چرخه زندگی فعال انگل اِکینو کوکوس گر فلوئوسوس و شیوع این بیماری انگلی در سگ‌ها و سایر گوشتخواران منطقه سیستان می‌باشد.

واژه‌های کلیدی: گاو، هیستوپاتولوژی، کبد، ماکروسکوپیک، گاوهای کشتار شده