DOI: 10.22059/IJVM.2022.344655.1005285 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

Evaluation of the Presence of Hairballs in the Gastrointestinal Tract and Urinary Stones in Razi Institute Laboratory Rabbits

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Abstract

BACKGROUND: Usually, the daily self-grooming by rabbits lead to fur accumulation in the animal's stomach. Since rabbit hair is looser than other animals and they constantly licking their body the fur can be pulled out easily. On the other hand rabbits are susceptible to urinary stone formation. **OBJECTIVES:** This study was designed to investigate the presence of hairballs and urinary stones in Razi Institute laboratory rabbits.

METHODS: During the one-year period the albino Dutch laboratory rabbit colony, in research, breeding and production of laboratory animals department of Razi Institute, including 106 males, 287 females and 166 neonates, were monitored. After necropsy of the selected animals, the gastrointestinal tract (stomach and intestines) were examined for the presence of hair and hairballs, and then the urinary system (kidneys, ureter, urinary bladder and urethra) were examined for the presence of any urinary stones.

RESULTS: No symptoms of anorexia, lethargy, abdominal pain, weight loss, decrease and abnormal stools were observed in them and also no mortality occurred in the whole colony. Stomach in all samples was full which indicates eating has been enough. No gas or congested spots or hemorrhage were observed in the intestines. The amount and consistency of stool in the intestines were normal. In none of the samples, hairballs were observed, but in most rabbits' stomachs (both sexes), a small amount of hair was observed in the stomach contents. Also, in the colony of the studied rabbits, no symptoms of urinary stones were observed.

CONCLUSIONS: Balanced diet and supply nutritional requirements as well as the absence of any stressors in breeding environments, has played a key role and has prevented many diseases such as hairballs and urinary stones. No observation of urinary stones in this study could lead to the hypothesis that infection with the bacteria that cause urinary stones in the studied rabbits were eliminated or non-pathogenic, which could be an indicator for specific pathogen free animals. However, bacterial and other infectious agents monitoring should be specialized.

KEYWORDS: Gastrointestinal tract, Hairballs, Rabbit, Stones, Urinary tract

Introduction

Rabbits are herbivorous animals; also due to the special high-fiber diet, they named fibrevores animals. In la boratory rabbits, food is available to the animal without restriction; therefore, the animals' stomach is never empty and occupies more than 15% of the gastrointestinal tract contents (Quesenberry and Carpenter, 2011). Usually, the daily self-grooming by rabbits lead to fur accumulation in the animal's stomach. Since rabbit hair is looser than other animals and they constantly licking their body the fur (also known as hair, cony, coney, comb or lapin) can be pulled out easily. Therefore some hair may enter to the mouth and pace through the stomach. Also the fur spilled on the food is always eaten. On the other hand, hair plucking in the last third of pregnancy to make a nest box is common and some of the plucked hairs may be eaten. Trichophagia is a condition in which the amount of hair eaten is excessive (Quesenberry and Carpenter, 2011). Eaten hair in the stomach becomes hairballs or fur balls that after a while, due to enlargement in the stomach, remain and cannot pass through the pyloric valve to enter the duodenum. This condition is also called trichobezoar (Figure 1) (Mondal *et al*., 2006; Quesenberry and Carpenter, 2011). Rabbits with trichobezoar develop anorexia, lethargy, movement inability, reduced mobility, abdominal pain, weight loss, abdominal distention, lack of self-cleaning, and stool pieces become smaller than usual (Fukumura *et al*. 2012; Nowland *et al*., 2015).The formation of hairballs in the stomachs of other animals such as sheep is also common, especially in woolly sheep. In humans, hair eating occurs following a mental disorder called rapunzel syndrome (Godara *et al*., 2015).

Urinary stones may be found in the kidneys, urinary bladder, and urinary tract of rabbits of any age and breeds (mostly middle-aged and older rabbits) (Figure 2).

Rabbit urine is concentrated and sometimes creamy due to its calcium carbonate and ammonium magnesium phosphate crystals and sometimes dark red due to the existence of porphyrin and may be mistaken for hematuria due to blood in the urine (Lee *et al*., 1978; Pinto Filho *et al*., 2016).The pH of rabbit's urine is alkaline and is between 8 and 9. The alkalinity of rabbit urine is due to the high alkaline carbonates in the diet formulation. The constituents of urinary stones are including magnesium, ammonium, phosphate, oxalate, carbonate as well as uric acid, urate and cysteine. The urinary stones are usually made up of calcium carbonate and oxalate. Urinary stones are more common in male rabbits due to the length of the urinary tract (King, 2006; Nowland *et al*., 2015). Among the causes of urinary stones in rabbits genetic factors, hormonal and nutritional imbalances, infections, especially the presence of bacteria in the urinary bladder, and kidney diseases are the most important causative factors (Quesenberry and Carpenter, 2011). On the other hand, some nutritional deficiencies such as Vitamin B6 deficiency also increases oxalate secretion and consequently lead to urolithiasis (Quesenberry and Carpenter, 2011). It is well documented that some breeds, such as the dwarf lop, are susceptible to urinary stone formation. Therefore, laboratory strains should not be obtained from them (White, 2001).

Urease-producing bacteria, such as *Proteus mirabilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Serratia* are always associated with stone formation and recurrence .These bacteria break down urea into ammonium and carbon dioxide, causing the urine to become alkaline and form phosphate salts. Intestinal microflora could prevent and/or induce the formation of kidney stones. *Oxalobacter formigenes* is an anaerobic gram-negative bacterium that reduces oxalate in the gastrointestinal tract and has been shown to prevent

calcium oxalate stones. In patients with kidney stones, bacteria of the genus *Bacteroides* are found to be 3-4 folds more than normal. Therefore, there is a possibility of their role in the occurrence of stones. Gram-positive bacteria of *Eubacterium* has shown the opposite effect on oxalate formation and *Escherichia coli* has the opposite effect on citrate formation (Wang *et al*., 2021). Clinical signs of rabbits with urinary stones include lethargy, anorexia, loud painful gritted teeth, hunching, reluctance to move, pushing the abdomen to the ground, reduced neonatal care, force and painful urination and the presence of blood in the urine (hematuria) can lead to death (Quesenberry and Carpenter, 2011). Also, in obese animals, due to the presence of fat around the kidneys and urinary ducts, the natural contraction of the urinary bladder and its normal position is disturbed and the bladder does not have a contractile state for complete excretion of urine. Therefore, a significant amount of urine remains inside the bladder and provides the basis for stone formation. On the other hand the resulted pain from arthritis, pododermatitis and spinal problems lead to incomplete urination and caused to urine retention (White, 2001).

High dietary calcium is involved in the formation of urinary stones. Prolonged consumption of high-calcium diets causes calcification (calcium accumulation) in the kidneys and aorta (Quesenberry and Carpenter, 2011). Urine is the main way of excreting calcium in rabbits. If dietary calcium is high, urinary calcium excretion is also increased. In rabbits, 45-60% of the calcium that enters their body is excreted in the urine and 20% of it, is excreted in the feces. In other mammals, such as rats, 93% is excreted in the feces and only 2% in the urine. Also, blood calcium in rabbits is 30-50% higher than other mammals (Quesenberry and Carpenter, 2011).

Oxalate is metabolized in the intestines by bacteria. Therefore, insufficient or destroyed intestines microflora consequently lead to accumulation of oxalate in the kidneys and urinary tract which can cause stones (Wang *et al*., 2021).

This study was designed to investigate the presence of hairballs and urinary stones in Razi Institute laboratory rabbits.

Figure 1. Types of rabbit hairballs in pathogenic condition (Nowland *et al*., 2015).

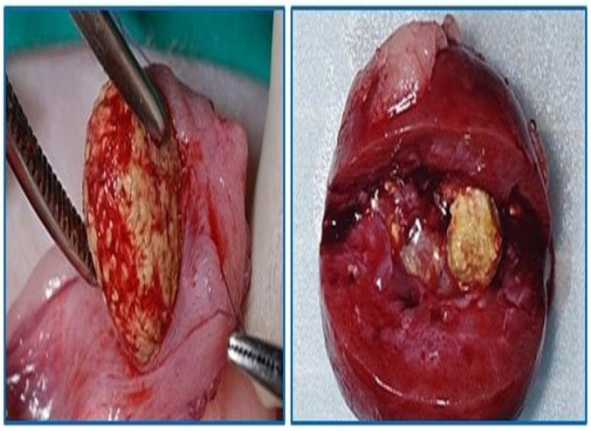

Figure 2. Urinary stones in rabbit kidney and urinary bladder (White, 2001).

Materials and Methods

During the one-year period in (June 2021- February 2022), the albino Dutch laboratory rabbit colony, in Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute, including 106 males, 287 females and 166 neonates, were monitored. In two turns with an interval of 6 months, 45 female and 12 male rabbits aged at 9-10 months (2-2.2 kg) were randomly selected. The animals were apparently healthy and negative for external parasites and had no specific disease. The rabbits were fed with the standard pellet of laboratory rabbits and they had access to water *ad libitum*. The rabbit breeding system was conventional and the material of the cages was aluminum with mesh floor and the tray under it was made of stainless steel. There was one female rabbit with neonates in each cage, and the male rabbits were kept in separate cages, individually. The temperature of the breeding room was 22-24°C, with humidity in a range of 45-55%. The light/dark cycle was 12:12 hours per day and the light intensity was less than 325 Lux.

The selected animals were transferred to the necropsy room and first, according to the ethical principles, euthanasia (by combining ketamine at 600 mg/kg and xylazine at 30 mg/kg by intramuscular injection) was performed (Baneux *et al*., 1986). After necropsy, the gastrointestinal tract (stomach and intestines) were examined for the presence of hair and hairballs, and then the urinary system (kidneys, ureter, urinary bladder and urethra) were examined for the presence of any urinary stones. Also, the diets used by the studied rabbits were analyzed to compare the nutritional requirements of this animal.

Rabbit nutritional requirements and diet analysis

160 Table 1 shows the nutritional requirements of laboratory rabbits and the analysis of diet used by the rabbits in this study.

Table 1. Nutritional requirements of laboratory rabbits and analysis of diet used by the studied rabbits (Circella *et al*., 2021).

Material	Rabbit nutritional requirements	Analysis of rabbit diet
Crude protein (%)	15-17	18.2
Energy (Kcal/lb)	950	912
Crude fat (%)	2	2.4
Crude fiber (%)	10-12	11.17
Calcium (%)	0.45	0.5
Phosphorus (%)	0.37	0.31
Arginine (%)	0.76	1.02
Histidine (%)	0.38	0.39
Isoleucine (%)	0.76	0.89
leucine (%)	1.2	1.34
Lysine (%)	0.92	0.88
Methionine (%)	0.6	0.57
Phenylalanine (%)	0.76	0.84
Threonine (%)	0.61	0.63
Tryptophan (%)	0.23	0.23
Valine (%)	0.84	0.88
Vitamin B6 (mg/kg)	1	3
Vitamin B2 (mg/kg)	4	4.85
Choline (g/kg)	1.2	1.34

165

Results

Hairballs

During one year of examination, in the colony of albino Dutch rabbits, no symptoms of anorexia, lethargy, inability to move, decreased mobility, abdominal

pain and distension, weight loss, lack of self-cleaning, abnormal stools, were observed and also no mortality occurred in the whole colony. Of 45 female and 12 male rabbits, no abnormalities were observed in the contents of the stomach and intestines after necropsy. Stomach in all samples was full which indicates eating has been enough (Figure 3). No gas or congested spots or hemorrhage were observed in the intestines. The amount and consistency of stool in the intestines were normal. After opening the stomach and various parts of the intestines, in none of the samples, hairballs were observed, but in most rabbits' stomachs (both sexes), a small amount of hair was observed in the stomach contents (Figure 4).

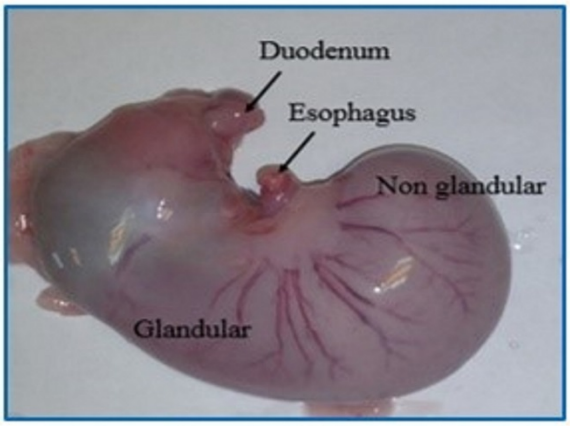

Figure 3. The stomach of all rabbits examined was normal and full.

Figure 4. The presence of hair in the contents of the rabbit's stomach

Urinary stones

The recorded data revealed no symptoms of urinary stones, including lethargy, anorexia, painful gnashing of teeth, hunchbacked, inability to move, pushing the abdomen to the ground, reduced neonatal care, painful urination were observed. In necropsies of selected animals, concentrate and creamy urine and sometimes dark red were observed (Figure 5). In none of the samples of kidney, urinary bladder and urinary ducts in all the studied rabbits, no stones and gravel were observed even in obese animals (Figure 6).

Figure 5. Rabbit concentrated and cream-colored urine when prepared directly from the bladder.

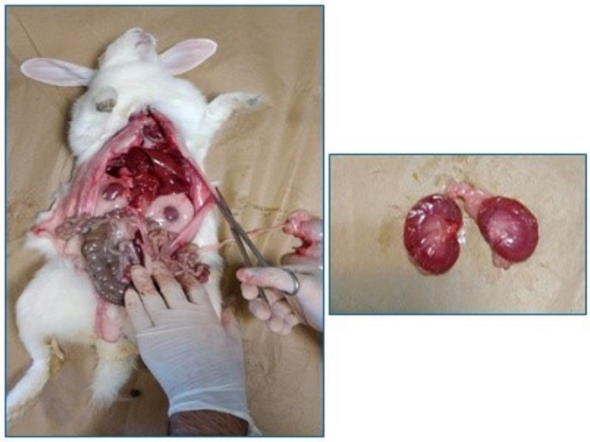

Figure 6. Absence of stones and gravel even in the kidneys and urinary tract of obese rabbits

Discussion

Causes of trichophagia in rabbits include low fiber and essential amino acids, high carbohydrates, starches and sugars in the diet, as well as the presence of any stressors, lack of mobility, decreased water intake, anorexia and concurrent diseases especially coccidiosis, enterotoxemia and *E. coli* infections (Circella *et al*., 2021; Mondal *et al*., 2006; Quesenberry and Carpenter, 2011). Reducing fiber reduces the movement of ingested substances in the digestive tract. Eating and gastrointestinal motions are interdependent. When movement decreases or stops, stool production also decreases or stops. Therefore, if the rabbit does not eat for only 12 hours, this issue should be investigated urgently (Quesenberry and Carpenter, 2011). In the case of domestic rabbits, carpet fur may also be eaten. In Angora rabbits, trichophagia is much more common and even leads to death

(Mondal *et al*., 2006). Mondal *et al.* examined the prevalence of trichobezoar in four breeds of angora rabbits in sub-temperate himalayan conditions in a 5-year study and found that 28.6% of rabbit deaths were due to trichobezoar and its complications. In that report, in most of the samples, trichobezoar was a large, single mass in the stomach of rabbits, and the contents of the stomach in affected animals with trichobezoar were watery, and the amount of stool in the intestines was low and very firm. Hairballs obstructed the pyloric valve and pathomorphological lesions were observed in the stomach, liver, lung, heart and kidneys (Mondal *et al*., 2006).The amount of crude fiber required in the rabbit diet is 10-14% (National Research Council Subcommittee on Laboratory Animal, 1995). In rabbit nutrition, both digestible and non-digestible fibers are required and have metabolic and physical benefits. Long-strand fibers, as in hay, promote healthy dentition because the plant silicates and large particles keep the constantly growing molar surfaces in proper occlusion.The long fibers also propel ingested fur through the digestive tract and reduce the risk for trichobezoars in the stomach. The larger particles from non-digestible fiber stimulate gut motility and enterocyte turnover (Nowland et al., 2015).Wang *et al.* published a report on recent developments in the mechanisms of urinary stone formation (Wang *et al*., 2021). Circella *et al.* published a report of the occurrence of 0.5-2 cm stone in the urethra in the preputial sac of a rabbit, which was made of calcium carbonate and was successfully removed by surgery (Circella *et al*., 2021).

In this study, in all necropsied rabbits, the stomach was completely full, indicating adequate appetite and complete eating. No hairballs were observed in the contents of the stomach and intestines, even in small sizes. Only a small amount of hair was observed in the contents of the stomach, which was normal and usual. In the

analysis of the diet, it was found that the amount of fiber, essential amino acids, calcium and phosphorus and vitamins, especially vitamin B6 are in the normal range and supply the nutritional requirements of rabbits. Also, the amount of energy in the diet was not more than normal, so the amount of carbohydrates in the diet was not high. All the rabbits had normal mobility, water and food intake. The amount, size and strength of rabbits' daily stools are normal, indicating proper digestive activity. In none of the samples of kidney, urinary bladder and urinary ducts in all the studied rabbits, no stones and gravel were observed even in obese animals. During the examination period, symptoms of anorexia, lethargy, gnashing of teeth, reluctance to move, abdominal distension and pain, pushing the abdomen to the ground, painful urination and the presence of blood in the urine (hematuria), decrease weight and any special diseases were not observed in rabbits and the environmental conditions of breeding salons were favorable and far from stressors.

Conclusion

Balanced diet and supply nutritional requirements especially protein, energy, fat, fiber, minerals, amino acids and various vitamins as well as the absence of any stressors in breeding environments, has played a key role and has prevented many infectious and non-infectious diseases such as hairballs and urinary stones (Fallahi, 2021; Fallahi & Mansouri, 2017; Fallahi, Mansouri & Modir roosta, 2020; Fallahi & Eslampanah, 2021). The absence of urinary stones in this study could lead to the hypothesis that infection with urease-producing bacteria that cause urinary stones in the studied rabbits were eliminated or non-pathogenic, which could be an indicator for specific pathogen free animals. However, bacterial and other infectious agents monitoring should be specialized.

Ethics

All study protocols have been approved by the ethics committee of Razi Vaccine and Serum Research Institute.

Conflict of Interest

The authors declare that they have no conflict of interest.

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بررسی وجود توپ‌های مویی در دستگاه گوارش و سنگ‌های ادراری در خرگوش‌های آزمایشگاهی مؤسسه رازی

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زمینه مطالعه: خرگوش‌ها در طول روز تمیز کردن خود را انجام می‌دهند که این کار غالباً منجر به تجمع مو در معده حیوان می‌گردد. از آنجا که موهای خرگوش نسبت به سایر حیوانات سست‌تر است و دائماً بدن خود را می‌لیسند، خزهای بدنشان به راحتی کنده می‌شود. از طرفی، خرگوش‌ها مستعد تشکیل سنگ‌های ادراری هستند.

هدف: این مطالعه به منظور بررسی وجود توپ‌های مویی و سنگ‌های ادراری در خرگوش‌های آزمایشگاهی مؤسسه رازی انجام شد.

روش کار: طی دوره یک ساله، کلنی خرگوش‌های آزمایشگاهی نژاد داچ آلبینو بخش پرورش حیوانات آزمایشگاهی مؤسسه رازی شامل 106 سر حیوان نر و 287 سر ماده و 166 نوزاد، تحت نظر قرار گرفته شدند. پس

از کالبدگشایی، دستگاه گوارش (معده و روده‌ها)، از نظر وجود مو و توپ‌های مویی مورد بررسی قرار گرفته و سپس سیستم ادراری (کلیه‌ها، میزنای، مثانه و میزراه) از نظر وجود هر گونه سنگ ادراری مورد بررسی قرار گرفتند.

نتایج: هیچگونه علائمی از بی‌اشتهایی، بی‌حالی، درد شکمی، کاهش وزن، کاهش و غیر طبیعی شدن مدفوع، در آنها مشاهده نگردید و نیز در کل کلنی هیچ تلفاتی رخ نداد. معده در تمام نمونه‌ها، پر بود که نشان‌دهنده خوردن غذا به مقدار کافی بوده است. در روده‌ها گاز و نقاط پرخون و یا خونریزی مشاهده نگردید. مقدار و قوام مدفوع در روده‌ها طبیعی بودند. در هیچکدام از نمونه‌ها، توپ‌های مویی مشاهده نشد ولی در اکثر معده خرگوش‌ها (هر دو جنس)، مقدار کمی مو در محتویات معده مشاهده شد. همچنین در کلنی خرگوش‌ها، هیچگونه علائم ناشی از ابتلا به سنگ‌های ادراری مشاهده نشد.

نتیجه‌گیری نهایی: متعادل بودن جیره غذایی و تأمین نیازهای غذایی و نیز عدم وجود هر گونه عامل استرس‌زا در محیط‌های پرورشی، نقش اساسی داشته و از بروز بسیاری از بیماری‌ها نظیر توپ‌های مویی وسنگ‌های ادراری جلوگیری به عمل آورده است. عدم مشاهده سنگ‌های ادراری در این بررسی، می‌تواند این فرضیه را مطرح نماید که عفونت به باکتری‌های مؤثر در ایجاد سنگ‌های ادراری منتفی و یا در حد غیر بیماریزا بوده‌اند که می‌تواند به عنوان شاخصی برای حیوانات عاری از عوامل بیماریزا مطرح باشد. گرچه باید پایش‌های باکتریایی و سایر عوامل عفونت‌زا بصورت تخصصی صورت گیرد.

واژه‌های کلیدی: توپ‌های مویی، خرگوش، دستگاه ادراری، دستگاه گوارش، سنگ‌ها

390