ISSN: 2583-9365

A study on hematological, serum biochemical and microbiological parameters in wild caught rodents

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Abstract

Laboratory mice constitute the most popular animal models used in biomedical research today. Like all animals, even mice housed in 'barrier' facilities are prone to infection. Wild mice (*Mus musculus*) living near vivaria could serve as a source of infection or infestation in laboratory mouse colonies, although little is known about the prevalence of infectious diseases in wild mouse. Very few surveys have been conducted on wild rodent populations to determine the prevalence of viruses and bacteria and also health monitoring of the wild rodents. Pathogens (bacteria, parasites) often gain entry into colonies through the introduction of infected animals or infected animal products, but viral dissemination via contaminated fomites or local wild rodents has also been implicated in the spread of disease. Hence, a preliminary study was undertaken to determine the prevalence of rodent pathogens and health status in wild mouse populations in and around and Bangalore. The present study revealed that none of the animals tested were positive for viral pathogens. However, bacterial pathogens and parasites were found in wild caught mice. Hematological and serum biochemical parameters were comparable to the colony bred anim

Key words: serum biochemistry, hematology, microbiology, wild rodents

Introduction

Mouse has been used in biomedical research since the early 20th century and it is a powerful system for basic research and plays a key role in mammalian genetic and biomedical research. The house or wild mouse (*Mus musculus*) is a small, slender rodent that has a slightly pointed nose; small, black, somewhat protruding eyes; large, sparsely haired ears; and a nearly hairless tail with obvious scale rings (Timm, 1994). The rodent fauna of India consists of 46 genera and 126 species. Of this, about 18% species are pests of agriculture in cultivated fields, and warehouses in both rural and urban environment.

Wild animals frequently carry endemic, low virulence infections or parasite loads with no overt clinical effects. However, these infections may cause significant population effects through alterations in fecundity, vulnerability to predation or potentiation of other stressors such as poor nutritional status. Several endemic diseases of wild rodents may affect reproducibility of results in behavioural, physiological or clinical research using laboratory rodent, and a limited number pose a zoonotic risk such as Leptospirosis, Lymphocytic choriomeningitis virus (LCMV) etc. Importantly, uncontrolled and unmonitored infection in laboratory mice has long been recognized as a difficult factor in experimental investigations, and as a potentially deleterious factor in animal welfare. Singleton *et al., (*1993) surveyed the prevalence of viral antibodies in house mice in southern Australia and found that the mice were seropositive for mouse hepatitis virus (MHV), rotavirus, minute virus of mice (MVM), mouse adenovirus, reovirus (reo 3), and murine cytomegalovirus (MCMV). They also reported that the sero-prevalence of MVM and reo3 were low when host survival was high and high sero-prevalence when host survival was low. Several wild populations of *M. domesticus* in southeastern Australia were tested by Smith *et al.,* (1993). They found serologic prevalence was high for MCMV (99%), murine corona virus (95%) and murine rota virus (74%) in all mice collected at different sites. He also reported serologic prevalence of mouse adenovirus strain K87 (37%), parvo virus (33%), reo virus type 3 (28%), LCMV (9.6 %) and Sendai virus (1.8%). The frequency of sero-positivity in *M. domesticus* fluctuated significantly between months and varied from as low as 45% in June 1995 to as high as 97% in April 1996. Sero-prevalence to MCMV was lowest during mid-to-late 1995 in the sampled population of *M. domesticus* and corresponded to a high rainfall and a high host density year compared with 1994 and 1996 (Moro *et al.,*1999). Becker *et al.,* (2006) reported high proportion of seropositive for MHV (86%), MCMV (79%), MTV (78%), MAV (68%), MPV (59%) and MVM (41%). Bacteriology of the oral cavity of BALB/c mice revealed 18 different species.

The predominant species of total cultivable flora were *Lactobacillus murinus* on mucosa (38%), *Staphylococcus aureus* in saliva (37%), *Streptococcus faecalis* in tooth samples (8%), *Staphylococcus sciuri* (4%) and *Escherichia coli* (3%) (Trudel *et al.,* 1986).

Mazzaccara *et al.,* (2008) reported age related changes in hematological parameters like RBC, HGB, HCT and PLT of C57BL/6J, 129SV/EV and C3H/HeJ mouse strains. Ingle *et al.,* (2011) reported the hematological values of mice and rat strains. They also concluded that the wide range of values may be due to the result of genetic changes, change in the feed, housing conditions, technique and equipment used or management conditions.

The presence of pinworms in mouse such as *Syphacia obvelata, Syphacia muris* and *Aspicufuris tetraptera* in the caecum and colon of the intestine was reported by Taffs (1976). This is a small, white, cylindrical worm found in the caecum and colon of mice, rats and hamsters. Bradford *et al.,* (2002) investigated the presence of parasites in rodents that cause infestations which could complicate research. They found out the parasites of mice which included protozoans such as *Pneumocystis carinii*, *Cryptosporidium muris*, *Toxoplasma gondii*, *Giardia muris, Spironucleus muris*. Michael *et al.,* (2009) investigated the consequence of an outbreak of *S. obvelata*, a murine pinworm gastrointestinal nematode in a transgenic barrier facility. The present study was undertaken to determine the hematological, serum biochemical parameters in addition to bacterial and viral pathogens in wild caught rodents.

Materials and methods:

Animals

The wild mice trapped at different locations in Bangalore city were used in this study. The trapping was done by placing the mouse trap near the active movement locations of mice. Mice were differentiated from the wild rats based on their tail length, body weight and size. The approximate age of the animals was also determined based on the above criteria. A total of ten animals were used in this study of which five were males (1-5) and five were females (6-10).

Anesthesia

Animals were anesthetized by using Ketamine at the dose rate of 80 mg/kg b.wt. and were euthanized humanely by using Carbon dioxide..

Blood collection methods

Blood samples were collected (300 µl) from retro-orbital plexus. A 100 µl of blood samples were collected in 2% EDTA for hematological analysis and 200 µl of blood samples without anticoagulant for serum biochemical estimations and virology.

Procedures

1. ELISA (for viral agents)

The serum samples were diluted 50 times in serum diluent i.e.1:50 (1 µl of serum sample and 50 µl of serum diluent) before using them for serology. Test was done as per the instruction given in the kit (Xpress Bio, USA). The viral pathogens determined include Lymphocytic chorio meningitis virus (LCMV), Mycoplasma, Minute Virus of Mice (MVM), Mouse Hepatitis Virus (MHV) and Sendai virus.

2. Hematology

Approximately 200 µl of blood sample was collected aseptically in 2 % EDTA coated vials. Samples were then analyzed in an automatic hematology analyzer (Sysmex K4500, SYSMEX Corporation, Kobe, Japan). The hematological parameters determined include: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, Platelets, Lymphocytes, Monocytes, Eosinophil and Basophils.

3. Serum biochemistry

All biochemical tests performed in this experiment were kit based which were developed according to particular method that is mentioned below (Table 1) and the procedures were carried out as per the directions of the kit manufacturer. Samples were analyzed in a semi-automatic analyzer (Chem-7, Erba Mannhei

Table 1: List of biochemical parameters

Biochemical parameters	Methodology
Glucose	Trider's method
Total protein	Biuret method
Albumin	BCG Dye method
Calcium	Moorehead and Briggs method
Bilirubin(direct)	Diazo method
Bilirubin (total)	Diazo method
Creatinine	Jaffe's method
Cholesterol	Trider's method
Urea	Kinetic, enzymatic method
BUN	Kinetic, enzymatic method
Phosphate	Ammonium molybdate method
SGOT	Modified IFCC method
SGPT	Modified IFCC method
Triglycerides	GPO-PAP method

4. Isolation and identification bacterial agents

Fresh fecal samples were collected and inoculated into nutrient broth and then onto the nutrient agar, subsequently subjected to differential / selective media based on gram staining and different biochemical tests (Michael *et. al.,* 2009) to determine the gut microflora.

5. Parasitological examination

Cellophane tape impressions from perianal region were collected and subjected for direct microscopic examination to detect the presence of any parasite or its ova.

Results:

Serum Biochemistry

The serum biochemical parameters were determined in all the ten animals and results are presented in Table 2. It can be seen from the results that the glucose levels varied from 60.53 mg/dl to 127.10 mg/dl. The total protein values ranged from 5.39 g/dl to 7.73 g/dl. Similarly, the values ranged from 0.22 to 0.93 mg/dl for bilirubin (direct), 0.28 to 1.29 mg/dl for bilirubin (total), 0.60 to 1.32 mg/dl for creatinine, 10.82 to 25.18 mg/dl for calcium, 06.59 to 15.82 mg/dl for phosphorous, 21.64 to 72.33 mg/dl for cholesterol, 32.97 to 86.70 mg/dl for urea, 14.41 to 40.51 mg/dl for BUN, 63.77 to 164.50 mg/dl for triglycerides, 88.40 to 443.80 U/L for SGOT and 34.14 to 140.4 U/L for SGPT.

Table 2: Serum biochemical parameters in wild caught mice

Parameters	Animal no.									
	Males					Females				
	1	2	3	4	5	6	7	8	9	10
Glucose (mg/dl)	60.53	127.10	76.22	121.00	69.63	70.11	88.54	106.10	109.00	60.98
Total protein (g/dl)	5.95	5.79	6.43	5.88	5.39	6.25	6.89	6.10	7.01	7.73
Albumin (mg/dl)	3.24	4.48	3.91	4.24	4.49	4.17	5.71	4.64	3.72	5.24
Bilirubin (direct) (mg/dl)	0.59	0.35	0.72	0.31	0.28	0.22	0.53	0.32	0.93	0.44
Bilirubin (total) (mg/dl)	0.34	0.69	1.06	0.78	0.80	0.28	1.29	0.69	1.24	1.06
Creatinine (mg/dl)	1.10	1.05	0.75	0.99	1.32	0.77	1.08	0.87	0.60	0.87
Calcium (mg/dl)	18.63	17.53	21.51	12.74	10.82	17.86	11.07	23.57	25.18	20.36
Phosphorous (mg/dl)	13.71	12.00	8.54	14.8	14.51	6.59	15.82	10.53	14.31	11.65
Cholesterol (mg/dl)	29.85	21.64	57.70	23.47	43.14	58.88	63.06	41.81	72.33	47.07
Urea (mg/dl)	80.32	57.45	30.85	55.32	86.70	35.72	37.92	32.97	35.97	41.21
BUN (mg/dl)	37.53	26.84	14.41	25.85	40.51	16.69	17.71	15.40	16.69	19.25
Triglycerides (mg/dl)	64.95	115.6	164.5	118.8	69.87	63.77	67.14	80.15	75.95	86.82
SGOT (U/L)	88.40	180.50	305.90	443.8	411.90	238.70	233.40	408.40	413.70	334.20
SGPT (U/L)	54.81	35.36	51.27	92.70	81.33	35.36	61.88	34.14	95.47	140.40

Hematology

The hematological parameters were determined in all the ten animals and results are presented in Table 3. WBC counts ranged from 4.1 to 9.0 thousands whereas, RBC counts varied from 5.23 to 7.92 millions. Similarly, hemoglobin values ranged from 9.4 to 16.1 g/dl and PCV ranged from 32.6 to 55.7 %. The lymphocyte values ranged from 61.9 to 92.9 %

Table 3: Hematological parameters in wild caught mice

Parameters	Animal no.									
	Males					Females				
	1	2	3	4	5	6	7	8	9	10
WBC x 10^3/cc	4.50	8.40	7.10	4.30	6.30	9.00	8.80	4.10	6.00	5.40
RBC x 10^6/cc	7.81	6.42	7.61	6.41	7.92	7.55	7.20	5.23	7.60	6.48
Hb g/dl	12.90	11.10	12.90	12.00	13.30	15.00	11.80	9.40	13.40	16.10
PCV %	44.40	40.80	45.60	38.50	47.00	47.00	44.40	32.60	46.00	55.70
MCV fl	56.90	63.60	59.90	60.10	59.30	62.30	61.70	62.30	60.50	58.80
MCH pg	16.50	17.30	17.00	18.70	16.80	19.90	16.40	16.10	17.60	17.00
MCHCx10^3/ml	29.10	27.20	28.30	31.20	28.30	31.90	26.60	25.80	29.10	28.90
Lymphocyte %	92.90	81.70	62.60	86.90	75.80	91.10	78.60	90.10	61.90	85.40

Isolation and identification

Fecal samples were cultured and two different colonies were isolated based on their morphology and gram's reaction. The results are presented in Table 4. Subsequently, various biochemical tests were used to identify different organisms like *Escherichia coli,* Pseudomonas, Bacillus, Salmonella, Proteus and Shigella. The results are presented in table number 5.

Table 4: Morphological determination of organisms

Animal #		Shape	Colour	Edge	Elevation	Gram's reaction
01	a	Round	White, translucent	Entire	Raised	-ve rod
	b	Round	Green, Opaque	Entire	Raised	-ve rod
02	a	Irregular	White, Opaque	Undulate	Raised	+ve rod
	b	Round	Cream, Translucent	Entire	Raised	-ve rod
03	a	Round	White, Translucent	Entire	Raised	-ve rod
	b	Irregular	White, Opaque	Curled	Convex	-ve rod
04	a	Round	Cream, Translucent	Entire	Raised	-ve rod
	b	Irregular	White, Opaque	Undulate	Raised	+ve rod
05	a	Round	Green, Opaque	Entire	Flat	-ve rod
	b	Irregular	White, Opaque	Undulate	Raised	+ve rod
06	a	Round	Cream, Translucent	Entire	Raised	-ve rod
	b	Round	White, Opaque	Entire	Convex	-ve rod
07	a	Irregular	White, Opaque	Curled	Convex	-ve rod
	b	Irregular	White, Opaque	Curled	Convex	-ve rod
08	a	Round	White, Translucent	Entire	Raised	-ve rod
	b	Round	Cream, Translucent	Entire	Raised	-ve rod
09	a	Round	White, Translucent	Entire	Raised	-ve rod
	b	Round	Cream, Opaque	Entire	Convex	-ve rod
10	a	Round	Green, Opaque	Entire	Raised	-ve rod
	b	Round	Green, Opaque	Entire	Raised	-ve rod

Note: a and b are different colonies

Table 5: List of biochemical tests carried out in wild caught mice

Animal #		Indole	MR	VP	Citrate	Gelatin	Catalase	Oxidase	TSI	Lactose Utilization	Organism
01	a	+	+	-	-	+	-	-	A^+/A^+	-	*Escherichia coli*
	b	+	-	+	+	+	+	+	A^-/A^-	-	Pseudomonas
02	a	-	-	+/-	-	-	+	+	A^+/A^+	+	Bacillus
	b	+	+	-	-	+	-	-	A^+/A^+	-	*Escherichia coli*
03	a	+	+	-	-	+	-	-	A^+/A^+	-	*Escherichia coli*
	b	+	+	-	-	+	+	-	A^-/A^-G	-	Proteus
04	a	+	+	-	-	+	-	-	A^+/A^+G	-	*Escherichia coli*
	b	-	-	+/-	-	-	+	+	A^+/A^+	+	Bacillus
05	a	+	-	+	+	+	+	+	A^-/A^-	-	Pseudomonas
	b	-	-	+/-	-	-	+	+	A^+/A^+	+	Bacillus
06	a	+	+	-	-	+	-	-	A^+/A^+G	-	*Escherichia coli*
	b	+	-	+	-	+	-	-	Yellow butt red slant $/G^+$	-	Salmonella
07	a	+	+	-	-	+	+	-	A^-/G^-	-	Proteus
	b	+	+	-	-	+	+	-	A^-/A^-G	-	Proteus
08	a	+	+	-	-	+	-	-	A^+/A^+G^+	-	*Escherichia coli*
	b	+	+	-	+	+	-	-	Yellow butt red slant $/G^-$	-	Shigella
09	a	+	+	-	-	+	-	-	A^+/A^+G	-	*Escherichia coli*
	b	+	-	+	-	+	-	-	Yellow butt red slant $/G^+$	-	Salmonella
10	a	+	-	+	+	+	+	+	A^-/A^-	-	Pseudomonas
	b	+	-	+	+	+	+	+	A^-/A^-	-	Pseudomonas

Note: a and b are different colonies, A- =Acid negative, A+ = Acid positive, G- = Gas negative, G+ = Gas positive

Serology

Serum samples were used for detection of various pathogens like LCMV, Mycoplasma, MVM, MHV and Sendai virus by using ELISA. The results are presented in table no. 6 and none of the animal is positive for any of the pathogen tested. The OD values above 0.30 were considered as positive as per the instructions of the kit manufacture

Table 6: OD values for various viral pathogens

Animal #	Pathogens				
	LCMV	MYCO	MVM	MHV	Sendai
1	0.290	0.042	0.049	0.010	0.002
2	0.150	0.004	0.026	0.013	0.011
3	0.139	0.138	0.037	0.014	0.011
4	0.006	0.018	0.025	0.005	0.012
5	0.023	0.016	0.001	0.012	0.028
6	0.007	0.010	0.031	0.017	0.013
7	0.016	0.007	0.025	0.045	0.006
8	0.000	0.002	0.096	0.033	0.018
9	0.022	0.003	0.009	0.005	0.036
10	0.011	0.034	0.025	0.044	0.013
Positive control	0.350	0.400	0.350	0.300	0.350

Parasitological examination

Out of 10 animals, four animals were positive for parasitic infestation (pinworm) and six animals were free of any parasites or eggs. Results are presented in Table 8

Table 7: Results of parasitological evaluation

Animal #	Observations
01	Presence of pin worm eggs
02	No parasites/eggs
03	Presence of pin worm eggs
04	No parasites/eggs
05	No parasites/eggs
06	Presence of pin worm eggs
07	No parasites/eggs
08	No parasites/eggs
09	No parasites/eggs
10	Presence of pin worm eggs

Discussion:

The present study was undertaken to determine the health status of the wild caught mice. It is generally believed that wild mice are the potent source of infection in laboratory animal facilities. Majority of the laboratory animal facilities across the globe follow very strict control measures to prevent wild rodent menace inside the facilities. There are very few reports on comprehensive health monitoring of wild mice. Hence, the study was undertaken to assess the hematologial, biochemical, bacterial, viral and parasitic load of wild mice. Ten wild mice (five males and five females) were trapped and used for this study.

The serum biochemical parameters were determined in all the ten wild caught mice and the values were comparable to colony bred mice (Fernández *et al.,* 2009). There was no significant difference observed between the colony bred mice and wild caught mice. Hematological parameters were determined and were found to be comparable to colony bred mice. No significant differences were observed between colony bred and wild caught mice.

The faecal samples were cultured as per the standard procedures and various pathogens like *E.coli*, Salmonella, Proteus, Shigella, Pseudomonas and Bacillus spp. were detected by using appropriate morphological and biochemical tests. Our results confirm with the observations of previous reports by various investigators. Faecal samples were subjected to parasitological examinations and four out of ten animals were found positive and six mice did not show any parasitic ova/eggs/adult worms. Serum samples were used for the detection of various viral pathogens like LCMV, MHV, Sendai, MVM and Mycoplasma by using ELISA methods. Intrestingly, none of the animals tested were positive for any of the viral pathogens. However, this is in contrast with the earlier reports by various investigators who reported various viral pathogens in wild mice (Smith *et al.*, 1993; Becker *et al.,* 2006).

It is concluded, that none of the wild caught mice were postive for tested viral pathogens. However, they harboured various bacterial pathogens in the gut. Few animals were infested with parasites but all the hematological and serum biochemical parameters were in the normal range and comparable to colony bred mice.

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