Vet. Med. J., Giza. Vol. 57, No.3. (2009):345-356.

Effect of different quarantine periods on performance, behaviour and physiology of laboratory guinea pigs.

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Received: 07/09/2009
Accepted: 07/10/2009

SUMMARY

A total of 60 adult guinea pigs of homogenous weight (250-350g) were used for evaluation of the appropriate quarantine period (1-5, 6-10 and 11-15 days post arrival) of laboratory guinea pigs before using the animal for experimental work. On arrival, the animals were allotted into 6 cages (10 animals of the same sex / cage). Feed intake, body weight and weight gain, growth rate, feeding behavior, and cortisol level were recorded for the animals during the different periods. Guinea pigs were also tested as experimental animal for evaluation of X-product toxicity; as indicated by two indicies (weight gain and mortality) during one week post injection, The highest stressful effect of transportation appears mainly during the first few days post arrival (1-5d), where guinea pigs recorded significantly the worst performance and behavior, and the highest cortisol level. However, during the second period (6-10d), the animals tended to modulate themselves and acclimate successfully to the surrounding environment through increased feed intake, attaining more weight, with lower morbidity and cortisol level. Meanwhile, by increasing the quarantine period to more than 10 days , the performance of the animal tended to redecrease again to values lower than that of the second period (6-10d) but still better than those of the first one (1-5d). Safety test results indicated that, the best performance was met in animals injected 10 days post arrival, while the lowest was obtained when the animals injected after 15 days post arrival. It can be concluded that, the stress of transportation and receipt of animals into new environment extended for at least 5 days post arrival, The period from 6-10 days is the best suitable period of acclimatization and

stabilization of the animal behaviourally and physiologically. Increasing the quarantine period for more than 10 days is not preferable as the animal may be exposed to adverse management conditions that reduce its performance again. Therefore, a quarantine period not less than 5 days or more than 10 days with average 7 days post arrival is recommended for successful acclimatization of the laboratory guinea pig before its use in experimental purposes.

Key words: Guinea pigs, acclimatization, behaviour, performance, safety test.

INTRODUCTION

Guinea pig has been employed as a research animal for human medical conditions such as juvenile diabetes, tuberculosis, Scurvy and pregnancy complication (Harkness et al., 2002 and Vanderlip 2003). Knowledge of the basic biology of laboratory animals used should be obvious for experimental workers (Hicks et al., 1998. and McEwen, 2002 and Stemkens-Sevens, et al., 2009).

Many experiences with research animal lifetime can be expected to cause some degree of stress, such as bad management practices, environmental changes, transportation and experimental procedures (Brown et al., 1999 and Harkness et al., 2002). Transportion may cause mild to moderate stress in animals during and for a period after the transportation process. This stress results from perturbations in the environment, fluctuations in temperatures during transportation, short-term food and water deprivation, noise, or other physical aspects of shipping (Grandin, 1997 and Pekow, 2005).

The stress of transport and receipt of animals into a new environment mandate the need for a period of stabilization and acclimation according to procedures appropriate for the species and the circumstances (Loew, 1980; IACUC 2001); as utilization of these transported animals before normalization and stabilization of their physiological and behavioral states can have considerable and unintended effects on research results (CCAC, 1993). This often occurs in conjunction with the quarantine period and permits a stress "recovery" period.

From the welfare point of view, acclimation and stabilization are important to prevent overwhelming the animal's normal coping mechanisms which maintain homeostasis and minimize animal distress (Wolfer et al., 2004). Therefore, it is essential for the newly received animal to be allowed to acclimate to the new environment (Capdevila et al., 2007 and Jennifer and Baldwin, 2006).

However, the period for equilibration of each animal and between species varies (AALACC, 1996) and Lack of awareness of this period can lead to serious effect on experimental data (Linden and McEachern 1985). Although literature on effects of transportation on rodents is limited, it generally includes information on the length of time necessary for different physiological parameters to normalize (Toth and January 1990).

Generally, primary mediators of a stress response (i.e., catecholamines and glucocorticoids) return to normal concentrations within 24 hr of transportation (Nyberg et al., 1988 and Dalin et al., 1993). Accordingly, IACUC (2001) recommends 3 days; preferably 5 days acclimated period for animals prior to any experimental manipulation. Moreover, University at Buffalo (2006) advised seven (7) days quarantine period for a chronic study and a minimum of 3 days for an acute study.

However, Hassimoto et al. (2004) mentioned that the minimum 2-wk period may not be enough to allow animals to acclimate for some commonly assessed physiologic parameters. Therefore this study was conducted to clarify and establish an appropriate quarantine period after arrival before using the animal for experimental work.

MATERIALS AND METHODS

Animals and location

This study was carried out in the Experimental Animal unit of the Egyptian Company for Production of Serum and Vaccine (EGYVAC). A total of 60 adult guinea pigs (30 male and 30 female) of homogenous weight (250-350g) were used for this experiment during the period from January to May 2008.

Housing

The animals were housed in Stainless steel cages (80 x 50 x 10 cm). Cages were well bedded with Clean saw dust (1-2 cm thick) and equipped with J- shaped feeder, inverted watering bottle and a removable metal debris tray. Guinea pigs were fed and watered ad-lib. Commercial balanced pelleted ration (18% crude protein, 2% fat, 10.5% crude fiber and 2600 kcal energy) was offered. Fresh clean tape water containing vitamin C (2mg/l) daily was available all time (Sutherland and Festing 1987).The whole unit was environmentally controlled to provide a constant temp. of (20-22°C) and RH. of (50-53%) and a lightening program of 8 hours /day with light intensity range of 450-550 lux (North, 1999).

Experimental procedures

After arrival, the animals were allotted into 6 cages (10 animals of the same sex / cage) in the quarantine room of the unit. For evaluation of the effect of the

different quarantine periods (5, 10, 15 days) on performance and physiology of guinea pigs, the following parameters were recorded daily "for the different animals" from the day of arrival and through the following periods; 1- 5, 6- 10, 11-15 days post arrival:

A- Performance data

Daily feed intake (g), Individual weight and weight gain (g) and the growth rate were recorded. Morbidity and mortality rates during each quarantine period were calculated (Del Barrio et al., 1993, Hicks et al., 1998 and Jeffrey et al., 2006).

B) Behavioral measurement

3 animals / cage were identified to act as a focal sample for measuring of feeding behavior. Feeding frequency and duration was recorded for each focal animal during a continuous 5 minute samples, twice per day, 3 days per each quarantine period (Sachser and Lick 1991, Terril, 1998) . Daily observation was done between 8.30 and 10.30 a.m then between 14.00 and 16.00 p.m, where the overall mean values were calculated for the animals during each period.

C) Physiological parameter

Three animals / cage were identified for blood sampling. Blood samples were collected on the day of arrival then at the 5^{th}, 10^{th}, 15^{th} day post arrival (at the end of each quarantine period). Serum was collected for assessment of cortisol hormone level (Nyberg et al., 1988 and Tuli et al., 1995).

D) Safety test evaluation:

At the end of each quarantine period "at the 5th, 10th, 15th days post arrival" two animals from each cage were injected with X- product for evaluation of its toxicity as indicated by weight gain and mortality %within one week post injection. The injected animals were followed for one week where the weight gain and mortality rate were recorded. Safety test is used "according to WHO recommendations" for evaluation of toxicity and safety of some biological products and vaccines, and the products only allowed for human use when all animals survive with no signs of toxicity (Louisiana (2000).

Statistical Analysis

The results were statistically analyzed by using of one way ANOVA test and T test completely randomized (student-Neuman –Kelus Test), SPSS. According to(Snedecor and Cochran, 1989).

RESULTS

Table (1): Effect of different quarantine periods on performance of guinea pig

Qp.(days) / Parameters	P1	P2	P3
Daily feed consumption (g)	23.84 ± 1.2a	31.65 ± 1.7b	25.11 ± 1.4a
Daily body wt. gain (g)	7.14 ± 3.4a	16.32 ± 3.3b	15.33 ± 3.6b
Growth rate (%)	2.87 ± 1.8a	6.48 ± 1.3b	5.87 ± 1.2b
Morbidity (%)	20.02	2.86	11.11
Mortality (%)	4.6	0.0	3.3

[a,b]: Means with different superscript within the same raw indicates significant difference between different groups at ($P<0.05$)
P 1: period from 1-5 days post arrival
P 2: period from 6-10 days post arrival
P 3: period from 11-15 days post arrival
Qp.:Quarantine period.

Figure 1: Effect of different quarantine periods on feeding behaviour of guinea pig

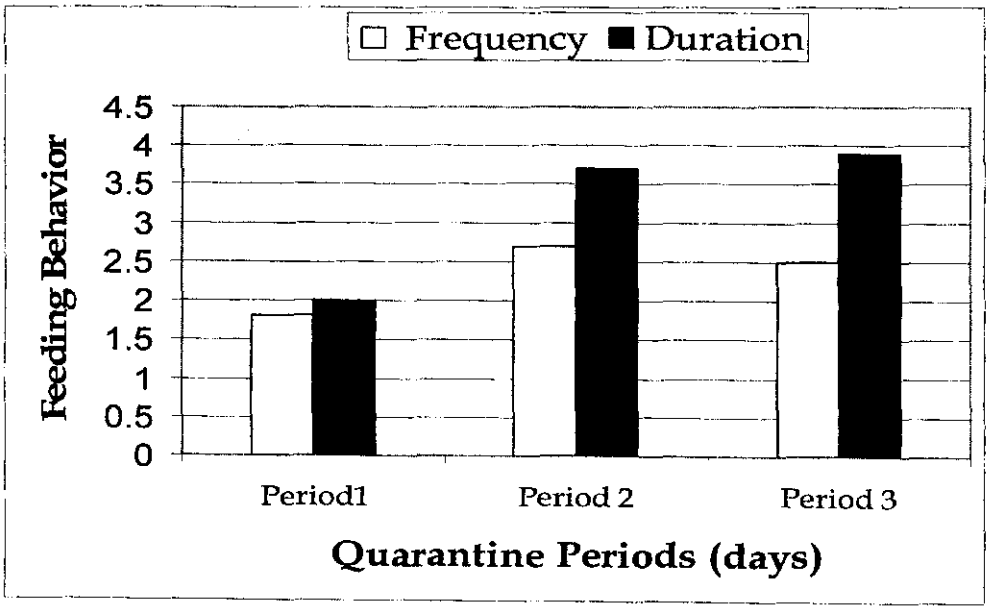


Figure 2: Effect of different quarantine periods on blood cortisol level (ng/L)

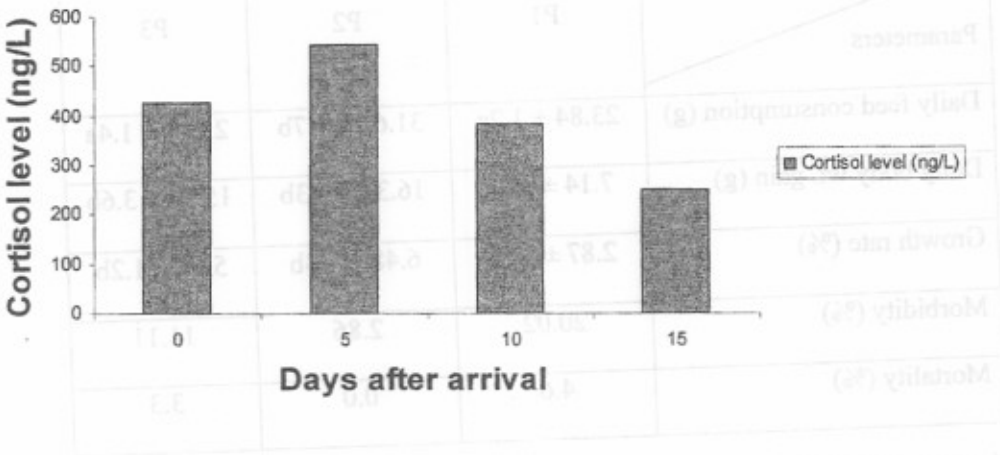
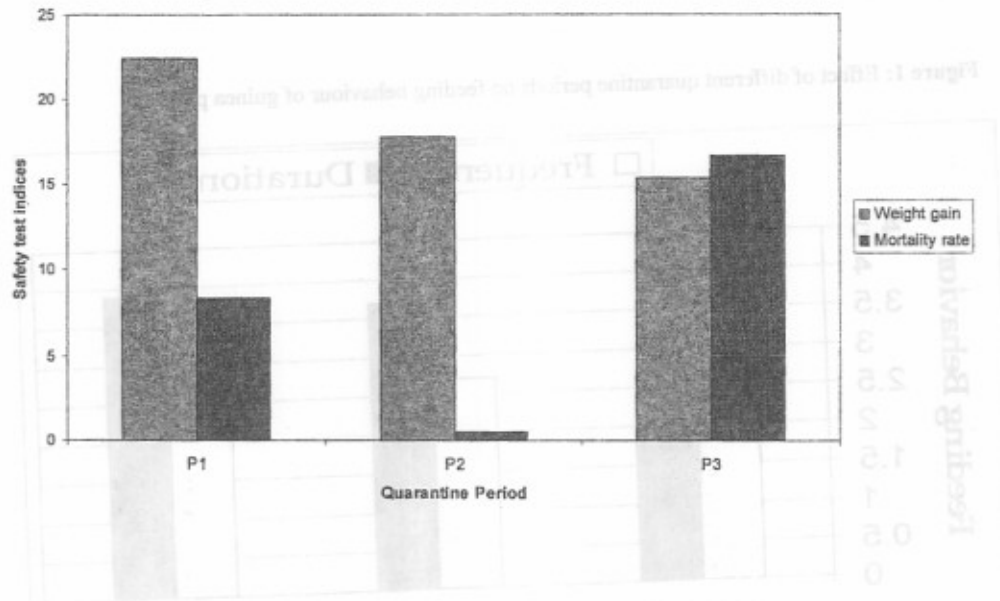


Figure 3: Effect of Different Quarantine Periods on Safety test indices



DISCUSSION

Quarantine periods and physical performance:

Data in table (1) revealed that, the highest stressful effect of transportation appears mainly during the first few days post arrival (1-5d), where guinea pigs recorded significantly ($p<0.05$) the lowest daily feed intake (23.84 ± 1.7g), the worst average daily weight gain (7.14 ± 3.3 g) and accordingly, the lowest growth rate (2.87 ± 1.3%) among the different groups. All these factors together resulted in the highest morbidity (20.02 ± 13.35) and mortality rate (4.6 %) during this period . The lower feed intake at the early post arrival period attributed to the fact that non acclimatized animals were not accustomed to feeding or drinking in the novel housing environment.

However, the most favorable values were achieved during the second period (6-10d), and the animals tended to modulate themselves and acclimate successfully to the surrounding environment through a significantly ($P<0.05$) increased feed intake, better weight gain (16.32 ± 3.3 g), consequently the highest growth rate (6.48 ± 1.3 %), with lower morbidity and mortality rate. Meanwhile, by increasing the quarantine period to more than 10 days ,the animal may be subjected to unfavorable management conditions that affect negatively its performance and health condition and the performance of the animal tended to redecrease again to values lower than that of the second period (6-10d) but still better than those of the first one (1-5d). Similar results were obtained by (Hurst et al., 1999 and Jeffrey et al. 2006) who found a significant decrease of body weight within a week after arrival of the guinea pigs.

Quarantine periods and Feeding behavior:

Regarding feeding behavior, it was clear that, the lowest feeding frequency (1.81 ± 1.56) and shortest duration (2.09 ± 2.68 sec) were recorded by animals quarantined for 5 days. Later on, after the 5th day the animal tended to modulate its behavior towards normal levels where the feeding frequency and duration increased to (2.72 ± 1.81, 3.71 ± 3.45) respectively in the second quarantine period (6-10 day) and (2.50 ± 1.46, 3.94 ± 1.94) in third quarantine period (11-15 day) with no significance between 2nd and 3rd periods (fig.1). The improved performance of the quarantined animals during the second period may be explained that a sort of stabilization and acclimation of the animal to the new environment has been occurred, and reflected in improved performance with better immune and health condition of the animal. The reduced feeding activity (frequency and duration) during the first five days post arrival could be attributed

to the stressful effect of transportation and the new environment. After the 5th day, the animal was acclimatized with the new environment and was established behaviorally and physiologically, this reflected in higher feeding activity. Similar results were obtained by Sachser and Lick, (1991).

Quarantine periods and Cortisol level:

It was clear that, cortisol level which is a good indicator of stress increased from (425.85± 114.5 ng/l) on day 0 "day of transportation" to reach the peak (543.3 ± 114.5 ng/l) at 5 days post arrival (Period 1) indicating highly stressed animal during this period. Later on by increasing quarantine period, it decreased gradually and tend to normalize to (383.3 ±114.5 ng/l) then (247.65 ± 114.5 ng/l) at 10^{th} and 15^{th} day post arrival (second and third periods respectively). {Figure 2}. IACUC (2001) indicates that the stress of shipment may impact a number of physiological parameters, (e.g.corticosteriod levels), Therefore, recommends that animals must be allowed to acclimate after transport for a period of 5 days prior to beginning any experimental procedures, regardless of their nature, University of Buffalo (2006).

The highest level of cortisol during the first period (1-5 days) could be attributed to the stress of transport process and receipt of animals into new environment and it coincides with lower feed intake, reduced growth performance and higher morbidity that occur during the same period. However, later on by increasing the quarantine period (6-10 days post arrival), a stress recovery occurs and the animal returns to its normal physiological levels that was parallel to the improvement in the animal performance and behavior as noted in the second and third periods. However, Hassimoto et al. (2004) mentioned that the minimum 2-wk period may not be enough to allow animals to acclimate for some commonly assessed physiologic parameters. Also, Jennifer and Baldwin (2006) found that elevated concentrations of cortisol can generally return to baseline within 1 to 7 days of transportation. Therefore, It's recommended that animals must be allowed to acclimate after transport for a period of 5 days prior to beginning any experimental procedures, regardless of their nature, University of Buffalo (2006).

Safety test:

By testing the animals for evaluation of X-product toxicity " safety test", it was clear that, the best performance as indicated by the highest weight gain (22.27 ± 3.4g) and lowest mortality (0%) during week post injection, were recorded in animals injected 5 days post arrival, while The lowest weight gain (15.33 ± 0.5g) and highest mortality (16.66%) was obtained when the animals injected after 15 days post arrival. {Figure3}.

Prasad et al.(1978) proved that, increased body weight during general safety tests could be obtained after a 1-week conditioning period.

Conclusion

It can be concluded that, the period from 6-10 days post arrival is the best suitable period of acclimatization and stabilization of the animal behaviourally and physiologically. So a quarantine period not less than 5 days or more than 10 days with average 7 days post arrival is recommended for successful acclimatization of the laboratory guinea pig before its use in experimental purposes.

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تأثير فترات العزل المختلفة بعد النقل على بعض الانماط السلوكية و الفسيولوجية و الكفاءة الأنتاجية فى الخنزير الغينى فى المعمل

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أجريت هذه الدراسة على عدد 60 أرنب هندى بالغة لمعرفة تأثير فترات العزل المختلفة (Quarantine) بعد نقل الحيوانات مباشرة و قبل استخدامها لإجراء التجارب المعملية عليها عند وصول الأرانب إلى وحدة حيوانات التجارب تم توزيعها على عدد ستة أقفاص (10 أرانب لكل قفص) و تم قياس كمية الأكل و معدل الزيادة اليومية فى الوزن و معدل النمو و السلوك الغذائى كما تم قياس مستوى هرمون الكورتيزول للحيوانات عند الوصول و فى نهاية الفترات (1-5 ،6-10 ،11-15 يوماً بعد وصولها) كما تم اختبار هذه الحيوانات كحيوانات تجارب فى نهاية كل فترة من فترات العزل و ذلك بأستخدامها فى تقييم مصل الكوليرا بواسطة اختبار(Safety test) عن طريق تسجيل معدل الزيادة فى الوزن و نسبة النفوق بعد حقن الحيوانات بهذا المصل و خلال أسبوع من الحقن

و قد أوضحت النتائج أن أعلى تأثير عن عملية النقل كان خلال الخمسة أيام الأولى بعد عملية النقل مباشرة، حيث انخفض معدل الأكل اليومى و سلوك الغذاء و سجلت الحيوانات أقل زيادة فى الوزن و معدل النمو و ارتفع مستوى هورمون الكورتيزول .بينما استطاعت الحيوانات أن تتكيف مع البيئة الجديدة بنجاح فى الفترة من6-10 أيام بعد الوصول ، حيث زادت كمية الأكل و معدل النمو و انخفض مستوى هرمون الكورتيزول فى الدم و قلت نسبة النفوق و الأصابات المرضية فى هذه المدة . بينما سجلت الحيوانات خلال المدة من11-15 يوم بعد الوصول انخفاضاً فى كمية الأكل اليومى و النمو و الزيادة فى الوزن لمستويات أقل من تلك المسجلة فى الفترة(6-10يوم) و لكنها أفضل من مثيلاتها المسجلة فى الفترة الأولى (1-5يوم). و لقد أظهر اختبار (Safety test) أن أفضل أداء لهذه الحيوانات فى هذا الاختبار (ممثلاً بأقل معدل للنفوق و أقصى زيادة فى الوزن) كان عند حقن الحيوانات بالمصل بعد خمسة إلى عشرة أيام من الوصول بينما كان أقل أداء لهذه الحيوانات عند حقنها بعد 15 يوم من الوصول.

و قد خلصت الدراسة الى أن الاجهاد الناتج عن عملية نقل الحيوانات إلى مكان اجراء التجارب يمتد الى فترة لا تقل عن خمسة أيام، المدة من (6-10أيام) تمثل أفضل فترة للتكيف مع البيئة الجديدة سلوكياً و فسيولوجياً و أوضحت الدراسة أن زيادة مدة العزل إلى 15 يوم غير مفضل فربما تتعرض الحوانات إلى عوامل بيئية او رعاية غير مناسبة و التى قد تؤثر سلباً على كفاءتها كحوانات تجارب ، و لذا فقد اوصت الدراسة بفترة عزل (Quarantine period) لا تقل عن 5 أيام ولا تزيد عن 10 أيام (متوسط 7 أيام) للأرانب الهندى بعد نقلة

مباشرة و قبل استخدامه فى التجارب المعملية و هى مدة مناسبة لتكيف الحيوان (فسيولوجياً و سلوكياً) مع الظروف البيئية الجديدة قبل استخدامه كحيوان تجارب.