MEDDOCS Open Access Publisher

ISSN: 2640-1223

Journal of Veterinary Medicine and Animal Sciences

Open Access | Research Article

Establishment of a Breeding Colony of *Peromyscus Yucatanicus*: Comparison of Reproductive Performance Between Wild- and Laboratory Born Mice

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Received: May 28, 2020

Accepted: Jul 15, 2020

Published Online: Jul 21, 2020

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Peromyscus yucatanicus; Breeding colony; Reproductive performance.

Abstract

In the Peninsula of Yucatan, Mexico, Localized Cutaneous Leishmaniasis (LCL) is a wild zoonotic disease. The Yucatan deer mouse, *Peromyscus yucatanicus*, is one of the three known reservoirs of the parasite, *Leishmania (Leishmania) mexicana*. The study of reservoir species is of great importance to understand the immune response of a resistant host and to search for a vaccine. However, to become an experimental animal has first to be adapted to captivity. Very little is known about the requirements of captive *P. yucatanicus*. The objective of the present research was to establish a breeding colony of *P. yucatanicus* and to compare the reproductive performance between wild- and laboratory-born mice. The breeding colony was derived from 37 wild-born *P. yucatanicus*. The fertility rate was maintained during ten years and was not significantly different among birth origins. However, laboratory-born mice took more time to produce a litter (46 days versus 39 days), were more aggressive to their mate (7.9% mortal fight) than wild-born ones (0%), produced smaller litter (2.7 versus 3 pups per litter) and cannibalized more pups (litter size at weaning 2.2 versus 2.6). Although the reproductive performance of wild-born *P. yucatanicus* was better than laboratory-reared mice, reproduction can still be enhanced improving husbandry to produce the number of animals needed for the study of *L. (L.) mexicana* infection.

Cite this article: Van Wynsberghe NR, Rivero-Cárdenas NA, Itzá-Ortiz MF, Sosa-Bibiano EI, Loría-Cervera EN, et al. Establishment of a Breeding Colony of *Peromyscus Yucatanicus*: Comparison of Reproductive Performance between Wild- and Laboratory-Born mice. J Vet Med Animal Sci. 2020; 3(1): 1026.

Introduction

Leishmaniases is a complex of zoonotic diseases of the Tropics and Subtropics [1]. The infective agent, *Leishmania* Ross, 1903 (Protozoa: Trypanosomatidae), is transmitted by phlebotomine vectors (Insecta: Psychodidae) to mammalian hosts [2-4]. Thus, *Leishmania* needs a mammalian species - a reservoir - to maintain the transmission cycle of the disease. In the Yucatan Peninsula of Mexico, one reservoir of *Leishmania (L.) mexicana* Biagi, 1953 emend. Garham, 1962, is the Yucatan deer mouse, *Peromyscus yucatanicus* J.A. Allen and Chapman 1897 (Rodentia: Cricetidae) [5-8]. The particularity of a good reservoir is that the animal harbors the parasite without much ill effect for the host. The studies of reservoir species are of great importance to understand the immune response of a resistant host. The study of both the biology of laboratory animals and their husbandry is primordial to any biomedical research.

The genus *Peromyscus,* known as deer mice, is one of the most widespread and geographically variable New World rodents [9]. *Peromyscus* are nocturnal and terrestrial [10]. They probably eat grains, seeds, fruits, and insects. Although most species of deer mice live in Mexico and Central America, very little is known about their behavior [11]. The monotypic species *P. yucatanicus*, as its name indicated, is restricted to the Yucatan Peninsula and is placed in the "*mexicanus*" species group which is an assemblage of about six species [12,13]. Hall stated that many species of deer mice acclimated well to captivity [9]. King made a thorough revision what is known about the maternal behavior in other *Peromyscus* spp [14]. However, only Lackey reproduced the Yucatan deer mouse in captivity but only for a year concluding that the pattern of reproduction and ontogeny in this species is apparently unique in the *mexicanus* species-group [11]. Thus, very little is known about the requirements of captive *P. yucatanicus*.

The objective of this study was to establish a breeding colony of *P. yucatanicus* and to compare the reproductive performance between wild- and laboratory-born mice. This colony was created to develop an animal model for LCL specifically for *L. (L.) mexicana*, to study the immune mechanisms involved in the pathogenesis and control of disease. Thus, the reproductive parameters of *P. yucatanicus* should be measured to produce at any time, the number of mice needed for research with the less possible stress to the animals.

Material and methods

The breeding colony was derived from 19 females and 18 males' *P. yucatanicus* captured in the medium-size forest from the municipalities of Escarcega and Xpujil, in the state of Campeche, Mexico. Those 37 wild-born deer mice were transported to the Center of Regional Research of the University of Yucatan, Merida, state of Yucatan. In quarantine, the starting diet of corn, sunflower seeds, fresh fruits, and vegetables was gradually changed to rabbit food with 16% protein (Provi, Mérida, Yucatan). At the end of the 40-days quarantine, the rodents received an ectoparasitic bath (0.15% Asuntol, Bayer of Mexico) and two days after were transferred into the animal care facility. All the progenitors were numbered by toe-clipping.

The colony was housed in a 31.2 m^2 animal care room. During a 12 hour light-cycle starting at 7:00 a.m., the room was illuminated by one 75-watt fluorescent tubes, an air extractor was functioning, and the temperature was regulated by an air-conditioning unit (12 b.t.u.) at 22 $\pm$ 3°C. During night-time, a ceiling ventilator (V.E.C.) replaced the air-conditioning. Average humidity was 80% $\pm$ 10%.

The reproductive females were permanently housed in medium-size cages (27 x 37 x 15 cm.) while the males were in small size cages (19 x 29 x 12 cm.). The cages had transparent acrylic bases, stainless steel tops with incorporated food distributor and water bottles. Wood shavings were used as bedding and cages were cleaned weekly except during lactation period (no cleaning during 15 days). Water bottles were cleaned monthly and cages tops every other month or earlier if needed. Each cage contained either cotton or hygienic paper for nest building, which was replaced weekly.

The basic rabbit-food and tap water were provided *ad libitum*. Fresh food was distributed twice a week: once 5 grains of corn or seeds of sunflower, and the second time one piece of banana, watermelon, papaya, zucchini, tomato, or carrot. However, after four months, the corn and sunflower seeds were removed from the diet of all rodents because of males getting overweight.

Pairing took place in the freshly cleaned cage of the female where the male was introduced. If a fight occurs within the first 10 minutes, the pairing was aborted. Young were born and raised in the female's cage. At weaning, the juveniles were housed individually in small-size cages. Since the colony of *Peromyscus yucatanicus* was established to produce animals for the study of leishmaniasis, the reproductive pairs were maintained together only the time needed. The colony was checked twice a week and the day of any pairing, partum, and weaning was recorded, as well as the origin of the pair (wild- *versus* laboratory-born), signs of fighting and identity of attacker (male or female) and target (pair or pup), the number of pups at both birth and weaning, and the survivor rate of pups to adulthood (6-months). Animals were handled according to Mexican Law for the use of laboratory animals [15].

Data analysis

The progenitor's origin was compared to dependent "reproductive success" such as fertility (producing a litter); readiness to reproduce (reproductive interval between pairing and the first litter, and between litter if the pair was maintained together; fighting: signs, intensity and target); production: litter-size at birth, at weaning, and survivorship to adulthood (6-months). The results were analyzed by Student t-tests (Mann Whitney test), Analyses of Variance either one-way ANOVA (either with repeated measures, Friedman test; or non-parametric, Kruskal-Wallis test) or two-way ANOVA (with Bonferroni *post hoc* test), Contingency table analyses (Chi-square and Fisher's exact test) and correlation for one grouping variable (Pearson r test) with the use of GraphPad Prism 5. Probability $\leq$ 0.05 was considered significant.

Results

The colony was originated from 37 *Peromyscus yucatanicus.* Fourteen females were captured pregnant and gave birth to 40 pups during the quarantine period from which 18 were translated into the animal care room, and used in the colony. During 10 years, 270 pairings took place from which 51 males and 54 females successfully produced 218 litters.

The behavior at pairing was informally observed during five minutes for the first 16 pairings. All pairing behaviors were basically the same. The male tried to approach the female. When

accepted, they stayed close together and the male cleaned his muzzle and ears. Then, he started to smell the ears of the female and her anal area (87.5% of the cases). This approaching behavior was repeated until the pair rested close together in a corner of the cage. Only two pairs fought from the start of the pairing introduction. The mice were immediately separated. At one of those instances, the female was already pregnant from a previous mating.

Fertility

Even so, 5 wild-born females and 3 males never reproduced compared to 8 captive-born females and 9 males, the percent of either male or female that reproduced successfully - pairing resulting in the birth of a litter - in the colony was not significantly different among birth origins (Chi-square test, p=0.8056; Table 1).

Table 1: Distribution of fertile males and females *Peromyscus yucatanicus* by its origin and their reproductive success (sample size).

Sex	Successful progenitors (total tried for reproduction)		
Born in	the wild	quarantine	animal care room
Female	73.7% (19)	66.7% (12)	80% (40)
Male	83.3% (18)	66.7% (6)	78.0% (41)

The percentage of successful pairing ranged from 58.82% (n=26) when the female was wild-born and the male captive-born to 46.98% when both progenitors were captive-born (n=190), however, the number of successful pairing was not significantly different among birth origins (Chi-square test, p=0.7126).

Readiness to reproduce

The mean time interval between pairing (defined as physically placing two mice in a cage) and the birth of first litter was 43.5 ± 28.61 days. The shortest interval was 22 days however this litter was lost soon after birth. Successful litters appeared at 26 days since pairing; however, their rate of rearing a litter to weaning for intervals less than 30 days was only 45.5%.

The mean time interval between pairing and first litter were significantly different among origins of the progenitor's pair (One-way ANOVA, p=0.0025; Figure 1). Wild-born females paired with laboratory-born males (48.40 ± 11.46 days) reproduced slower than either captive-captive (46.51 ± 34.45 days; p=0.0226), wild-wild (39.0 ± 27.55 days; p=0.0026) and captive-wild pairs (37.15 ± 16.77 days; p=0.0031). The latter reproduced faster than when both progenitors were captive born (P=0.0222).

Interestingly, the difference was mainly related to the origin of the male (Mann Whitney test p=0.0028). Wild-born male produced a litter (38.04 ± 22.38 days) faster than captive-born ones (46.54 ± 32.59 days). The time interval between consecutive litters was never significantly related to the origins of the progenitors.

During pairing, fight occurred in form of tail biting which in captive-born *P.yucatanicus* escalated to killing. Rates of aggression were significantly different among the origins of paired *P. yucatanicus* (p= 0.0442). Wild-born females never attacked their partner and all fights (n=28), except three, were performed by captive-born *P. yucatanicus* (Figure 2).

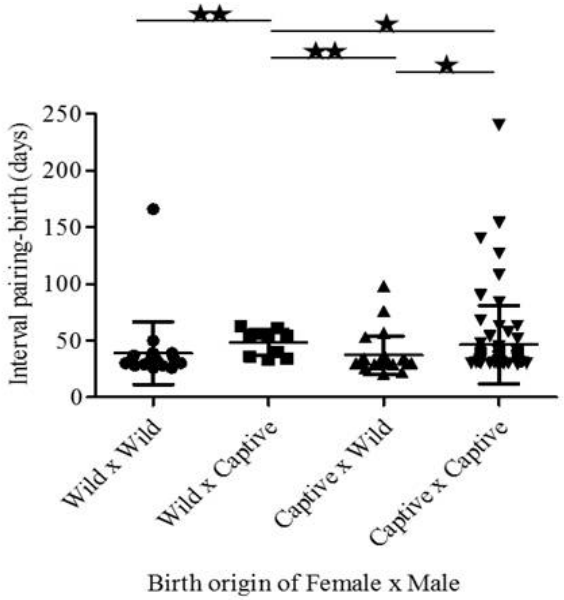

Figure 1

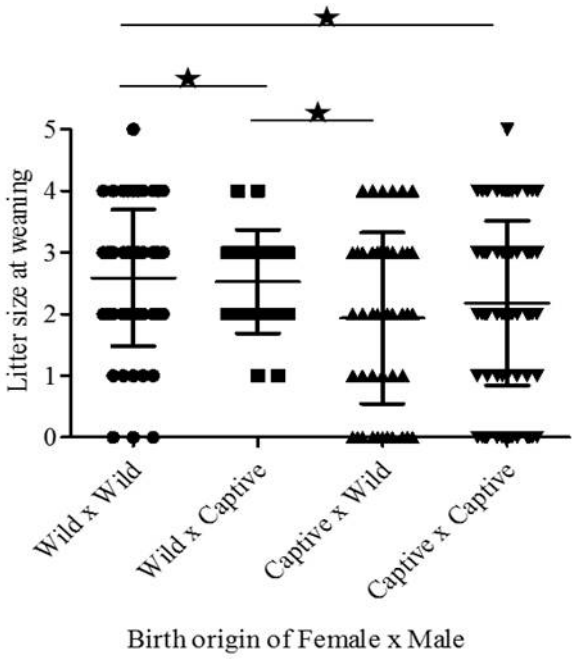

Figure 2

Production

Wild-wild pairs gave birth to larger litters (Mann-Whitney test: $\bar{x}$ =3.10 ± 0.79 pups per litter) than either wild-captive (2.58 ± 0.84 pups per litter; P=0.0208) and captive-captive pairs (2.70 ± 0.88 pups per litter; P=0.0104).

Wild-born females were better mothers (2.57 ± 1.04 pups per litter) than captive-born one (2.11 ± 1.35; P=0.0319). Pairs with both wild progenitors lost less pups (2.59 ± 0.15 weaners per litter) than either captive-wild (1.94 ± 0.21 weaners per litter; P=0.0113) or captive-captive pairs (2.18 ± 0.13; P=0.0378). Wild-captive pairs took better care of young pups (2.53 ± 0.19; P=0.0403) than captive-wild ones (Figure 3).

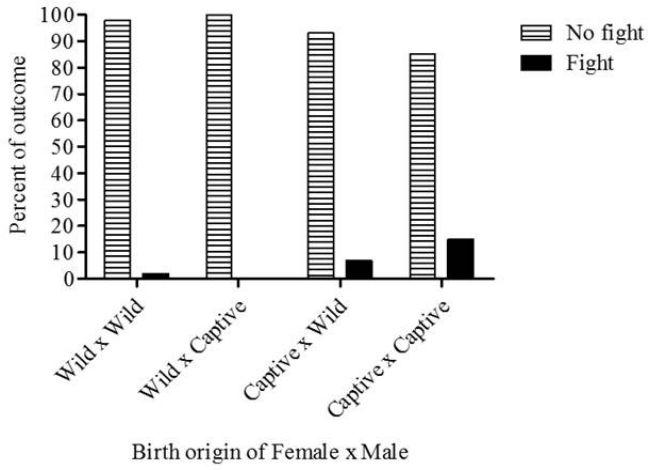

Figure 3

The number of pups reaching adulthood (6-months old) did not presented any difference among the pair origins. Similarly, the birth origins of the progenitors had no effect on the sex of pups, and on the aggression of the female to pups (23 pups with tail bites, 4.39%). Male's *P. yucatanicus* never attacked a pup.

Discussion

The reproductive success measured by fertility (producing a litter), readiness to reproduce (fighting and reproductive interval), production (litter-size at birth, at weaning, and survivorship to adulthood), in a natural environment is expected to be different that in captivity. Some reproductive variables like frequency to produce a litter, embryo resorption rate, and stress during rearing can change due to adaptation to environmental fluctuations [16]. Stressful situations are quite different, for example, food and water provided *at libitum* is no longer determinant for survival. However, confinement to small cages, handling, imposed mating partner, inability to avoid cage mates, and crowding during rearing can transform the animal behavior resulting in a kind of "natural selection in captivity" [17].

In the present colony, 31 *P. yucatanicus* never reproduced (n=136). Lackey reported that sterility does occur in captive *P. yucatanicus:* seven pairs never produced a litter in more than a year; but the author did not precise either the origin of the pair or the number of pairing performed. In a colony of old-field deer mice (*P. polionotus),* 74% of wild females (N=54) produced pups and 80% wild males (N=61) were fertile but in the F1 generation the fertility rate decreased to 64% for females and 75% for males [18]. However, individual *P. yucatanicus* does not seem to display such change in fertility since the apparent sterility found was not related to the progenitors' origin (wild- or laboratory-born, Table 2). Thus, the Yucatan deer mouse seemed to adapt well to captive environment.

Table 2: Recapitulation of the reproductive success by the origin of both progenitors of *Peromyscus yucatanicus.*

Origin of	N	Successful	Mean interval (days) between		Fighting rate		Mean litter size	
female x male	Pairing	pairing	Pairing to birth	Birth to birth	Tail biting	Mortal	at birth	at weaning
Wild x Wild	79	51.06%	39.0	37.3	2.1%	0.0%	3.0	2.6
Wild x Captive	26	58.82%	48.4	38.6	0.0%	0.0%	2.6	2.5
Captive x Wild	74	48.15%	37.2	41.7	3.7%	3.7%	2.7	1.9
Captive x Captive	190	46.98%	46.5	46.4	7.2%	7.9%	2.7	2.2

In the present study, successful pairings (producing a litter) was not related to the progenitors' origin. Price's found that, in the North-American deer mouse *P. maniculatus,* only 67.4% of the wild-caught pairs were fertile, whereas deer-mice bred in captivity for 17 years had a 93.3% fertility rate [17]. Similarly, in white-footed deer mouse *P. leucopus*, Millar and Threadgill found that all wild-born females reproduced but only 33 to 82% of the laboratory-born pairs bred [19]. In contrast, Botten *et al*., establishing a colony of the *P. maniculatus*, obtained a first fertility rate of 85% (n=26 wild-caught pairs) decreasing slightly to 73% (N = 59) in laboratory-reared pairs [20]. However, most of these successful pairing rates were much higher than those of the present study, demonstrating the need to improve the husbandry of *P. yucatanicus*, for example, improving the diets witching from rabbit to rodent pellets.

Gestation time in *Peromyscus* spp. ranges from 21 to 27 days [21]. Base on the shortest interval observed between pairing and birth, Lackey postulated that the gestation period of *P. yucatanicus* was much longer, 30 days [11]. In the present colony, the 30-days gestation of Yucatán deer mouse could be confirmed, since only 45.5% of litter born before that time survived. *Peromyscus yucatanicus* seems to have the longest gestation length in its genus.

In the present study, the mean time interval between pairing and first litter were significantly different among origins of the progenitor's pair (p=0.0025). Interestingly, the difference was mainly related to the male's origin (p=0.0028), wild-born male produced a litter faster (mean=38.04 days) than laboratory-born ones (mean= 46.54 days). Since gestation length is constant, those results demonstrated that wild-born male *P. yucatanicus* more readily accepted an imposed mate and successfully copulate than captive-born ones.

In the *P. yucatanicus* colony, wild-born females always accepted the mate presented to them and only three wild males fought, in contrast to 25 aggressions by captive-born mice. Tail biting is a good sign of rejection and as soon as the first tail lesion ever occurred, the mice should be separated to prevent mortality. Moreover, the use of cage enrichment, such as cardboard tubes and paper, to create in this limited space both places to avoid cage mates and building nest occupations, seems to be necessary with *P. yucatanicus*. Fighting might partly explain the present low rate of pairing success (Table 2); apparently the Yucatan deer mouse does not always accept the imposed mating partner thus more behavior observations are needed.

Lackey estimated the litter size of *P. yucatanicus* in the wild to 2.8 pups per litter. In the present study, wild-born pairs produced larger litter (3.1 pups per litter) than captive-born ones (2.7) [11]. Similarly, Botten *et al*. found a decrease in mean litter size from 4.3 in wild-caught to 4.0 in captive-born female *P. maniculatus* [20]. These results contrasted with those of *P. polionotus* where litter size increased under domestication, from 3.1 in the wild to 3.5 in wild mice in captivity and to 3.7 in laboratory-born females [18]. However, more than a difference of mean litter size, Price's revealed that captive deer mice had a higher variance in number of pups per litter [17]. Lack put forward the theory that natural selection in the wild favors an optimal clutch size with very little variability [22]. The significant differences in litter size of the present *P. yucatanicus* might be to uncontrolled factors probably resulting from Price's "natural selection in captivity".

Wild-born *P. yucatanicus* were better mothers than captive-born ones (Table 2). Similarly, pup's cannibalistic behavior of *P. maniculatus* increased from 5% in founders to 26% in captive-reared pairs [20]. A possible explanation is that in the wild, mice generally live a short time and produce few litters. For example, *P. maniculatus* females produce an average of three or four litters in their life, thus a natural selection against both cannibalism and desertion of the young should exist in nature [23]. In contrast, captivity mice live longer producing more litters in their life span. Moreover, due to the necessity to maintain genetic diversity in an outbred colony, offspring of cannibalizing female have nearly as good a chance of being used for breeding as those of a non-cannibalizing female. Thus, a quite different natural selection exists between wild and captive environment. One of the reasons for offspring desertion and the following cannibalism might be higher restlessness of females in captivity (Price, 1967) [17]. Once more, the use of enrichment to increase the heterogeneity in the cage and place for the mom to rest without being "bugged" by her pups is highlighted. Another explanation might be nutritional deficiencies; however, attempts to prevent it with supplemental diets have been unsuccessful as in the present study with the addition of fruit and vegetable [24].

Martin postulated that since the well-being of an animal depends on its familiarity with its surroundings, those adapted from several generations in captivity should reproduce better than those freshly captured [25]. The present outbred colony of *P. yucatanicus* obtained the opposite, wild-born male mated faster than captive ones, wild mice fought less and wild-born females produced and reared larger litter. This research highlighted the necessity to better understand the needs of this species such as diet and the addition of physical and occupational enrichment. Although the reproductive performance of wild-born *P. yucatanicus* was better than laboratory-reared mice, reproduction can still be enhanced improving husbandry to produce the number of animals needed faster and with less stress and aggression for the study of *L. (L.) mexicana* infection.

Acknowledgements

To the personnel from the animal housing facilities of the Centro de Investigaciones Regionales Dr Hideyo Noguchi, Universidad Autonoma de Yucatan, for their continuing help.

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