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ANNUAL CYCLICITY OF GALL STONE PREVALENCE IN DEER MICE (*PEROMYSCUS MANICULATUS GAMBELII*)

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ABSTRACT: The prevalence of gall stones (100% cholesterol) in a deer mouse (*Peromyscus maniculatus*) population located in Siskiyou County, California (USA) was studied on a monthly basis from February 1985 through January 1988. During each year we documented a pronounced annual cyclicity with peak prevalence (31 to 53%) during the winter and low prevalence (2 to 3%) during late summer. Gall stone prevalence was not related to sex or age of the animal. The earliest onset of gall stone production and the maximum prevalence achieved were associated with the greatest abundance of deer mice; lower levels of population abundance were associated with later onset of gall stone production and lower peak prevalences. This association of gall stone prevalence with both season and population abundance levels suggests that the causative factor(s) is/are related to the quality and availability of the diet.

Key words: Gall stones, cholesterol, *Peromyscus maniculatus*, annual cycle, field study, prevalence, dietary factors.

INTRODUCTION

Although absent in certain rodents and ungulates with mainly cellulose diets, such as the pocket gopher, rat and horse (Vorontsov, 1967; Davenport, 1982), most mammals possess gall bladders to concentrate and store bile. Possession of a gall bladder does, however, increase the likelihood of gall stone formation. These stones may consist primarily of the calcium salt of unconjugated bilirubin (pigment stones) or of cholesterol (cholesterol stones). The propensity for gall stone formation under natural environmental conditions appears relatively widespread among primates, such as marmosets, baboons, orangutans and humans. Other mammals including the hedgehog, ox, rabbit and elephant are known to occasionally produce gall stones (Gurll and Denbesten, 1979).

Cholelithiasis has also been documented in several species of rodents, including the hamster, guinea pig, house mouse and ground squirrel, in response to diet manipulations under laboratory conditions (Gurll and Denbesten, 1979). However, we are aware of only a single report of gall stone formation in wild rodents (Pence et al., 1978). Therefore, we were surprised during the initial phase of a long-term study of liver parasites in deer mice (*Peromyscus maniculatus gambelii*) to find that gall stones were common. We studied this phenomenon over a 3 yr period during which we documented a pronounced, repeatable, annual cycle of gall stone prevalence. In this report we document the annual cyclicity of gall stone prevalence, discuss this on the basis of age and sex, and suggest an explanation for the seasonality of gall stone formation.

MATERIALS AND METHODS

All deer mice examined during this study were captured along dike roads on the Tule Lake National Wildlife Refuge, Siskiyou County, California (41°50'N, 121°30'W) at an elevation of 1,230 m. The habitat, 40 m wide and 7 km long, is bounded along the long axis by Tule Lake on one side and by an irrigation ditch on the other, and at both ends by contiguous habitat. The most prominent weedy vegetation consists of summer cyprus (*Kochia scoparia*), tansy-mustard (*Descurainia Sophia*), tumble-mustard (*Sisymbrium altissimum*), giant wildrye (*Elymus cinereus*), lamb's-quarters (*Chenopodium album*) and nettle (*Urtica holosericea*). These plants form a relatively dense cover during the spring and early summer and then die; during the fall and winter the area is sparsely covered by dead-down vegetation. Climatically, the area

is characterized by warm summers and relatively cold winters. Frost can occur during any month of the year. Meteorological conditions during this study were normal for the area and are recorded at Headquarters, Tule Lake National Wildlife Refuge (Tulelake, California 96134, USA).

The deer mice were captured in rolled oat-baited Sherman (H. B. Sherman Traps, Inc., Tallahassee, Florida 32316, USA) live traps spaced at 8 m intervals along sections of the dike roads. Trapping was conducted at the new moon phase of each lunar cycle (approximately 28 day intervals) from February 1985 through January 1988 (collections made on the 1st and 30th of December 1986 are reported as a single monthly sample). Deer mice captured during the 36 consecutive monthly samples were killed, weighed, measured, and sex and reproductive status determined. The mice were then classified as adult (body length ≥ 90 mm) or subadult (body length < 90 mm). The intact liver with gall bladder was removed from each animal and placed in 10% formalin. Although the gall stones were easily seen with the unaided eye in fresh gall bladders, all were examined under a dissecting microscope. The chemical composition of the gall stones was determined through infrared spectrophotometry by Arawak Laboratories, Inc. (Los Angeles, California 90036, USA).

FIGURE 1. Gall bladders from *Peromyscus maniculatus*. Conditions illustrated from top to bottom are no gall stones present, compacted with gall stones of similar size, partially filled with gall stones of various size, filled with cholesterol sheet and many small gall stones, filled with several large (approximately 1 mm) gall stones.

RESULTS

During the 3 yr of this study we examined the gall bladders of 3,915 deer mice. Of these, 846 (22%) contained gall stones. Analysis showed these gall stones were entirely composed of cholesterol. The stones varied in size and shape from flat sheets a few micrometers in diameter to well-developed whitish spheres approximately 1 mm in diameter (Fig. 1). These stones often filled the gall bladder, occasionally to the extent that they blocked the common bile duct.

The data on monthly numbers of each age and sex of mice examined and gall stone prevalence is presented in Table 1. A significant ($P < 0.05$) sex-related difference in gall stone prevalence occurred only once in the adult deer mice during the 36 consecutive months of this study (October 1987, $\chi^2 = 4.26$, df = 1, $P < 0.05$) and twice in the subadult age class (March 1986, $\chi^2 = 4.35$; and May 1986, $\chi^2 = 6.24$, df = 1). In each case gall stone prevalence was higher among the males than in females. Since significant differences between sexes in either the adult or the subadult component occurred so rarely, the sexes were grouped, and the monthly sample compared for potential age-related differences in gall stone prevalence. This occurred in only three of 36 comparisons (May 1985, $\chi^2 = 3.92$, df = 1, $P < 0.05$; and in January and February 1987, $\chi^2 = 4.66$ and 5.23, df = 1, respectively, $P < 0.05$). Thus, the prevalence of gall stones in this deer mouse population was essentially independent of age- or sex-related factors.

The prevalence of gall stones in the population was not stable over time during any

TABLE 1. Relationship of gall stone prevalence to age, sex, season of year and relative abundance of deer mice. Given are number of mice examined (n), the number of these with one or more gall stones (+) and the gall stone prevalence (%) of the entire sample during each of 36 consecutive months. The relative abundance (% trap success) could not be accurately determined in January 1988 because of inclement weather.

	Adult				Subadult					
	Male		Female		Male		Female		Gall stone prevalence	Relative abundance
Date	n	+	n	+	n	+	n	+	(%)	(%)
1985										
Feb	43	25	35	13	5	3	4	2	49	98
Mar	54	18	43	23	8	3	9	1	40	98
Apr	37	12	66	16	12	3	7	2	27	98
May	43	12	36	5	19	1	22	2	17	100
June	47	4	57	4	6	0	8	1	8	95
July	36	3	58	2	14	1	5	0	5	96
Aug	27	1	29	3	41	5	22	1	8	93
Sept	32	0	36	2	36	0	15	1	3	97
Oct	38	15	39	10	22	5	21	5	29	56
Nov	50	23	32	12	14	6	23	8	41	63
Dec	40	20	32	9	20	11	27	9	41	61
1986										
Jan	62	33	38	18	11	4	22	15	53	60
Feb	68	34	28	13	7	3	12	3	46	57
Mar	55	20	33	8	8	5	19	4	32	67
Apr	50	13	43	7	2	1	11	2	22	41
May	37	7	50	7	5	3	10	0	17	48
June	29	2	73	7	3	1	2	0	9	28
July	21	1	45	5	21	0	12	0	6	19
Aug	10	1	22	1	50	0	18	0	2	13
Sept	20	0	40	1	33	1	6	0	2	15
Oct	23	3	33	2	41	4	10	1	9	15
Nov	32	9	21	3	32	6	32	11	25	41
Dec	98	39	87	33	9	2	15	4	37	43
1987										
Jan	44	24	45	19	7	0	9	3	44	95
Feb	41	16	31	8	8	2	20	1	27	90
Mar	38	8	31	8	14	3	16	1	20	97
Apr	35	8	31	12	18	4	23	9	31	89
May	34	8	32	5	14	1	26	3	16	24
Jun	41	4	56	3	5	0	6	0	7	15
July	38	3	48	5	10	0	6	0	8	10
Aug	19	2	31	0	38	1	11	0	3	16
Sept	11	1	18	1	52	2	15	1	5	7
Oct	32	5	30	0	24	3	17	2	10	6
Nov	44	4	28	2	20	1	8	0	7	28
Dec	13	0	13	1	7	0	6	1	5	28
1988										
Jan	38	13	25	6	4	1	5	2	31	

of the three annual cycles (χ^2 = 206.30, 192.21, and 90.36, $P < 0.001$, df = 11 for years one through three, respectively; Fleiss, 1981). However, the seasonal cyclicity of gall stone prevalence exhibited a pronounced year-to-year consistency. For example, peak prevalence levels invariably occurred during the winter months and annual lows during the late summer or early fall (Table 1, Fig. 2). However, in

spite of a similar seasonal cyclicity, gall stone prevalence during year one was significantly higher than during year two during April, October, and November ($\chi^2 = 4.30$, $P < 0.05$; $\chi^2 = 13.98$, $P < 0.001$; $\chi^2 = 7.16$, $P < 0.01$; df = 1 for all, respectively). Further, monthly gall stone prevalence among the three annual cycles was significantly different from October through March ($\chi^2 = 21.22$, $P < 0.001$; $\chi^2 = 44.32$, $P < 0.001$; $\chi^2 = 17.75$; $P < 0.001$; $\chi^2 = 9.93$, $P < 0.01$; $\chi^2 = 13.90$, $P < 0.001$; $\chi^2 = 9.28$, $P < 0.01$; df = 2 for all, respectively). Nonetheless, except for the lower amplitude during February and March of year three, the decreasing seasonal prevalence of gall stones was remarkably similar during all years of the study (Fig. 2). Conversely, the chronology of gall stone recrudescence was not consistent between years. The most pronounced increase in gall stone production occurred from September to October in year one, from October to November in year two, and from December to January in year three (Fig. 2). Further, peak prevalence levels of 53% (January, cycle 1), 46% (February, cycle 2), and 31% (April, cycle 3) document additional significant differences ($\chi^2 = 13.40$, $P < 0.001$, df = 2) in the gall stone cycles.

The annual relative abundance of deer mice (based on monthly trap success) varied from 85% (56% to 100%) during year one to 40% (13% to 95%) and 46% (6% to 97%, excluding January 1988 when inclement weather prevented an accurate assessment of population abundance) during years two and three (Table 1). Population abundance during the time of increasing gall stone formation (September–January) averaged 68%, 31%, and 14% for years one to three, respectively.

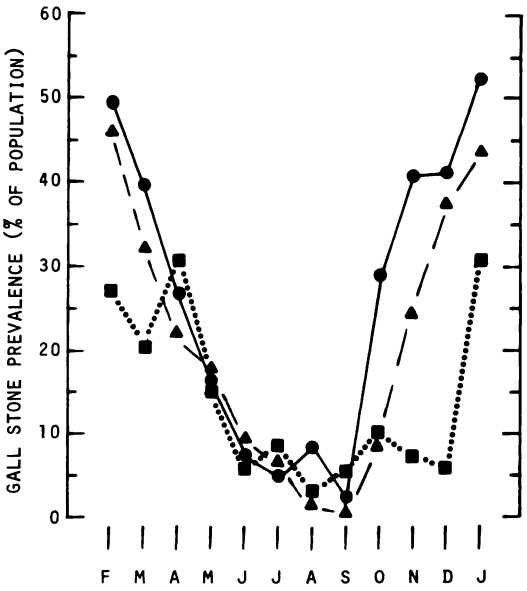

FIGURE 2. Consecutive annual cycles of gall stone prevalence in a population of deer mice, *Peromyscus maniculatus*. Year one (beginning February 1985) is indicated by the circles, year two by the triangles, and year three by the squares. The total number of deer mice examined was 3,915.

DISCUSSION

There are several dietary regimens known to induce gall stone formation in laboratory rodents. A feature common to all these dietary manipulations is supersaturation of the bile with cholesterol relative to the levels of bile salts and phospholipids which normally solubilize the cholesterol. When this occurs, cholesterol crystalizes out of the solution, agglomerates and ultimately forms gall stones. Bile may become supersaturated with cholesterol as a result of several conditions including (1) a decrease in the bile salt pool, (2) decreased bile salt secretion rate, (3) excessive cholesterol secretion in the bile, (4) biliary stasis, (5) infection in the biliary system, and (6) infrequent or incomplete emptying of the gall bladder (Gurll and Denbesten, 1979).

While it is possible that two or more of these factors could act synergistically to produce supersaturation of the bile with cholesterol, the cyclical prevalence of gall stones in these deer mice makes it unlikely that conditions outlined in (4) through (6) are involved. However, dietary alterations can markedly affect conditions (1) and (2) because a portion of the bile salts secreted into the intestine is subsequently resorbed

and returned to the liver through the portal circulation. These resorbed bile salts help to maintain the bile salt pool and may also inhibit cholesterol metabolism. The presence of dietary fibers, particularly those with a high lignin content, can cause retention of bile salts in the intestine, thereby reducing the bile salt pool (Story, 1981). Thus, both the availability of recycled bile salts for immediate secretion as well as the negative feedback mechanism of bile salts on cholesterol metabolism can be affected by the nature of the intestinal contents. We believe that this diet-related mechanism is a plausible explanation for the seasonal and magnitudinal fluctuations of gall stone formation in this deer mouse population.

Deer mice are omnivorous and are known to augment their herbivorous diet by eating invertebrates. On numerous occasions, particularly from late spring to early fall, the mice were observed feeding on insects. However, such foodstuffs would be much less available during the colder portions of the year. Deer mice do not hibernate and, because of relatively low availability of other foods on our study area during winter, it is likely that the winter diet consists primarily of seeds and/or dried plants. Thus, it may not be coincidental that periods of potentially high lignin content in the diet coincide with periods of high gall stone prevalence. This is to some extent substantiated by evidence that gall stone formation can be induced in the hamster, guinea pig, mouse, prairie dog and ground squirrel via dietary manipulations (Gurll and Denbesten, 1979). For example, guinea pigs held on a weight-losing diet form cholesterol stones when fed 1% cholestyramine which binds the bile salts. Conversely, hamsters form gall stones after being fed a diet high in sucrose or glucose but free of fat and fiber. In this case, cholesterol stone formation can be decreased by adding cellulose, polyunsaturated fatty acids, or uncooked starch to substitute for the sugar in the diet. Thus, there appears to be several, perhaps even species-specific, dietary mechanisms whereby production of cholesterol gall stones can be induced.

Although the factor(s) causing the annual cycle in gall stone prevalence in this deer mouse population is (are) not presently known, they might well be associated with other cyclical parameters. Initially, we examined the relationship between the annual gall stone and population abundance cycles. The annual population abundance was high and relatively little seasonal fluctuation occurred during the first year of this study (Table 1). In contrast, the subsequent 2 years were characterized by lower annual abundance and more pronounced seasonal fluctuation. Nonetheless, a pronounced annual cycle in gall stone prevalence occurred each year. This suggests that abundance-related factors may not play a pronounced role in gall stone production during periods when both the quality and availability of food are high. Conversely, abundance-related factors may be more involved in gall stone production when the diet is of less quality and/or is less available. This is supported by the similarity between annual gall stone cycles from May through September when foodstuffs are abundant and of high quality compared to the relatively asynchrony of the cycles during seasons when food is less available and of lower quality.

Thus, the most suspect of the environmental factors potentially causing the gall stone cycles is a season-related change in the diet. Such a change could involve both the nutritional quality and, depending on the population abundance, the amount available per individual. The hypothesis that abundance-related dietary factors may seasonally be major determinants of gall stone formation in this population of deer mice is supported by the differential chronology of recrudescence and peak levels of gall stone prevalence during the three cycles. For example, the decreased availability of a high quality diet resulting from the relatively high deer mouse abundance (68% trap success) is a plausible explana-

tion for the relatively early recrudescence and higher peak prevalence of gall stones in year one.

The change in diet hypothesis also predicts that a more suitable diet/individual potentially resulting from the lower deer mouse abundance during year two (31% trap success) and year three (14% trap success) would be associated with increasingly later onset of gall stone recrudescence and lower peak levels. Our hypothesis that seasonal change in the quality/quantity of the diet is the major factor causing the annual cycle in gall stone prevalence is supported by the role diet is known to play in gall stone production in other rodents (Portman et al., 1975; Story, 1981).

Additional evidence that diet may be the paramount factor inducing gall stone production in wild rodents has been documented by Pence et al. (1978). They found cholesterol gall stones in 71% of cottonrats (*Sigmodon hispidus*) collected during winter from a xeric site in Lubbock County, Texas (USA) where the sparse vegetation consisted primarily of annual forbs and grasses and the stomach of these cottonrats was usually distended with coarse dried plant material (stems, roots and leaves). Conversely, they did not find gall stones in cottonrats collected during the winter and early spring from relatively mesic areas. The stomachs of these animals were partially filled with green forbs. These results are ecologically comparable to those of our study as the study area is essentially xeric during the winter and mesic during the summer.

Under certain conditions the formation of gall stones can occur at a rapid rate. Mice fed 1% cholesterol and 0.5% cholic acid will form cholesterol stones after about 2 mo on this diet. Prairie dogs and ground squirrels fed a high (1.2%) cholesterol diet develop biliary cholesterol crystals within 5 days after treatment and discrete stones after 14 days (Gurll and Denbesten, 1979). Our documentation of gall stones in very young deer mice also suggests a very rapid rate of formation. We found gall stones in a 9.7 g female and an 8.2 g male with body lengths of 74 mm and 69 mm, respectively. These animals were among the smallest captured and probably were not yet completely weaned. Certainly they had begun to incorporate hard foodstuffs in their diet no longer than 2 wk previous to capture. Therefore, it is possible that deer mice born during the winter may ingest lithogenic milk resulting from the females' diet during this season. Such milk may contribute to gall stone formation through its effect on the bile salt pool in neonatal mice (Subbiah and Hassan, 1982). It would be interesting to learn if such milk affects the growth, survival, and activity patterns of winter-born neonatal deer mice. This and other potential effects of gall stones on the biology of this deer mouse population warrant further investigation. However, because there is such great variation between mammalian species as regards their cholesterol metabolism (Portman et al., 1975), ecological studies should be augmented by specific laboratory investigations on the effect of diet on gall stone production in this population of deer mice.

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