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Renal Amyloidosis in Cattle: An Abattoir-Based Pathological Study in Urmia, Iran

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Introduction

Abstract Serum-associated amyloidosis is the most common form of amyloidosis in domestic animals and can lead to death. In this study, 1294 cattle (with a total of 2588 kidneys inspected) were inspected at the Sahand Abattoir, Urmia, Iran, over one year. Of these, 2462 kidneys were healthy (95.1%) while 126 kidneys had lesions (4.9%). There was no significant correlation between the frequency of gross lesions in the kidney and the sex or breed of the cattle (p>0.05). Additionally, the frequency of macroscopic changes in all cattle aged 4.5 years and older was significantly higher than that of younger ones and calves (p<0.05). Microscopic examination using hematoxylin and eosin, periodic acid schiff, and congo red tissue staining methods revealed the relative frequency of kidney lesions as follows: hemorrhage (27%), hydropic degeneration (16.7%),congestion (15.9%), interstitial nephritis (8.7%), glomerulonephritis (7.1%), acute tubular necrosis (6.3%), hydronephrosis (4.8%), cysts (4%), fibrosis (3.2%), amyloidosis (1.6%), pyelitis (1.6%), and renal lipidosis (0.8%). In two kidneys with amyloid deposits, one belonged to a bull with localized pulmonary tuberculosis, while the other belonged to a different bull with no other lesions detected in any organs. The most involved structures were glomeruli (98%), and only 2% of cortical tubules had amyloid deposits. Although renal amyloidosis is rare in slaughtered cattle in Urmia city, its heat resistance and potential transmission through food, especially to individuals with chronic inflammatory diseases, require careful inspection in slaughterhouses and condemnation of suspicious carcasses.

Amyloid is a pathological protein material deposited between cells in different tissues and organs of the body in a wide range of clinical conditions. While there are various components in the deposit, the primary constituent of the amyloid material is the amyloid filament protein. Depending on the type of amyloidosis and different clinical forms, the protein composition is different. Despite similarities in the components and staining characteristics of amyloid, it is biochemically considered a heterogeneous substance. Based on its deposition in the tissues and organs of different domestic and wild animals, different types and clinical forms of amyloidosis are known. The two main types of amyloidosis in animals include LA (light chain amyloid) and SAA (serum-associated amyloid). Type LA light chains, originating from unstable monoclonal immunoglobulins produced due to plasma cell dyscrasia, result in the formation and production of amyloid fibers. In type AA amyloidosis, the levels of SAA are increased, which is common in inflammatory conditions. However, elevated SAA levels alone are not necessarily sufficient to cause amyloid deposition leading to amyloidosis. The deposition of SAA can occur due to defects in the breakdown of monocyte-derived enzymes genetically or determined abnormalities in the structure of the SAA molecule [1]. Although renal amyloidosis is not common in cattle, it is diagnosed as an isolated disease in most dairy herds. Bovine renal amyloidosis usually affects one cow in the herd, and clinical symptoms do not appear until the animal is 4 years old or older [2]. Recent findings that AA amyloidosis indicate can be transmissible. Similar to the pathogenesis of transmissible prion diseases, this process involves seeding, which may lead to the formation of AA amyloidosis [2]. In 2005, renal amyloid deposition was confirmed in 15 out of 302 cows in Japan, resulting in an incidence rate of 5%. This rate was significantly higher compared to previous reports from Japan and other countries, which ranged from 0.4% to 2.7%. AA amyloidosis poses a life-threatening risk to patients with chronic inflammatory diseases, and these individuals should avoid consuming foods that may contain AA amyloid fibrils. However, more detailed information on bovine amyloidosis is needed to ensure food safety [3]. Bovine tissues designated for human consumption may harbor AA amyloid deposits. The ramifications of this phenomenon for human health are potentially extensive. Numerous animal experiments have demonstrated that AA amyloidosis can arise from the oral ingestion of AA fibrils. The previous results underscore the necessity for additional investigations to assess the biological risks associated with the consumption of food products containing amyloid [4]. Substantial evidence indicates that the pathology of murine and avian AA amyloidosis can be propagated through oral transmission, suggesting that this mechanism may also apply to additional species [5].

In this study, we aimed to collect kidney waste from slaughtered cows at the Urmia abattoir based on observed changes in color, size, and consistency. The collected kidneys were fixed in 10% formalin buffer, and tissue sections were prepared. These sections were stained with hematoxylin and eosin (H&E) and evaluated microscopically. Given that the incidence of amyloidosis is associated with chronic diseases, the objective of this study is to investigate the presence, severity, and extent of amyloid deposits in the renal structures of damaged kidneys, specifically glomeruli and intertubular spaces.

Materials and methods

This investigation was conducted over the course of one year, from September 1, 2023, to August 1, 2024, with inspections occurring two days each week. The specific days were varied in subsequent weeks. The study focused on the Urmia slaughterhouse (Dashte-Sahand, Urmia city, Iran), particularly in the context of postslaughter carcass inspection. Attended cows and the kidneys of slaughtered cows that exhibited changes in appearance, such as alterations in color, size, and texture consistency, or that had exudative secretions like pus or mucus, were collected after recording the age, sex, and, if possible, the breed of the animals. These samples were placed in a proportion of 1 volume of tissue to at least 10 volumes of 10% formalin buffer fixative. After transferring the samples to the pathology laboratory at the Urmia University, the tissues were left for at least 48 hours to undergo fixation. The tissues were then cut either longitudinally or transversely, depending on the type and location of the lesions. Following this, the tissue processing steps, including dehydration, clearing, and paraffin impregnation, were carried out using a tissue processor. The paraffin molds were then cut with a rotary microtome to a thickness of 6 micrometers and stained with hematoxylin and eosin (H&E). The prepared slides were evaluated using an Olympus optical microscope to assess the presence and determine the type of kidney lesions (6). Previous studies have reported the prevalence of bovine kidney amyloidosis to range between 5-10%. In the present study, the average prevalence rate is found to be 7%. Type 1 error ($\alpha = 0.05$), (p = 0.07 and d = 0.1), and the confidence level is 95% and (95% - Z Score = 1.96) be inspected. The sample size is calculated according to the following formula:

$$n = \frac{z_{1-\frac{\alpha}{2}}p(1-p)}{d^2}$$

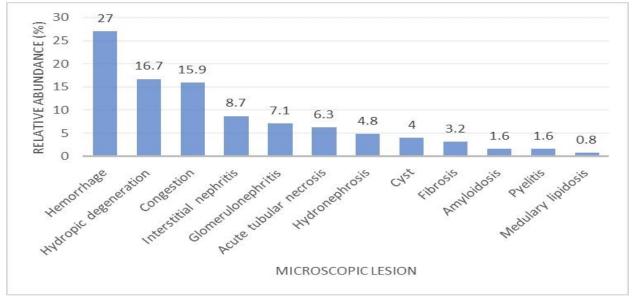
The abbreviations in the formula represent the following: *n* for sample size; *Z* for the standard score corresponding to the desired confidence level; α for the type I error; *P* for the estimated proportion of the population with the characteristic of interest; and *d* for the margin of error (precision level).

Given that the number of cows slaughtered at the Sahand abattoir in Urmia varied daily, ranging from 30 to 60 cattle, inspections continued after slaughter until the target sample size of 1294 pairs was achieved. In the microscopic examination of damaged kidneys, suspicious cases of glycogen or fat accumulation and amyloid deposition in the kidney, differential histochemical staining of periodic acid schiff (PAS) and Congo red were used.

Statistical analysis

Descriptive statistics of the studied variables, including absolute and relative frequencies, were calculated. Statistical analysis of the data was done using SPSS 27 software. Using Pearson's chi-square test and Fisher's exact test, we investigated the relationship between the demographic variables (age, sex, and breed) of slaughtered cows in Urmia city and the frequency of positive serum titers, as well as macroscopic and microscopic changes in kidney tissue. Significant differences between groups were determined using the Bonferroni post hoc test (p<0.05).



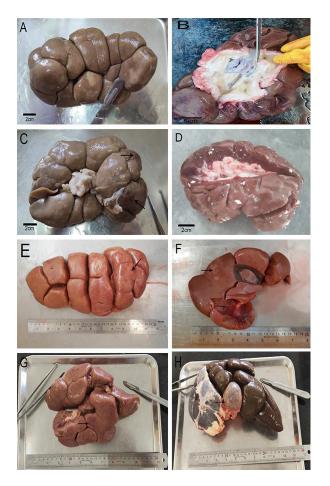


Results

Over the period from August 1, 2023, to July 31, 2024, the kidneys of 1294 cows slaughtered at the Sahand abattoir, Urmia, were subjected to macroscopic inspection for changes in color, consistency, and size. Among total of 2588 kidneys, the 1231 pairs of kidneys (2462

kidneys), all were normal in terms of color, consistency, and size. In contrast, 63 pairs of kidneys (126 kidneys) exhibited appearance changes, including paleness, darkness, staining, hardening, softening, and variations in size. Therefore, based on the results of the abattoir inspection, macroscopic changes were observed in 126 kidneys (5.1%), and no macroscopic lesions were detected in the kidneys of 2462 (95.1%) slaughtered cows.

The microscopic examination of bovine kidneys with macroscopic changes revealed the following frequency of microscopic lesions: medullary hemorrhage (34/126; 27%), hydropic degeneration (21/126; 16.7%), cortical and medullary congestion (20/126; 15.9%), interstitial nephritis (focal purulent or non-purulent) (11/126;



Discussion

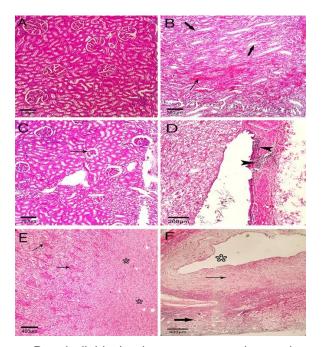
The results of this study showed that in the macroscopic examination (Figure 2) of the kidneys of 1294 cows (2588 kidneys), 2462 kidneys were healthy (95.1%) and 126 kidneys were damaged (4.9%). The results of the chi-square test indicated no significant relationship between the frequency of macroscopic kidney changes and the gender or breed of the slaughtered cows studied (p<0.05). Also, the

8.7%), glomerulonephritis (focal purulent or nonpurulent) (9/126; 7.1%), acute tubular necrosis (8/126; 6.3%), hydronephrosis (6/126; 4.8%), cyst (5/126; 4%), fibrosis (4/126; 3.2%), amyloidosis (2/126; 1.6%), pyelitis (2/126; 1.6%), and medullary lipidosis (1/126; 0.8%). In addition, no microscopic lesions were observed in three kidneys (3/126; 2.4%) (Figure 1).

Fig 2. Macroscopic renal lesions in slaughtered cattle (A): Kidney with pale areas extending to the medulla; microscopic examination showed focal congestion and hemorrhage in the cortex and medulla. (B): Hydronephrosis characterized by pelvic expansion and renal parenchyma atrophy, with surface indentation due to thinning parenchyma. (C): Kidney with focal darkening (arrow) in the cortex; microscopic examination revealed atrophied and cystic glomeruli (focal) in the cortex, focal hemorrhage in the medulla, and tubular hydropic degeneration and lipidosis of the medulla. (D): Normal bovine kidney with regular structures and no changes in color, consistency, or size. (E): Kidney with orange discoloration and pale/dark cortical spots (arrow), soft tissue consistency; microscopic examination showed amyloid deposits in glomeruli, fibrosis (glomerulosclerosis), and kidney parenchyma inflammation. (F): Kidney with focal cortical white spots (arrow); microscopic examination showed non-purulent focal interstitial nephritis with hyaline casts in cortical tubules. (G): Kidney with diffused paleness, white (arrow), and dark spots on the surface; microscopic examination revealed glomerular amyloidosis and renal parenchyma inflammation. No changes were detected in other organs. (H): Dark-colored kidney with a large cyst attached to the renal pelvis; microscopic examination showed pyelitis.

frequency of macroscopic changes in all cows aged 4.5 years and older was significantly higher than that of younger cows and calves (p<0.05).

The results of the present study are consistent with the results of Nourmohammadzadeh, et. al. regarding the effect of age on the occurrence of kidney lesions in cattle and the lack of effect of sex on their occurrence [7]. Microscopic evaluations of damaged kidneys revealed that the most common pathological change was hemorrhage, observed in 34 kidneys (27%). The least frequent pathological lesion was renal lipidosis, found in only one kidney (0.8%). Additionally, amyloid deposits were identified in two kidneys (1.6%). In this study, renal hemorrhage exhibited a higher relative frequency compared to other lesions (27%). In all cases of hemorrhage, no lesions such as renal infarction were present. Occasionally, hemorrhage was observed in kidneys with glomerulonephritis, interstitial nephritis, or renal lipidosis. In the remaining cases, hemorrhage was noted with congestion or without any other lesions in the



Renal lipidosis is uncommon in cattle; however, instances have been reported in pregnant cows or cows slaughtered immediately after parturition [9]. In the present study, only one case of kidney medulla lipidosis was observed, with an unclear cause. Hemorrhage was also present in parts of the medulla. Regarding the frequency of amyloidosis, it is noteworthy that out of two cases of renal amyloidosis in cattle, one was observed in a bull over 4.5 years old with local pulmonary tuberculosis, while the other was in a 3.5-year-old cow without any macroscopic symptoms in other organs. The results of the statistical analysis of the frequency of kidney. The reason for observing more cases of kidney hemorrhage (without the presence of other lesions) can be due to non-pathological reasons such as complications caused by electric shock before slaughter or manipulation and pressure of kidneys due to human factors. Of course, the frequency of cases of kidney hemorrhage with the presence of infarction has been reported in previous studies of cow kidney lesions in variable numbers and was in the range of 6.6% to 20.40% [8].

Fig 3. Photomicrograph of microscopic lesions in cow kidney (A): Normal kidney with regular glomerular and tubular structure. (B): Focal hemorrhage in the medulla (thin arrow) with tubular hydropic degeneration (thick arrow). (C): Atrophic and cystic glomeruli (arrow) in the renal cortex. (D): Pyelitis with local accumulation of inflammatory cells (arrowhead). (E): Hemorrhage in the medulla (arrow) with severe and diffuse vacuolation of the medulla (asterisk). (F): Hydronephrosis with pelvic dilatation (asterisk), fibrosis, hemorrhage, reduction in medulla thickness (thin arrow), and cortex atrophy with parenchymal inflammation (thick arrow); Hematoxylin and eosin (H&E) staining.

microscopic lesions (Figures 3 - 6) in all the studied cows by chi-square test and Fisher's exact test showed that there is no significant frequency relationship between the of microscopic lesions with the age, gender, and breed of slaughtered cows with macroscopic changes in all of them (p>0.05). Previous studies have indicated that the occurrence of amyloidosis is associated with chronic diseases within the body and have noted its sporadic occurrence in cows older than 4 years (10). In this regard, Tojo et al. have mentioned the occurrence of amyloidosis in cattle older than 6 years [3].

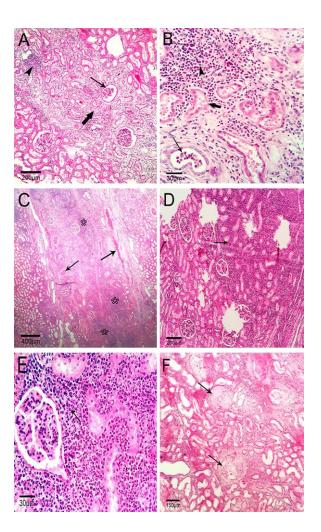


Fig 4. Continuation of microscopic lesions in bovine kidney (A): Acute localized non-suppurative glomerulonephritis with accumulation of inflammatory cells (arrowhead), degenerated tubules (thick arrow), and reduced number and atrophy of glomeruli (thin arrow). (B): Higher magnification of acute localized non-suppurative glomerulonephritis showing mononuclear (lymphocyte) inflammatory cells (arrowhead), degenerated tubules (thick arrow), and inflammatory cells within the lumen of some tubules (thin arrow). (C): Non-purulent chronic interstitial nephritis with destruction of tubules, replacement of inflammatory cells in tubular interstitial spaces (asterisk), protein hyaline casts (arrow) in some tubules, and presence of fibrosis in some areas. (D): Acute localized purulent glomerulonephritis with infiltration of purulent inflammatory cells (arrow) in tubular interstitial spaces and hypercellularity in glomeruli. (E): Higher magnification of acute localized purulent glomerulonephritis with predominantly neutrophilic inflammatory cells (arrow) in tubular interstitial spaces and inside glomeruli. (F): Kidney of a bull with tuberculosis showing amyloidosis with pink amyloid protein deposits in glomeruli (arrow) and inflammatory cells in tubular interstitial spaces; Hematoxylin and eosin (H&E) staining.

AA amyloidosis is the most common form of amyloidosis in domestic animals. This form is related to chronic inflammatory or neoplastic diseases (other than immunocytic dyscrasia) or may be idiopathic without any underlying disease. AA amyloidosis has been reported in domestic animal species, including dogs, horses, cats, cattle, pigs, sheep, goats, and avian species. This form is also used in chronic diseases of wildlife animals in captive cheetah (Acinonyx jubatus), Siberian tiger (Panthera tigris altaica), mink (Mustela vison), black-footed wild cat (Felis nigripes), black-footed ferrets (Mustela nigripes), gazelle (Gazella dorcas), mountain gazelle (Gazella gazella), bighorn and Dall's sheep, freeranging lions, swans and other lions have been described [1]. The results of a study have shown that cows with amyloidosis had chronic diseases such as chronic mastitis (6 cases) or chronic

pneumonia (4 cases), which are thought to be a causative factor for AA amyloidosis. However, in 5 cases of cows with amyloidosis, there was no chronic disease [11]. Another study, regarding the incidence of AA amyloidosis in cattle, shows that in cows with mastitis, metritis, and pododermatitis, there is a high prevalence of systemic amyloidosis in response to inflammation [12]. In another report, they detected the presence of systemic AL amyloidosis in a 5-yearold Holstein-Friesian cow in association with bovine leukocyte adhesion deficiency disease. In this study, amyloid deposits were identified in the perivascular and intercellular spaces of various visceral organs, including the liver, kidneys, pancreas, adrenal glands, and digestive system [13]. In addition, prion-induced amyloidosis has been observed in animals [14]. Although renal amyloidosis is uncommon in cattle, it is typically

diagnosed as an isolated condition in most dairy herds. Bovine renal amyloidosis usually affects only one cow within a herd, with clinical symptoms generally not manifesting until the cow is 4 years old or older [15]. Many retrospective studies have shown amyloidosis as a possible cause of animal death. Approximately 42 proteins in humans and 10 proteins in animals are recognized as amyloidogenic. Based on the location of the deposit, the cause can be systemic or local. Misdiagnosis of amyloidosis in animals may also increase the likelihood of zoonotic transmission. Therefore, evaluating the prevalence of amyloidosis requires considerable attention. Early diagnosis improves overall prognosis and reduces animal mortality [16].

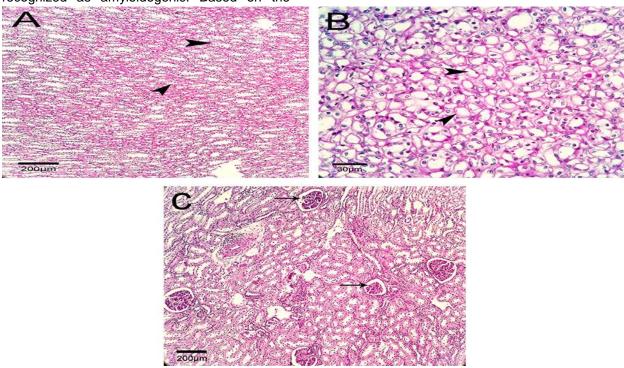


Fig 5. Microscopic photomicrograph of kidney lesions with specific staining (A): Tubular hydropic degeneration (arrow) with hemorrhage in the medulla. (B): Medullary lipidosis with PAS-negative fat-contained cells (arrowhead) and eccentric nuclei. (C): Localized acute glomerulonephritis with atrophic glomeruli and PAS-negative basement membrane (arrow), indicating proliferative glomerulonephritis; Periodic Acid Schiff (PAS) staining.

In reactive amyloidosis (AA), serum protein (SAA) forms deposits in mice, domestic and wild animals, and humans with a history of chronic inflammation. Extracellular deposition of amyloid fibers causes disease in affected animals. Recent findings suggest that AA amyloidosis can be transmissible. Similar to the pathogenesis of transmissible prion diseases, amyloid fibrils induce a process (seeding-nucleation) that may lead to the development of AA amyloidosis. AA amyloidosis develops in adult birds due to chronic inflammation, such as tuberculosis [2]. In mammals, including humans and cattle, AA amyloid deposition occurs in most organs through a process known as seeding. Misfolded SAA species catalyze the formation of fibrils, leading to the development of large extracellular amyloid aggregates. A previous report showed that AA amyloidosis may occur in Japanese cattle, which raises the question of whether this is the case in European cattle used for human consumption [4]. Another study conducted in Japan found that fecal transmission of AA amyloidosis in captive (caged) cheetahs contributed to the high incidence of the disease. In this study on animals with AA amyloidosis, it was discovered that the feces of cheetahs contained AA amyloid fibrils. These fibrils differed from those found in the liver in terms of their molecular weight, shape, and greater transmissibility. Therefore, the results of this study showed that feces are a transmission medium that may accelerate AA amyloidosis in a captive cheetah population. Thus, these results provide a pathogenesis for AA amyloidosis and suggest possible measures to save cheetahs from extinction [17]. AA amyloidosis typically deposits on the intestinal wall, potentially causing ulceration and amyloid fibril release. This transmission route is less probable in humans, raising questions about protective mechanisms. Seeding is protein-specific, yet cross-seeding by different fibrils can occur, especially among closely related proteins. It is plausible that

numerous protein aggregates with amyloid-like structures exhibit a preferential affinity for M cells. This affinity enhances their transport efficiency to locations where they can undergo further translocation [5]. The standard pH for in vitro assembly is, in fact, analogous to the conditions encountered during the digestive processes. Despite significantly lower temperatures, the hydrolysis of proteins is primarily facilitated by enzymatic action. There remains a paucity of knowledge regarding the potential assembly of degraded dietary proteins into amyloid-like formations throughout the digestive process. peptides Nonetheless, corresponding segments of human amyloid proteins have been demonstrated to enhance amyloid deposition in a murine model of AA amyloidosis [18].

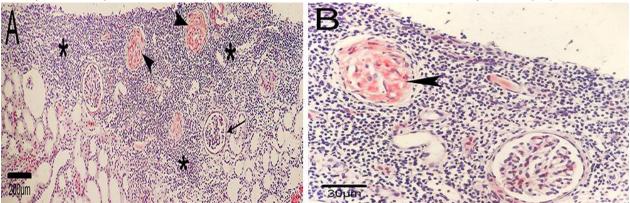


Fig 6. Photomicrograph of bovine kidney with amyloidosis **(A)**: Orange-colored deposits of amyloid protein inside the glomeruli (arrowhead), with inflammatory cell infiltration in the tubular interstitial spaces (asterisk) and periglomerular fibrosis (arrow). **(B)**: Further magnification of glomerular amyloidosis showing orange-colored deposits (arrow head); Congo red staining.

Concerning the heat resistance of amyloid protein, previous research showed that AA fibrils exhibit a considerable degree of thermal stability and, akin to prions, necessitate autoclaving at a temperature of 135 °C to eradicate their pathogenic properties effectively. These discoveries may play a pivotal role in mitigating the transmission of AA fibrils via alimentary substances to various animal species, particularly human populations [19]. In the current study, lesions such as glomerulonephritis and interstitial nephritis were observed focally. The macroscopically changes observed were primarily related to focal color changes and, occasionally, consistency changes (hardening in chronic cases), with no significant alterations in kidney size. These findings are consistent with the research of El-Mashad et al. The study noted that in cases of focal and diffuse interstitial nephritis, there were minimal changes in the kidney's appearance. The size of the kidneys remained normal or was slightly increased. Color changes manifested as red or gray spots on the capsule surface [8]. In domestic abattoir surveys, the primary reason for condemning cows' kidneys is multifocal or diffuse acute interstitial nephritis [6]. In this study, because lesions such as glomerulonephritis and interstitial nephritis were focal, local trimming was performed. Interstitial nephritis can present as diffuse, local, or focal and may occur in cattle following coliform septicemia, leptospirosis, malignant catarrhal fever, or lumpy skin disease. Conversely, glomerulonephritis predominantly is immunologically mediated, characterized by the involvement of antibodies and immune complex deposits in the glomeruli. It is also important to note that the administration of certain drugs, such non-steroidal anti-inflammatory drugs as (NSAIDs), aminoglycosides, vitamin K3, tetracycline, and amphotericin B, is nephrotoxic and can lead to various kidney complications. Therefore, it is necessary for veterinarian colleagues not to use these drugs, especially in cases of tissue dehydration or diseases associated with anorexia [20]. In the current study, interstitial nephritis and glomerulonephritis were focal. The causative agent was not identified due to the absence of bacterial culture.

Conclusion

The results of this study showed that in the macroscopic examination of 1294 cows (2588 kidneys) at the Sahand Slaughterhouse, Urmia over one year, 2462 kidneys were healthy (95.1%), while 126 kidneys were damaged (4.9%). Microscopic examination of kidneys with macroscopic lesions, using H&E, PAS, and Congo red tissue staining, revealed that hemorrhage, hydropic degeneration, congestion, interstitial nephritis, and glomerulonephritis were the most frequent, while renal lipidosis was the least frequent. Meanwhile, renal amyloidosis with a frequency of 1.6% was much less frequent than other lesions. Congo red staining revealed that in two cases of kidneys with amyloidosis, the glomeruli were the most affected structures (98%), while only 2% of cortical tubules had amyloid deposits. Although renal amyloidosis in slaughtered cows in Urmia city is guite rare, the potential for oral transmission, particularly to individuals with chronic inflammatory diseases, necessitates increased diligence in carcass inspections and the condemnation of any suspicious carcasses.

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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