

Study of Non-Specific Reactive Hepatitis in Stray Dogs

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Abstract

The purpose of this paper was to study the possible causes of Non-Specific Reactive Hepatitis (NSRH) in tissue samples of housed dogs that were collected from different cities of Andalucía (Spain). Histologically, this disease was characterized by the presence of lymphocytes and plasma cells spread throughout the liver parenchyma and in the portal stroma, with no evidence of hepatocyte necrosis. These animals showed non-specific chronic reactive hepatitis that varied from moderate to severe. In order of prevalence, the more common pathologies associated with NSRH were gastrointestinal and renal diseases, as well as pneumonia mainly of parasitic or infectious origin.

Keywords: Liver, Hepatitis, Dogs, Retrospective case series

Sokak Köpeklerinde Spesifik Olmayan Reaktif Hepatit Çalışması

Öz

Bu çalışmanın amacı, Endülüs'ün (İspanya) farklı şehirlerinden toplanmış olan barınak köpeklerine ait doku örneklerinde Spesifik Olmayan Reaktif Hepatit (NSRH) olası nedenlerini araştırmaktır. Hastalık, histolojik olarak, hepatosit nekrozu bulgusu olmadan, karaciğer parankimi boyunca ve portal stroma içinde yayılan lenfositlerin ve plazma hücrelerinin varlığı ile karakterize edildi. İncelenen hayvanlarda, derecesi orta ila şiddetli arasında değişen, spesifik olmayan kronik reaktif hepatit belirlendi. Görülme oranı yönünden değerlendirildiğinde, NSRH ile ilişkili olarak en sık görülen patolojiler gastrointestinal problemler ve böbrek hastalıklarının yanı sıra parazitik veya bulaşıcı kökenli pnömonilerdi.

Anahtar sözcükler: Karaciğer, Hepatit, Sokak köpekleri, Retrospektif olgular

INTRODUCTION

The liver is a vital organ to investigate in any individual animal, but also to determine the health status of a population of domestic or stray dogs, which may be reservoirs of diseases for humans, domestic and other free-living animals. Liver has important roles in homeostasis, metabolism, catabolism and immune function^[1]. Diseases within the hepatobiliary system for any reason could lead to decreased metabolism of toxins and therefore an increased risk of immune suppression or reproductive failure in the animal^[2,3].

Inflammation in the liver parenchyma is termed hepatitis and it can be classified as acute or chronic hepatitis^[3]. Acute hepatitis is characterized by random distribution

of necrosis and apoptosis, with neutrophils accumulate and minimal infiltrations of lymphocytes. This type of hepatitis is usually related to infectious organisms such as bacteria, like *Escherichia coli* that in neonates usually seed the liver via the umbilical veins or less often the portal venous or hepatic arterial systems^[3,4]. Chronic hepatitis is the most common form of hepatitis and is characterized by fibrosis, accumulation of mononuclear inflammatory cells, including lymphocytes, macrophages and plasma cells^[2-4]. In veterinary medicine, chronic liver disease may develop following chronic bile duct obstruction, infection with hepatotropic infectious agents, familial or hereditary metabolic diseases, or may be toxic, drug-induced, or possibly autoimmune in origin^[1-4]. Nonetheless, little information has been reported about this type of pathologies in domestic or stray dogs, among



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which parasitic infestations^[5], bacterial infections^[6], and viruses, such as those that affect the digestive system have been frequently described^[7].

Non-Specific Reactive Hepatitis (NSRH) is another form of hepatitis that has been well described in humans^[8], but little is known in domestic or free-living wild mammals^[2,9]. NSRH is distributed throughout the liver parenchyma in response to some systemic illness, most often in the gastrointestinal tract or as the residuum of prior liver inflammation. Typically, there is a mild inflammatory infiltrate in the portal tract and without evidence of necrosis^[8,9]. NSRH can be further classified into two different types according to the nature of inflammatory infiltrate; when there is an infiltrate of neutrophils within the connective tissue of the portal tracts it is called non-specific reactive active hepatitis; whereas when mononuclear cells, primarily lymphocytes and plasma cells are present it is termed as non-specific chronic reactive hepatitis^[4,8].

There are only few studies done on this new entity in veterinary medicine^[2,9-11]. The aim of this study was to analyze the histopathological changes and nature of the inflammatory infiltrates in dogs with NSRH and to correlate it with different extrahepatic diseases.

MATERIAL and METHODS

A total of 104 stray dogs that were housed in rescue shelters of Andalusia (Spain) were necropsied in the Faculty of Veterinary Medicine of Cordoba University between 2011 and 2016. They followed standard protocol that included macroscopic and microscopic evaluation of a range of organs, including the liver. The study was conducted with authorization of the Spanish Ministry of Interior (Protocol 2012) and the control of the Ethical Commission of Veterinary Medicine of the University of Las Palmas de Gran Canaria (agreement MV-2017/05).

Of these 104 carcasses, 58 cases were selected due to the evidence of macroscopic or microscopic liver changes noted in the reports. Cases with moderate to severe histologic distortion due to autolysis were eliminated. After review, only 23 cases of different breed, age and sex that fit the selection criteria were included in the study (*Table 1*). These criteria were used in other studies performed in marine and terrestrial mammals^[10-12], and included mild liver enlargement and presence of inflammatory infiltrates in the liver parenchyma. The tissue samples were fixed in 10% neutral-buffered formalin, dehydrated through graded alcohols, and embedded in paraffin wax. Sections (4 µm thick) were cut and stained with haematoxylin and eosin for histopathological examination. The number of infiltrating neutrophils, lymphocytes and plasma cells observed in the liver parenchyma were counted in randomly selected fields at 400x, which also included hepatic sinusoids and portal areas. NSRH was classified as mild (cell counts ranging from 20 to 50 cells per field), moderate

Table 1. Age, sex and species of dogs included in the study

Case	Age	Sex	Breed
1	Adult	M	Mixed-breed
2	Young	M	Mixed-breed
3	Young	M	French Bulldog
4	Adult	M	Golden Retriever
5	Young	F	Mixed-breed
6	Adult	F	Yorkshire Terrier
7	Adult	M	Yorkshire Terrier
8	Young	F	Spanish Water Dog
9	Young	F	Mixed-breed
10	Adult	M	Mixed-breed
11	Adult	F	Mixed-breed
12	Adult	F	Boxer
13	Young	M	Canary Mastiff
14	Young	M	Neapolitan Mastiff
15	Adult	M	Mixed-breed
16	Young	M	Mixed-breed
17	Young	M	English Bulldog
18	Young	F	Mixed-breed
19	Adult	F	Mixed-breed
20	Young	F	Mixed-breed
21	Adult	M	Labrador Retriever
22	Adult	M	Mixed-breed
23	Adult	F	Mixed-breed

(50 to 75 cells per field) or severe (more than 75 cells per field), following the WSAVA classification for liver inflammation^[12]. In addition, diagnoses of extrahepatic causes in animals with different NSRH severities were evaluated to see the possible relation with the degree of NSRH diagnosed.

Statistical Analysis

Variables were summarized as frequencies and percentages in each extrahepatic disease. Chi-squared test (χ^2) with Yates correction was used to compare prevalence between age (adult/young), sex, as well as the degrees (mild, moderate, severe) of the groups in the set of studied diseases. Differences were considered significant at $P < 0.05$.

RESULTS

The histopathological findings of the lesions presented in liver were in accordant with the diagnosis of this disease. All animals studied showed non-specific chronic reactive hepatitis. No other hepatic conditions such as hepatic lipidosis or pigment accumulation were seen. Two out of 23 were diagnosed as severe, five out of 23 as moderate and the rest of sixteen animals as mild NSRH. The histologic exam revealed an inflammatory infiltrate

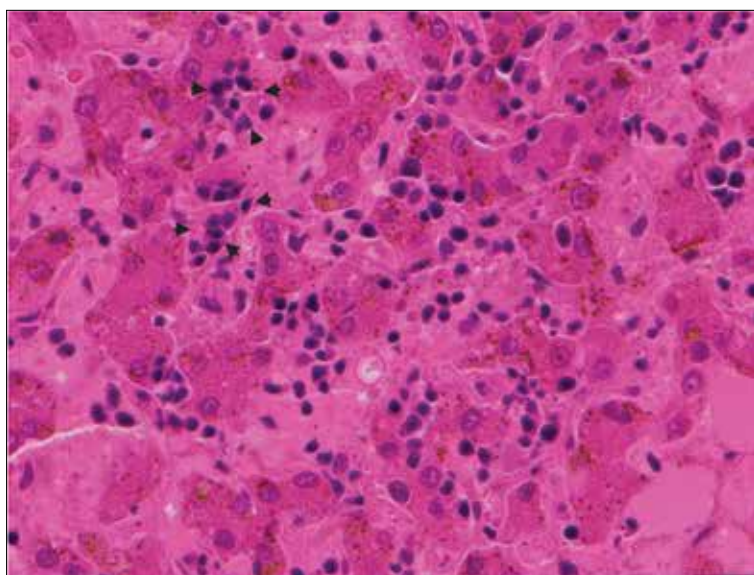


Fig 1. Moderate NSRH showing inflammatory infiltrate in portal spaces and hepatic sinusoids composed by lymphocytes and plasma cells (arrows), HE x20

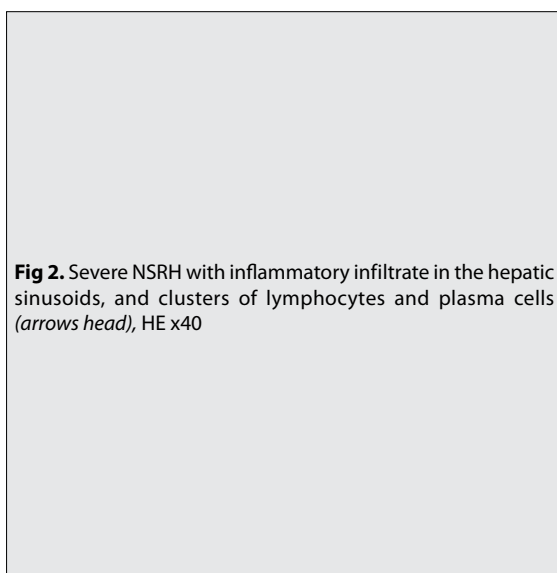
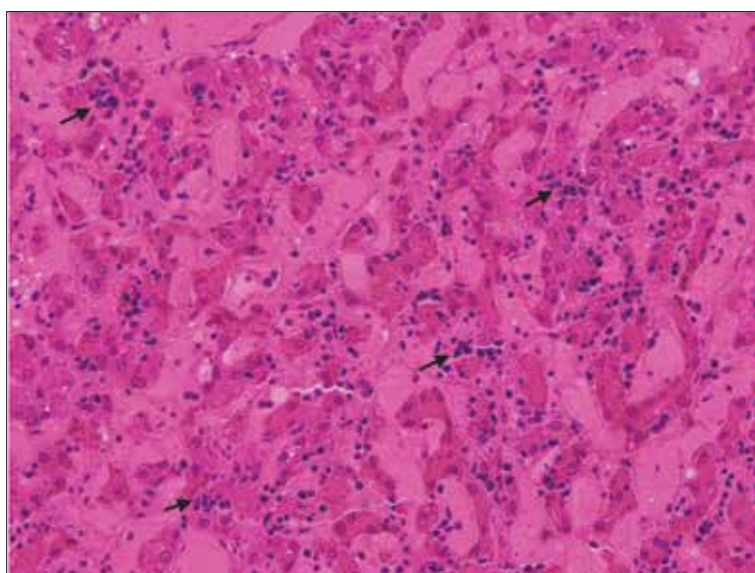


Fig 2. Severe NSRH with inflammatory infiltrate in the hepatic sinusoids, and clusters of lymphocytes and plasma cells (arrowheads), HE x40



composed of plasma cells and lymphocytes in the portal areas, around the hepatic veins and within the sinusoids, without evidence of hepatocellular necrosis or fibrosis (Fig. 1). Those dogs with severe NSRH also showed marked dilatation of the hepatic sinusoids as a result of increased number of erythrocytes, lymphocytes and plasma cells (Fig. 2).

These stray dogs showed some pre-existing extrahepatic disorders that might have accounted for NSRH (Table 2). Therefore, gastrointestinal diseases caused by parasites and viruses were the most common pathologies observed (10/23, 43.48%). Parasites involved in these processes were *Ascaris* spp. and *Ancylostoma* spp. (3/23, 13.04%), whereas Parvovirus (2/23, 8.7%) was the only virus related to gastrointestinal processes. The remaining five cases of gastrointestinal diseases were inflammatory diseases such as inflammatory bowel disease. Renal disease was the second extrahepatic pathology more commonly found

in this study (5/23, 21.74%), and consisted mainly of glomerulonephritis with thickening of the glomerular basement membrane and increased cellularity by proliferation of mesangial cells, and tubulonephritis with predominantly lymphocytic infiltrate associated with tubular degeneration and necrosis. Fibrinosuppurative pneumonia produced by *Pasteurella* spp. was also observed in 2/23 dogs. In addition, widespread granulomas due to fungal infection (*Cryptococcus neoformans*) were found in 2/23 animals. Other viruses identified were Adenovirus I (2/23, 8.7%) and Morbillivirus (2/23, 8.7%). Pathologies such as meningitis, demodicosis, *Ehrlichia canis* infection, melanoma and cirrhosis were also diagnosed in the dogs of the present study (Table 2).

The statistical analysis revealed no differences between age, sex, and degree of NSRH considering P values significant at $P < 0.05$. Gastrointestinal disease was the most prevalent process, with p value of 0.55 when trying to find differences

Table 2. Samples studied and overall prevalence of extrahepatic diseases in dogs according to age, sex and degree of NSRH

Extrahepatic Diseases	Age		Sex		Degree			Moderate - Severe (n=7)
	Adult (n=12)	Young (n=11)	Male (n=13)	Female (n=10)	Mild (n=16)	Moderate (n=5)	Severe (n=2)	
Fungal granulomas	8.3% (n=1)		7.7% (n=1)		6.3% (n=1)			
Adenovirus	8.3% (n=1)	9.1% (n=1)	7.7% (n=1)	10% (n=1)		40% (n=2)		28.57% (n=2)
Virus infection		9.1% (n=1)	7.7% (n=1)				50% (n=1)	14.3% (n=1)
Inmunodeficiency		9.1% (n=1)		10% (n=1)	6.3% (n=1)			
Gastrointestinal disease	33.3% (n=4)	54.6% (n=6)	38.5% (n=6)	40% (n=4)	43.7% (n=7)	40% (n=2)	50% (n=1)	42.9% (n=3)
Nematodes	8.3% (n=1)	18.2% (n=2)	15.4% (n=2)	10% (n=1)	12.5% (n=2)	20% (n=1)		14.3% (n=1)
Parvovirus		18.2% (n=2)	7.7% (n=1)	10% (n=1)	12.5% (n=2)			
Others	25% (n=3)	18.2% (n=2)	23.1% (n=3)	20% (n=2)	18.75% (n=3)	20% (n=1)	50% (n=1)	28.57% (n=2)
Renal disease	33.3% (n=4)	9.1% (n=1)	15.4% (n=2)	30% (n=3)	25% (n=4)	20% (n=1)		14.3% (n=1)
Heart failure		9.1% (n=1)	7.7% (n=1)		6.3% (n=1)			
Meningitis		9.1% (n=1)	7.7% (n=1)		6.3% (n=1)			
Foreign body obstruction	8.3% (n=1)		7.7% (n=1)		6.3% (n=1)			
Demodicosis		9.1% (n=1)	7.7% (n=1)		6.3% (n=1)			
Morbillivirus		9.1% (n=1)		10% (n=1)		20% (n=1)		14.3% (n=1)
Pneumonia	8.3% (n=1)	9.1% (n=1)	7.7% (n=1)	10% (n=1)	6.3% (n=1)	20% (n=1)		14.3% (n=1)
Peritonitis		9.1% (n=1)		10% (n=1)		20% (n=1)		14.3% (n=1)
Mycotic tracheobronchitis		9.1% (n=1)		10% (n=1)	6.3% (n=1)			
<i>Ehrlichia canis</i> infection	8.3% (n=1)		7.7% (n=1)				50% (n=1)	14.3% (n=1)
Cirrhosis	8.3% (n=1)		7.7% (n=1)		6.3% (n=1)			
Melanoma	8.3% (n=1)			10% (n=1)	6.3% (n=1)			

according to age, and 0.9 and 0.67 in the case of gender and degree of NSRH, respectively.

DISCUSSION

Histologic features observed in the 23 stray dogs were similar to those described in cetaceans [10,11] and dogs with reactive hepatitis [9]. Thus, NSRH was the only hepatic change observed in the present study. Identical results were described in other studies of the canine liver [9] or in numerous studies of humans with the hepatitis C virus [13]. Although this form of hepatitis is one of the most common forms of hepatopathy, only a few detailed descriptions

have been reported [9-11]. Interestingly, this process is often not taken in consideration and misdiagnosed as chronic hepatitis. All these authors describe NSRH as an unspecific reaction of the liver that may result from a variety of causes, such as bacterial endotoxins resulting from sepsis, or a bystander reaction of the liver to any systemic reaction, as well as febrile illnesses and inflammation in the splanchnic bed [2,8,11].

Non-specific chronic reactive hepatitis was the unique form of NSRH detected in these dogs. They were seen in the different age, sex and breed groups of the study population, and often associated with chronic inflammatory lesions

in other organ systems, most commonly gastrointestinal disease (10 cases), renal disease (5 cases) or pneumonia (2 cases). Similar results were seen in other studies performed in dogs [2,9]. Nevertheless, the other form of NSRH termed as non-specific reactive active hepatitis was only observed in cetaceans [11], but not in any of the animals of our study.

The degree of inflammation observed in the dogs of the present study was mild to moderate and the distribution of the inflammatory infiltrates in this organ was diffuse or periportal. Therefore, most of the hepatitis cases had mild portal inflammatory infiltrates (16/23, 69.57%). This pattern was also a common finding in a case series of hepatic lesions in cetaceans stranded on the Canary Islands [10,11], as well as in other studies performed in dogs [9], where they described slight to moderate infiltration of neutrophils in acute cases and plasma cells and lymphocytes in chronic cases [9-11]. In contrast, 2 out of 23 animals of our study showed severe inflammatory infiltrate in the hepatic sinusoids forming clusters. It has been suggested that it could be associated with local or systemic antigen stimulation of the immune system caused by infection or infestation [11]. In other reports done in large number of animals, similar hepatitis was found in combination with other hepatic findings such as lipidosis or peracute hepatitis [1]. Nonetheless, this type of hepatic findings was not found in any animal of our study. Only one animal of 23 showed hepatic cirrhosis that was related to exposure to toxic substances. Moderate (5/23, 21.74%) and severe (2/23, 8.7%) NSRH were often associated with inflammatory and non-inflammatory diseases in other organ systems. Nevertheless, cases with mild inflammatory infiltrates were associated with gastrointestinal and renal disease, pneumonia, and other pathologies like melanoma, dermatitis, *Ehrlichia canis* infection or meningitis. However, other reports associated reactive hepatitis with a primary problem in the heart such as cardiac insufficiency, diseases of the reproductive system as endometritis and endocrine disorders, mainly diabetes mellitus [9].

Studies done in large number of stray dogs have also showed that high number of animals were parasitized with these helminths [5], or similar viruses [7]. Despite this fact, it is interesting to highlight that no information has been described about NSRH in stray dogs, among which parasites [5], bacteria [6], and viruses, such as those found in the digestive tract have been frequently reported [7]. Moreover, recent studies emphasized the relevance to evaluate stray dogs since can have an epidemiological importance in relation to different zoonotic diseases [14] due to they share the same environment with humans and thus can be host or reservoir of a wide variety of zoonosis [15].

The statistical analysis showed no significant differences between age or gender and degree of NSRH in each extrahepatic disease.

In conclusion, the results of the present study pointed that disorders of a wide variety of organs inside and outside the splanchnic bed could be associated with NSRH. Hence, within this collection of 23 cases of liver disease, there was evidence NSRH of variable severity that could be related to different stages of extrahepatic disorders such as parasitic or infectious diseases. The grading system used in this study provided more detailed information than was typically described in the case record and has the added value of uniform designations of lesion classification and severity. Larger studies must be done in order to obtain a better estimation of the relation between NSRH and extrahepatic diseases, as well as to learn about the specific mechanism underlying these phenomena.

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