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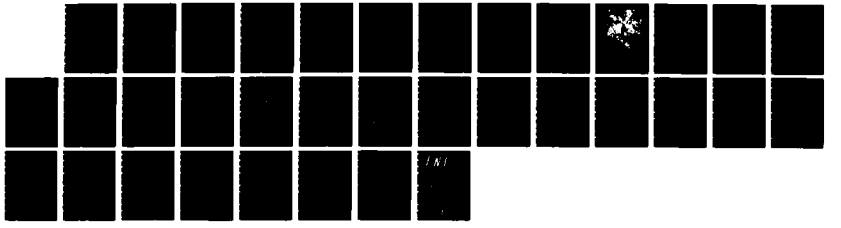

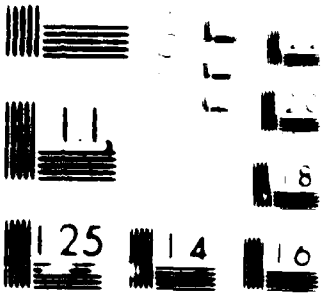
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EPIZOOTIOLOGY OF HANTAAN AND RELATED VIRUSES IN BALTIMORE

Annual Report

29 June 1985

Keerti Shah, M.D.

Supported by

U.S. ARMY MEDICAL REEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MARYLAND 21701-5012

Contract No. DAMD17-84-C-4015

The Johns Hopkins University
Baltimore, Maryland 21218

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SECURITY CLASSIFICATION OF THIS PAGE

AD-A178311

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704 0188
Exp Date Jun 30 1986

1a REPORT SECURITY CLASSIFICATION Unclassified	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S)	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION The Johns Hopkins University 6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION
6c ADDRESS (City, State, and ZIP Code) 34th & Charles Street Baltimore, Maryland 21218	7b ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-84-C-4015
8c ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, Maryland 21701-5012	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62770A; PROJECT NO: 3M1627.70A871; TASK NO: AD; WORK UNIT ACCESSION NO: 358

11 TITLE (Include Security Classification)
(U) Epizootiology of Hantaan and Related Viruses in Baltimore

12 PERSONAL AUTHOR(S)
Keerti Shah, M.D.

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual Report	FROM 6/84 TO 5/85	1985 June 29	32

16 SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	13	

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
virus, serology, rodents, Hantavirus, Hantaan virus, Norway rat

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

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20 DISTRIBUTION AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	

22a NAME OF RESPONSIBLE NDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
Mrs. Judy Pawlus	301-663-7325	SGRD-RMI-S

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

19. Abstract (cont.)

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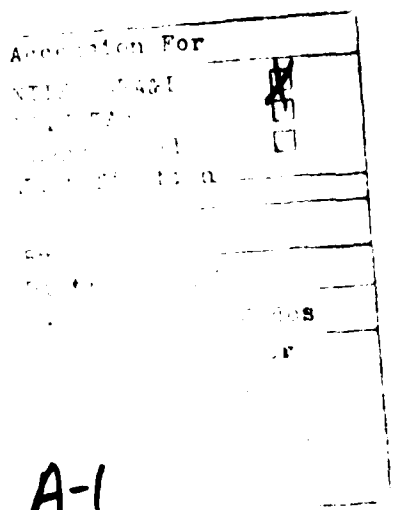
FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).



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ABSTRACT:

The mammal fauna within Baltimore City has been surveyed over a one year period for evidence of infection by Hantaviruses within resident species. Live trapping of the Norway rat using a mark-recapture protocol has been successfully employed at several different habitats within the city, and a combination of mark-recapture and removal methods have been used to sample six other small mammal species at a number of sites. Representative serosurveys have also been made of the resident cat, dog and human population. The Norway rat consistently demonstrates its importance as a reservoir of HTNV through its widespread geographic dispersion of infected populations, high titer and prevalence rates within adults for any given population, and a constancy of seropositivity throughout different seasons. The field vole, Microtus pennsylvanicus, appears to maintain a different viral agent, possibly related to Prospect Hill virus, in parkland and urban settings. Other species of small mammals co-occuring with either of these two species at selected study sites, also yield evidence of infection with Hantan viruses. We also demonstrate reciprocal titers against Hantaan virus in human serum collected within Baltimore City up to 2048. Differences in prevalence rates in Norway rat populations at different sites are discussed in relationship to the ecological and/or demographic characteristics of the infected populations.

INTRODUCTION:

This report summarizes all research activities conducted during the period 1 June, 1984 to 31 May 1985, pertaining to epizootiology of Hantaviruses (HTNV) in mammalian species of Baltimore City, Maryland, USA. These newly described viruses are believed to constitute a new genus, Hantavirus, within the Bunyaviridae family. Agents of this group are associated clinically with a hemorrhagic fever with accompanying renal syndrome (HFRS). The severity of symptoms in infected individuals ranges from subclinical to a fulminant hemorrhagic fever with mortality attributed to renal insufficiency.

Lee et al. (1978) isolated the prototype agent of this group (Hantaan strain 76-118) in Korea from lung tissue of the striped field mouse, Apodemus agrarius coreae. Virus isolates with demonstrable antigenic relatedness to Hantaan virus have since been made from rodent species distributed across the Nearctic and Palearctic. Based on cross neutralization studies, four broadly related subgroupings of agents are now recognized viz. Urban rat virus strains, the Nephropathia Epidemica strains, Hantaan strains and the Prospect Hill strains (Schmaljohn et al., 1985). These differ in geographic distribution, rodent reservoir species and pathogenicity for humans. In light of the taxonomic status of viruses within this grouping, we will employ the acronym HTNV to refer to the all strains constituting the Hantaviruses.

The brown rat and black rat (Rattus norvegicus and Rattus rattus, respectively) were identified as reservoir species for human cases of urban associated HFRS in Seoul, Korea (Lee, et al., 1982), and have been implicated in outbreaks of illness in laboratory personnel and animal handlers in Japan, England, Korea and Belgium. The cosmopolitan distribution of these species, as well as the potential dispersal of infected animals via international shipping, prompted investigators to survey rat populations in port cities of the United States for evidence of infection. Viral isolation or serologic evidence of infection has been reported from Norway rat populations in Philadelphia, Houston, New Orleans and Baltimore, USA. The rat isolates of virus share a high degree of antigenic similarity both within the United States and compared to Korean and Japanese strains of rat associated virus (LeDuc et al., 1984, Schmaljohn et al., 1985). Serologic evidence of infection in rats was found in populations at sites well removed from port facilities and widely distributed throughout the city of Baltimore. These findings indicated that the virus had not been recently introduced, and that further study of the epizootiology of the virus in this rodent species, as well as other peridomestic mammalian species would be of value in assessing potential or realized human involvement (Childs, et al., 1985).

The contract outlined five major areas of performance:

1. To determine the prevalence of antibody to HTNV in the small mammal populations of Baltimore City.

2. Determine any association of HTNV infection in small mammmals within different urban habitats (park land, residential, wharf, etc.).

3. Investigate the population dynamics of naturally infected populations of rats within Baltimore City.

4. Describe the distribution of viral antigen within tissues of infected mammals.

5. Isolate, identify, and immunologically characterize HTNV from different mammalian species.

We have concentrated our performance on the first three outlined objectives, and have initiated efforts at virus isolation and identification.

RESULTS:

STUDY SITES:

All sampling efforts during the past year have been confined to Baltimore City, with the greatest attention concentrated on locations within the eastern half of the city. Norway rats have been collected from 13 sites within the area, constituting a wide variety of potential urban habitats viz. waterfront, parkland, residential and institutional (Figure 1. and Table 1.) Sites up to 9.6 km from waterfront facilities were included (Western Run). Trapping of Rattus norvegicus began in early July, 1984 at six different sites (Charles Village, Winston-Govane, Western Run, McElderry, Granary Piers, Cherry Hill) to assess relative population size, prevalence rates and feasability for long term mark and recapture study. Three sites were chosen in early October for more detailed study based on representative rat population, occurence of seropositive animals and habitat type. Mark-release-recapture (MRR) protocol was begun at Cherry Hill (CH), Winston Govane (WG) and Chase-Milton (CM) study sites in October, 1984. The Chase-Milton site was chosen as a substitute for the McElderry site due to disturbance to the habitat at this locale. The substitute site was adjacent to a former study area, Port St., from which seropositive rats had already been identified. Cherry Hill study area comprised open abandoned parkland while the other two study areas were in moderate to heavily settled residential sites.

House mice (Mus musculus) were sampled from two residential settings, the Patterson Park study area and WG, one institutional site (JHU), and two parkland settings, HR and CH. Small mammals, exclusive of Mus, were surveyed at two parkland sites HR and CH. Urban stray cats were collected during routine activities by the Baltimore Municipal Animal Shelter (M.A.S.) personnel from either city-wide sources or one study area (RH), while sera tested from domestic dogs were collected at the Animal Shelter facilities from animals prior to euthanasia, and represent the city-wide populations.

RATS:

We have screened, by immunofluorescent antibody technique (IFAT), 426 rats captured at 13 sites within Baltimore City (Table 2). Three animals from Baltimore county are also included in the sample. Nine of the sites are located in urban residential areas, two are in urban park areas, one is in an urban commercial-industrial location. The county site is rural. Intensive trapping has been directed toward the urban residential and urban parkland areas.

Our trapping of rats shows that evidence of infection by HTNV is widespread throughout Baltimore. Every location sampled, regardless of sample size, contains individuals seropositive by IFAT and/or plaque reduction neutralization (PRN) tests. Crude prevalence rates (IFA titer $\geq$ 32) range from 22-67% in areas where more than 10 rats have been caught (N = 9 locations). Although the data show that some rats from all urban habitats sampled are infected, the two urban park areas (Cherry Hill and Western Run) have lower prevalence rates (21.6% and 28.6%) compared to the urban residential areas (38.5% to 66.7%). There is also a difference in the maximum antibody titers associated with the two land use

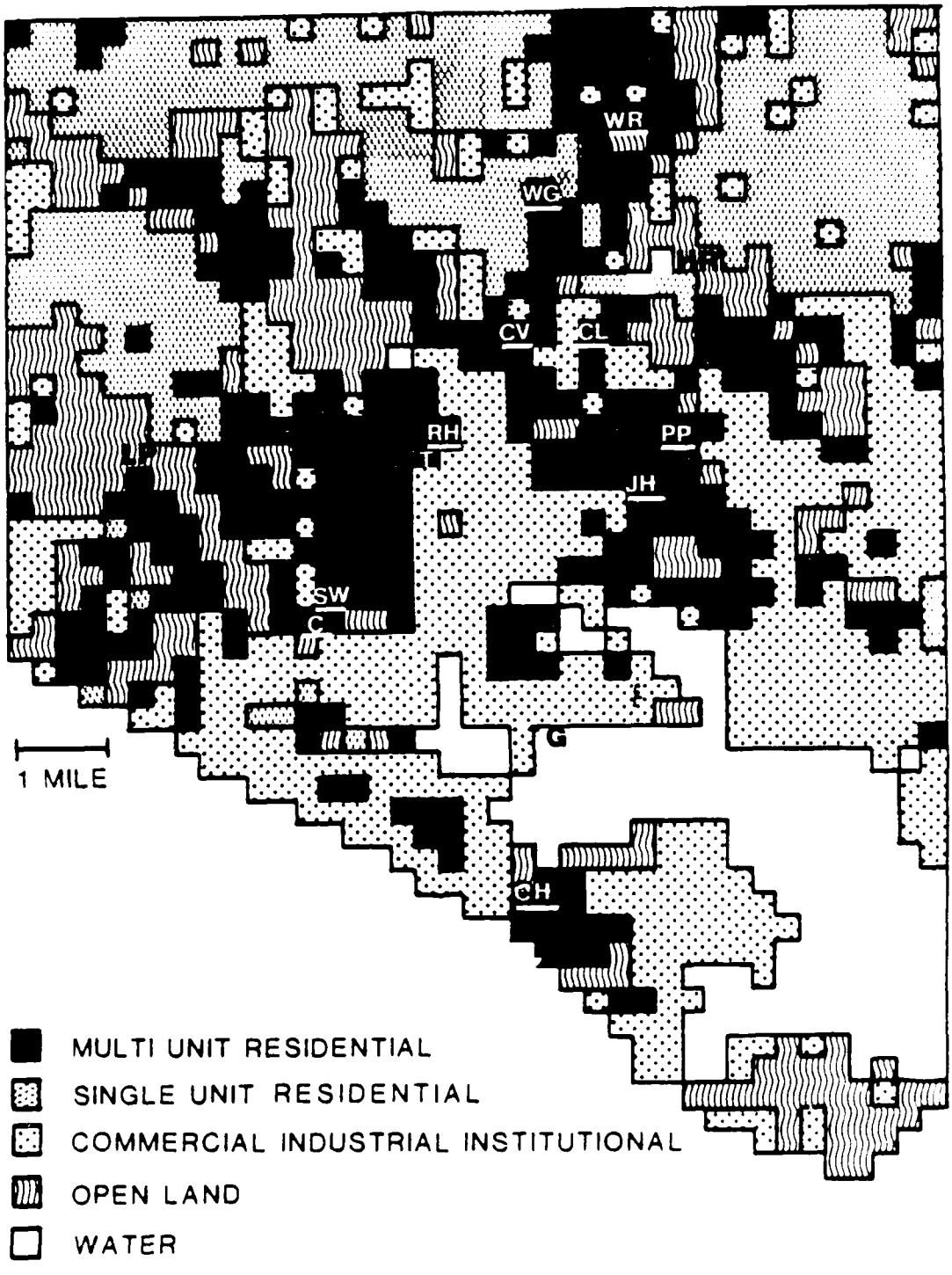

Fig. 1. Land use map of Baltimore showing location of study sites. Abbreviations as in Table 1.

Table 1.

Trapping locations screened for HTNV activities within Baltimore

Trapping location	Species	Habitat
Patterson Park Study Area (PP)		
Port St.	*Rattus*, *Mus*	Multi-unit residential
Biddle St.	*Mus*	Multi-unit residential
Milton St.	*Rattus*, *Mus*	Multi-unit residential
Chase St.	*Rattus*, *Mus*	Multi-unit residential
Montford St.	*Rattus*, *Mus*	Multi-unit residential
Herring Run (HR)	*Mus*, *Sciurus*, Small mammals	Urban Park
Leakin Park (LP)	*Mus*, *Sciurus*	Urban Park
Charles Village (CV)	*Rattus*, *Mus*	Multi-unit residential
Winston/Govane (WG)	*Rattus*, *Mus*	Multi-unit residential
Western Run (WR)	*Rattus*	Parkland
Castle (JH)	*Rattus*	Multi-unit residential
Cherry Hill (CH)	*Rattus*, *Mus*, Small mammals	Open land
Reservoir Hill (RH)	*Rattus*, *Felis*	Multi-unit residential
Smallwood (SW)	*Rattus*	Multi-unit residential
Granary A (G)	*Rattus*	Commercial/Industrial
JHU (JH)	*Mus*	Institutional
Christian (C)	*Rattus*	Multi-unit residential
Tiffany (T)	*Rattus*	Multi-unit residential
Chilton (CL)	*Rattus*	Multi-unit residential
Baltimore Co.	*Rattus*	Rural
City-wide	*Felis*, *Canis*	Mixed

Table 2.

Numbers, locations and prevalence rates of antibodies to HTNV in rats from Baltimore

Location	N	% ≥ 32	Highest titer
Port *	87	66.7	> 2048
Reservoir Hill	65	52.3	> 2048
Winston-Govane	65	49.2	> 2048
Chase *	51	45.1	> 2048
Cherry Hill	51	21.6	512
Charles Village	26	38.5	> 2048
Bradford *	19	73.7	> 2048
Castle	15	40.0	> 2048
Western Run	14	28.6	512
Smallwood	6	66.7	> 2048
Christian	5	40.0	> 2048
Baltimore County	3	33.3	128
Tiffany	2	100.0	> 2048
Chilton	2	100.0	> 2048
Granary A	1	100.0	512
TOTAL **	412	49.3	> 2048

* Streets within the Patterson Park study area
** 14 rats missing titer or location

areas. Titers from urban residential rats typically exceed 2048, occasionally reaching 32,768, while the highest titer from park rats is 512, a 4-64 fold lower level. The reasons for this difference are not clear but we suggest the following possibilities: 1) differences in virus strains; 2) differences in rat population age structure; 3) habitat variations that influence transmission. These possibilities are considered below or in the discussion.

As we have previously shown (see enclosed reprint) a significant association exists between rat weight and antibody prevalence. Table 3 summarizes the distribution of trapped rats by sex, 100 g weight class and the occurence of IFA titers $\geq$ 128. We only include titers $\geq$ 128 because titers above this level have significant neutralizing activity against prototype HTNV, and are believed to represent the best evidence of specific infection (see reprint). The prevalence of antibody by this criteria is 13.2% (N = 136) in rats less than 300 g, and 51.4% (N = 276) in larger animals.

Overall the mean weight for trapped rats is 352 g for males and 339 g for females. Considerable variation exists between study sites. Urban park areas contain smaller rats than the residential locations for all sites with sample sizes greater than 14 (range for males 234-271 g in parks; 321 - 424 g for residential areas: range for females 205 - 217 g in parks; 300-435 g in residential areas). Weight is an excellent indicator of Norway rat age up to 300 g, and is useful up to approximately 450 g (Calhoun, 1962). The differences in mean weights in residential versus park rats could indicate a younger population with correspondingly lower prevalence rates in park rats. However, the possibility exists that weight structure in park versus residential rats reflects differences in available food resources, and underlying age structure remains the same. We expect that mark-recapture studies will provide the data on survivorship necessary to distinguish between these alternatives.

The differences in weight specific prevalence rates is also apparent in geometric mean titers (GMT) (Fig. 2). This figure considerably underestimates the actual difference between weight class titers because all rats with titers > 2048 were assigned an endpoint titer = 2048 in the calculation of GMT, although we know that rat titers to HTNV may reach 32,768. Rats weighing < 300 g had a relatively constant GMT, ranging between 32 and 128, while larger animals had titers between 128 and 1024.

The shift towards higher antibody prevalence and titer occurs around 300 g of weight when rats are becoming mature adults. Few rats below 200 g are sexually mature as evidenced by pregnancy, lactation or perforate vaginas in females, or scrotal testes in males (Table 4). The observation of weight-age specific antibody prevalence rates, and associated increases in titers, indicate that rats may be acquiring HTNV infections at an early age (approximately 30-40 days of age, or at weaning; weight = approximately 100 - 150 g). However, the possibility exists that they are infected shortly after birth or transplacentally and are not expressing high antibody titers until a later age. Laboratory experiments that may help resolve these issues are underway.

The overall distribution of seropositive rat titers is shown in Fig. 8a. Rats are unique in their titer distribution, with approximately 50% of all seropositive animals having IFA titers $\geq$ 2048. No other species of mammal from Baltimore shows this pattern, although *Microtus* may ultimately demonstrate a similar titer distribution against its associated agent, Prospect Hill Virus (PHV).

Table 3.

Distribution of rat titers > 128 to HTNV
by location, sex and 100 gram weight class

Location	Sex	X wt	< 100	1-199	2-299	3-399	4-499	>500
Port	M	379	1/3	0/1	2/4	7/14	10/17	6/6
	F	353	1/4	0/4	0/4	3/10	12/14	5/6
Reservoir	M	424	-	-	0/2	1/10	9/14	4/5
	F	435	-	-	0/2	5/12	7/10	7/10
Winston-	M	345	1/4	2/3	0/1	5/12	3/10	3/3
Govane	F	307	1/6	2/4	0/4	2/7	1/7	1/4
Chase	M	358	0/4	0/2	0/1	2/7	9/14	3/4
	F	300	0/2	0/4	0/4	0/2	1/4	3/3
Cherry	M	234	0/2	1/10	1/12	1/7	1/1	-
Hill	F	205	-	2/9	1/9	0/1	-	-
Charles	M	321	0/1	0/3	0/3	1/3	2/6	-
Village	F	405	-	-	0/2	0/2	2/4	1/2
Bradford	M	442	-	-	0/1	1/2	4/5	2/2
	F	344	0/1	-	-	2/5	2/2	1/1
Castle	M	371	-	0/3	-	0/2	0/1	3/3
	F	357	-	1/1	0/1	0/1	0/2	1/1
Western	M	271	-	1/1	0/4	1/3	-	-
Run	F	217	0/1	0/1	0/3	0/1	-	-
Smallwood	M	356	-	0/1	-	-	-	1/1
	F	332	-	0/1	-	0/2	0/1	-
Christian	M	515	-	-	-	-	0/1	1/1
	F	442	-	-	-	0/2	-	0/1
Balt. Co.	M	63	0/1	-	-	-	-	-
	F	230	-	0/1	1/1	-	-	-
Tiffany	M	425	-	-	-	-	1/1	-
	F	475	-	-	-	-	1/1	-
Chilton	M	528	-	-	-	-	-	1/1
	F	351	-	-	-	0/1	-	-
Granary A	M	525	-	-	-	-	-	1/1
TOTAL	M	352	2/15	4/24	3/28	19/60	39/70	25/27
	F	339	2/14	5/25	2/30	12/46	28/45	19/28

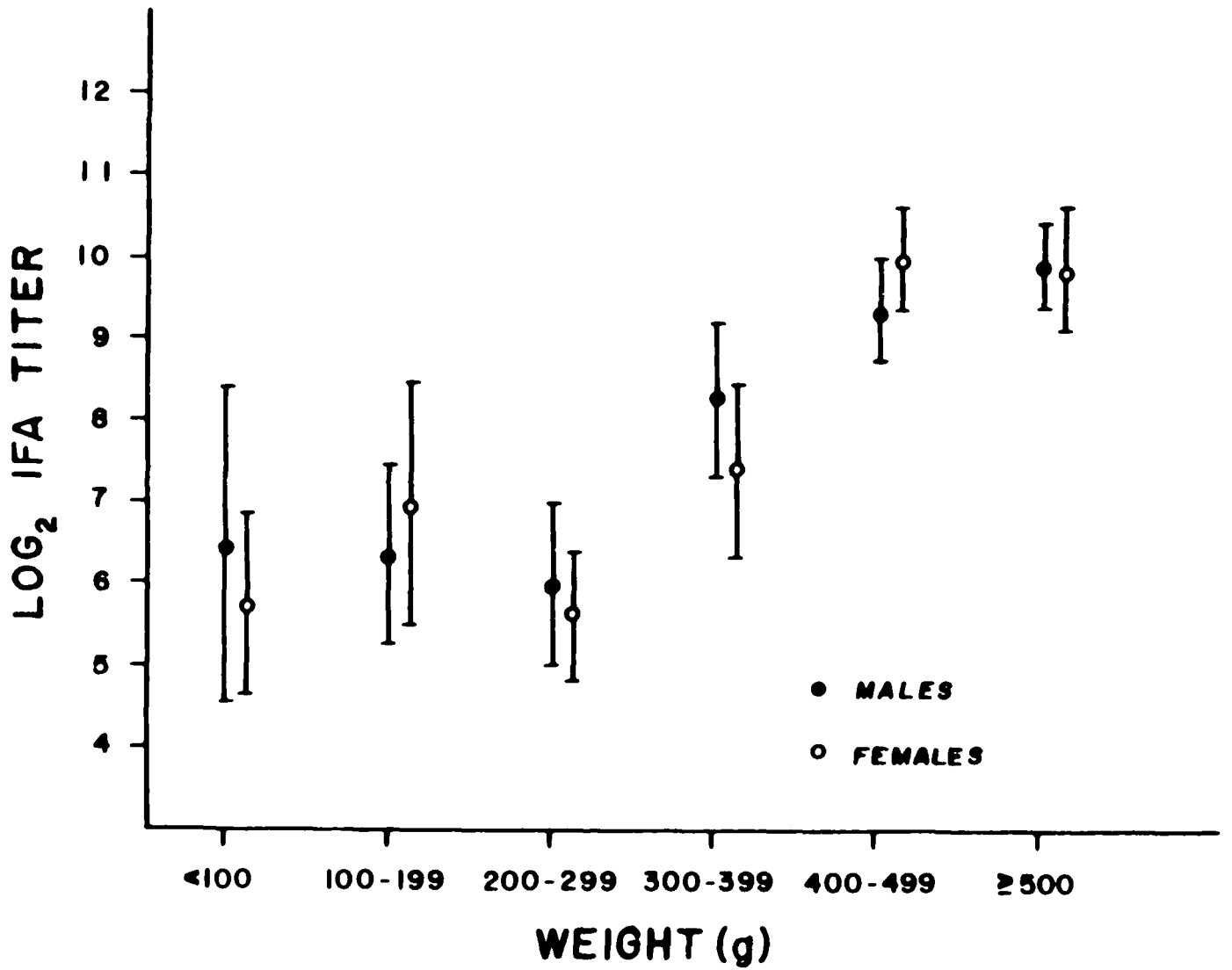

Fig. 2. Geometric mean titers (GMT) of male and female rats by 100 g weight classes (Mean ± 95% confidence interval).

Table 4.

Sexual characteristics of Baltimore rats

Sex	Character	weight (g)					
		< 100	1-199	2-299	3-399	4-499	>500
Male							
	Scrotal	1/10	13/22	26/26	54/56	60/61	25/25
Female							
	Perforate	1/13	10/23	21/24	40/40	40/40	28/28
	Preg./Lact.	0/11	0/17	2/10	10/33	10/35	17/23

The high prevalence rate of HTNV infections in Baltimore rats, coupled with the extremely high antibody titers, makes this rodent species the most significant reservoir in urban Baltimore. The widespread geographic distribution of seropositive rats throughout all urban habitats suggests that this infection has been enzootic in rats for a long period of time and is not recently introduced.

Of the 426 rats we have captured, approximately 120 have been marked with eartags and released. We have recaptured and rebled 13 rats at intervals of 1 - 6 months (Fig. 3). In addition, 10 rats have been recaptured at 1 - 3 day intervals, within a single trapping session, and were released without rebleeding. Twelve rats have been bled twice, and a single individual (rat 63) has been bled three times. Three tagged rats have died during handling, and a single individual was found dead in the field.

The longitudinal changes in IFA titers of the 13 recaptured rats is shown in Fig. 3. Seven rats were negative at first capture (titer < 32) and remained negative at recapture 1 - 4 months later. A single rat with titer = 32 remained unchanged at recapture 5 months later. Three rats showed moderate to severe decreases in titer (2-4 fold) over a 1 - 2 month period. One of these animals dropped from a titer = 128 to 32. Two rats showed significant increases in titer (4-32 fold) over a 1 - 6 month period. One of these animals (rat 74) showed a dramatic rise in titer from 512 to > 16,384 in a single month.

We have observed most possible combinations of change in titer, except for a shift from positive to negative, or a definitive seroconversion. We believe that rat 74, whose titer rose 32 fold, is clearly a recent seroconversion and we probably caught this animal during the phase of exponential rise in serum IgG. No IgM assays have yet been performed. The three animals with decreasing titers may be misleading. It is possible that antibody levels fluctuate naturally in rats, but our studies show that the IFA assay can vary 2 fold with different antigen slides used on different days. We plan to conserve our small amounts of recapture sera until the conclusion of our study, and then retiter all paired sera under identical conditions.

The recapture rate for rats approaches 20% (38 rats were caught and released in May and are not yet at risk for recapture), which is an excellent figure for the Norway rat. We believe that both capture and recapture rates will increase steadily throughout the summer and fall months, after having experienced a precipitous drop over winter (Fig. 4). Winter is obviously a stressful period for rats in both residential and park environments and populations are only now rebuilding. The sensational increase in captures at Cherry Hill can only be due to immigration of new rats from surrounding areas because few of these animals are newly weaned, and we know the population on this study grid was near zero over winter. The urban residential site at Chase shows a seasonal trend similar to Cherry Hill, while Winston-Govane shows a less smooth decline. Overall the trap success rates (rats per 100 trap nights or % success) at the three sites are comparable, with Winston-Govane having the highest (7.5%) followed by Cherry Hill (7.0%), and Chase having the lowest (4.0%).

Over the next year we expect to obtain survivorship data on rats that may help explain discrepencies in antibody prevalence rates between residential and park study sites. So far the longest continuous record of rat survival in park areas is one month, while seven animals from residential areas have survived a minimum of 2 - 6 months. If survivorship in parklands is low, this fact coupled with the observed low mean weights may indicate a younger age structured population. We would therefore expect lower prevalence of antibody and lower titers based on our observations of age-weight specific prevalence functions.

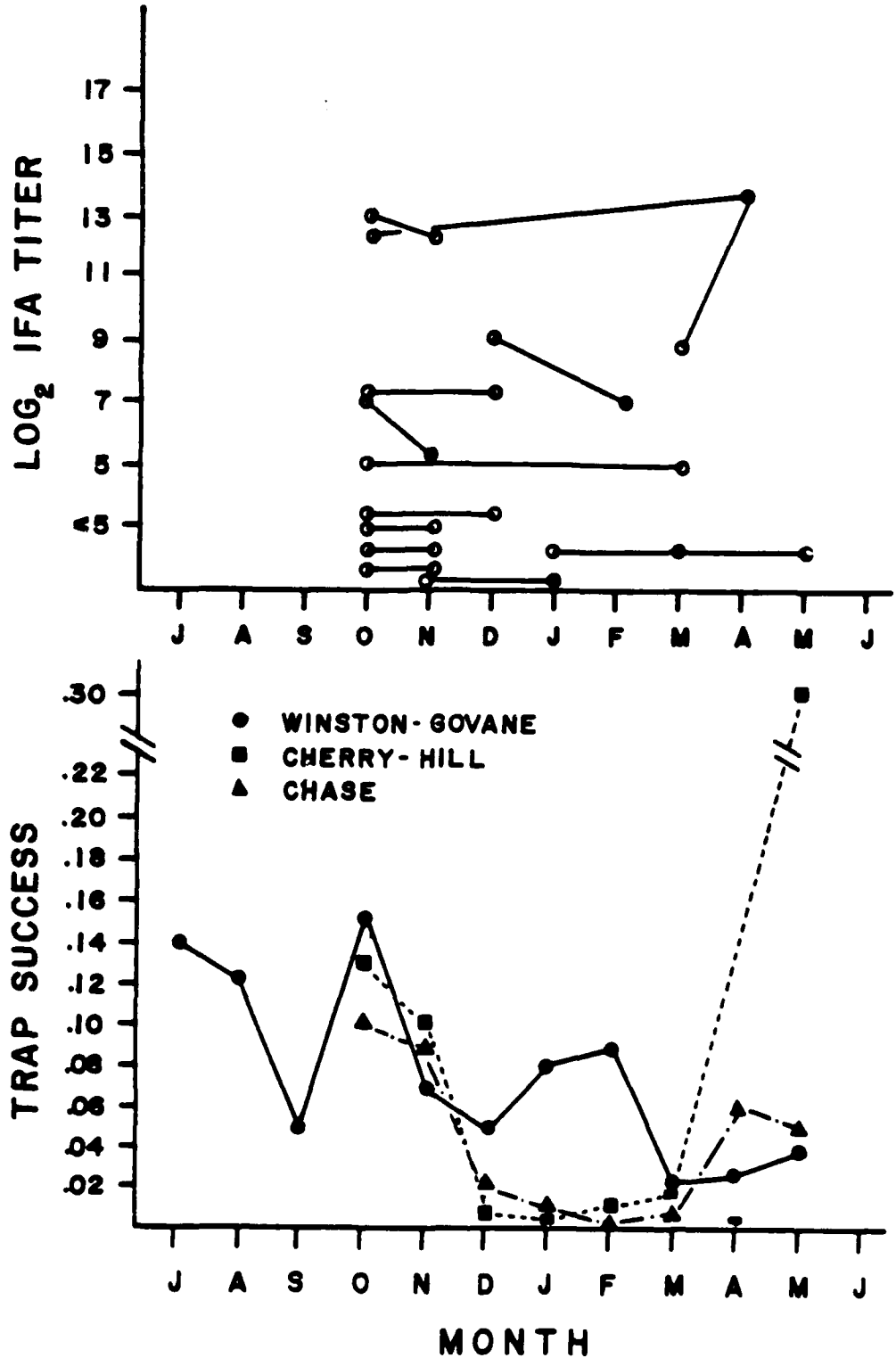

Fig. 3. (Top) - longitudinal changes in IFA titer of rats captured at least twice.

Fig. 4. Trap success for rats at two urban residential locations and one urban parkland.

Relative indices of population size can be obtained by comparing numbers of rats trapped per area per unit effort. When we plot such a measure against antibody prevalence rates (titer $\geq$ 128) in rats > 300 g (the adult population or the population at risk for being seropositive), we obtain some measure of how infection rates with HTNV vary in rat populations of different size (and possibly density). Because populations vary over seasons we have plotted these values for various study sites for each season for which we have trapped 10 or more rats > 300 g (Fig. 5). The resulting figure shows no trend for increasing antibody prevalence rates with increasing trap success. The prevalence rates show broad variations (range = to 37 - 78%), as does trap success (range = 4.0% to > 20.0%), but the regression slope is not significantly different from zero. In general prevalence rates do not vary within a site from season to season.

The best evidence to date suggests that HTNVs cause a persistant infection in their various rodent hosts, and virus is shed for long periods of time in urine, feces or saliva (Lee et al., 1981). The nature of this infection probably ensures that most members of a social species will come into contact with an actively infectious individual at some time, provided survivorship is long enough. If transmissability of HTNV is relatively great, than we may expect to see high infection rates in socially active species, such as rats, even when absolute population numbers are low.

HOUSE MICE:

We have sampled sera and/or tissues from 265 Mus musculus from five sites and have captured, marked and released an additional 55 mice at one location. A total of forty-six recaptures have been recorded for marked animals bringing the total number of captures of this species to 366. The distribution of captures per site is given in Table 5.

Ten percent of all individual Mus sera tested by IFAT have yielded specific antibody to HTNV at titers $\geq$ 1:32. The frequency histogram of specific titer to HTNV declines steadily with increased dilution (Figure 8b). Eighty-five percent of seropositive individuals had antibody titers in the range of $\geq$ 1:32 to 1:128, while 12% fell with the range of 1:128 - 1:512, and three percent had titers of 1:512. The prevalence of seropositives have ranged from 0.0% to 17.8% at various city trapping sites where ten or more individuals were sampled (Table 5). Titers of 1:128 or higher have only been seen at two locations, Port St. and Cherry Hill. These two locations, however account for 47% of all Mus sera examined. Four of eleven (36.4%) of seropositive Mus from Port St. and 1 of 13 (7.7%) of seropositive mice from Cherry Hill had IFA antibody titers of 1:128 or higher to HTNV. The difference between these sites was not significant (P=.24 Fischer's Exact Test).

A greater percentage of males were seropositive (11.8%) than females (8.3%), however the difference was not statistically significant (X^2 = 1.09, d.f. = 1, P = 0.7). Variation in the seropositivity rate between the two sexes was noted at most sites (Table 5). Unlike the pattern observed with Rattus, no relationship between weight and prevalence of HTNV specific antibody or titer was noted.

We have observed a rise in IFA titer to HTNV in three individuals based on serial serum sampling (Figure 6). The maximum increase has been a sixty-four fold rise in titer over a one-month period. Most individuals from whom a longitudinal series of sera were obtained have remained seronegative. A single

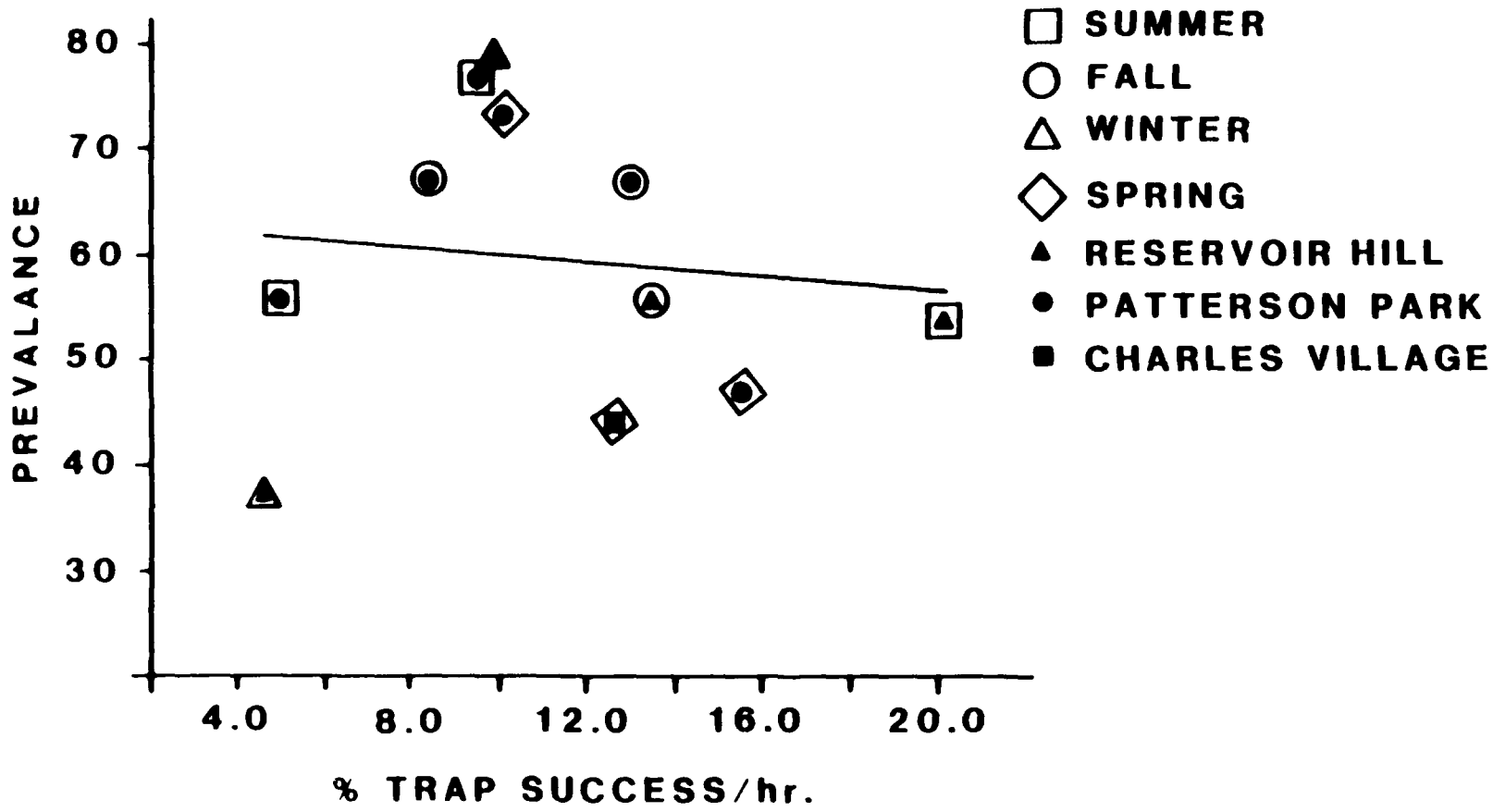

Fig. 5. Prevalence of antibody to HTNV in Baltimore rats as a function of trap success.

Table 5.

Summary of mice trapped in Baltimore and tested for antibody to HTNV

Location	Total N	% Titer ≥1:32	% males ≥1:32	% females ≥1:32	maximum titer
Port St.	79	(14)	11	17	512
Biddle St.	40	(7)	10	5	64
Milton St.	27	(7)	20	0	32
Chase St.	40	(5)	7	4	64
Montford St.	28	(4)	8	0	64
Herring Run	1	(0)	0	0	-
Leakin Park	4	(0)	0	0	-
Hopkins-Hygiene	19	(5)	9	0	32
Winston-Govane	31	(0)	0	0	-
Cherry Hill*	97	(18)	25	16	128
TOTAL	366	(10)	12	8	

* includes 46 instances of recaptures

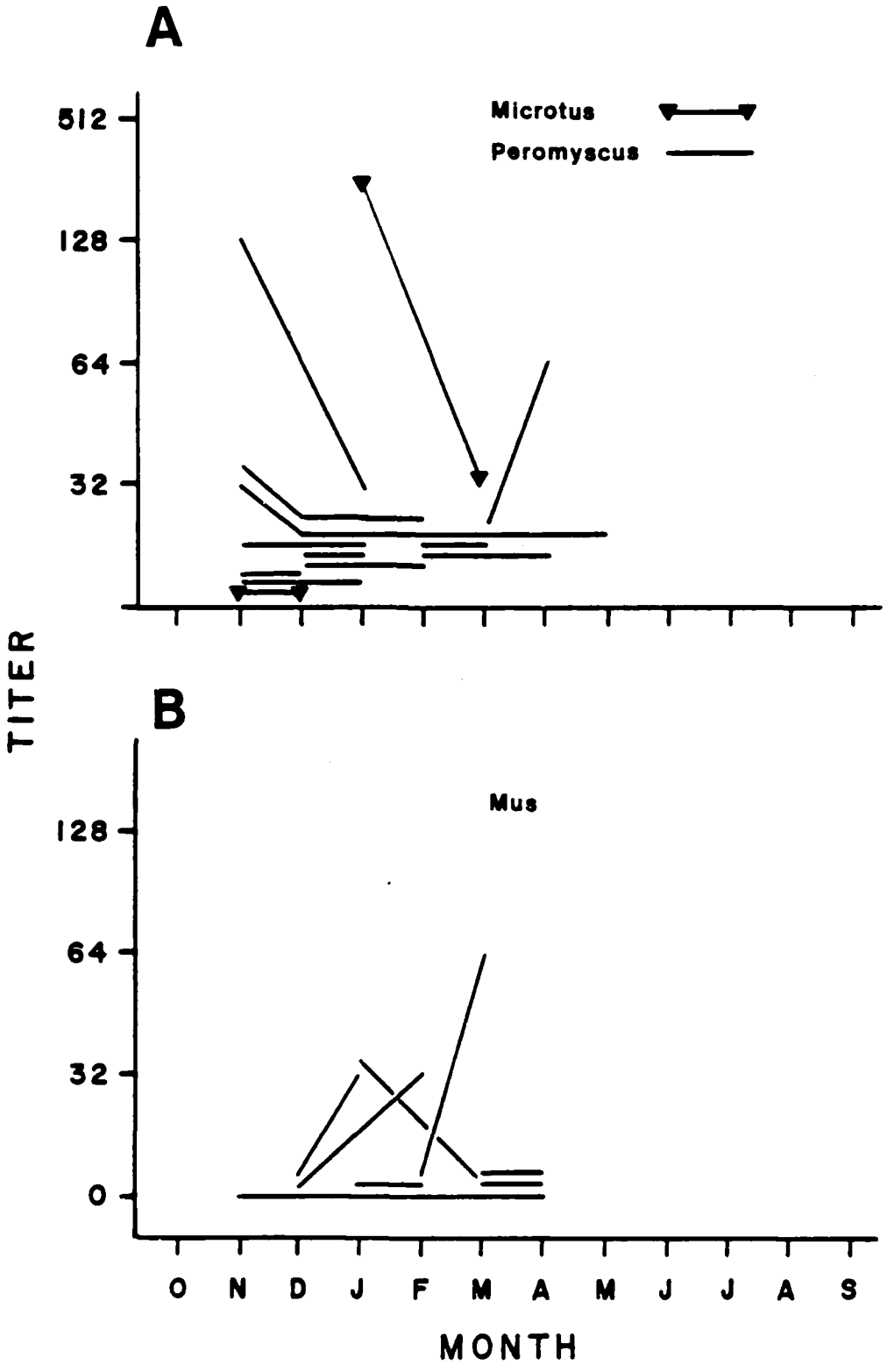

Fig. 6. Changes in IFA titers in small mammals captured and rebled at least twice. A) Microtus pennsylvanicus and Peromyscus leucopus. B) Mus Musculus.

decline in antibody titer over a three month period was observed.

The data indicate that Mus musculus does acquire infection with HTNV in many of the same areas from which infected rats have been sampled. The only exception to this general finding has been the lack of seropositive individuals from the Winston-Govane trapping area. None of the occupants at this residential sites have reported occurrence of Rattus norvegicus within their homes, while a great many have noted mouse infestations at one time. This contrasts with the Port St. trapping area where domiciliary rats infestation has been reported to occur. Seropositive mice have also been spatially associated with infected rat and vole populations in the parkland setting of Cherry Hill (See below). The prevalence rate, and associated antibody titers, are lower in Mus than in sympatric species such as Rattus or Microtus which may mean mice are opportunistic hosts of the viruses circulating in coexisting reservoir species.

Further evidence of this relationship will depend upon comparison of the virus strains infecting these species at a given site. At this point, it appears that mice are playing a minor role in transmission and maintenance of these viruses in urban settings.

OTHER SMALL MAMMALS:

Four additional species of small mammals were examined for evidence of infection by Hantaan-like virus including the field vole Microtus pennsylvanicus, the white footed mouse, Peromyscus leucopus, the chipmunk, Tamias striatus and the Eastern gray squirrel, Sciurus carolinensis (Table 8). These species have been sampled at one or more of three parkland sites within the city, i.e. Herring Run, Leakin Park or Cherry Hill. The sample sizes for the latter two species are low thus far, and no anti-HTNV titer at or above 1:32 has yet been observed.

VOLES:

Fourteen of 50 (26%) voles have evidence of specific antibody to HTNV virus by IFA, with titers ranging up to 1:512. The frequency distribution of antibody level based on four fold dilution classes (Fig. 8c) reveals a steady decrease in the proportion of individuals with positive IFA results at greater antibody dilution. Twenty percent of all seropositive Microtus however had IFA antibody levels of 1:512 or greater (Fig. 8c).

Prospect Hill virus was originally isolated from this species. We have begun to screen Microtus antisera against Prospect Hill antigen to compare reactivity with Hantaan virus (76-118). Preliminary results indicate that most Microtus antisera tested yield four to eight fold higher titers against the Propsect Hill virus than Hantaan virus. Several sera yielded identical titers against the two antigens used, while in a few instances the anti-Hantaan titer exceeded that for Prospect Hill.

No significant difference was observed between the two sexes in proportion of individuals with antibody titers equal to or greater than 1:32. Six of 26 female Microtus pennsylvanicus were seropositive by IFA, while seven of 18 males were seropositive at titers at or above 1:32. Sequential serum samples have been obtained from three animals (Fig. 6). In two instances a decline in serum titer have been observed over a two-month period, while no evidence of antibody production against HTNV was noted for the third animal over a one-month interval. Collection of more sera and tissues will allow greater definition of the transmission parameters of HTNV within this species. We will be

concentrating further effort on evaluating the relative contribution each agent makes to the infection status of the population, as well as the dynamics of the infection.

WHITE FOOTED MOUSE:

The prevalence rate of HTNV infection in Peromyscus leucopus, based on serologic data, is approximately 13% (Table 8; Fig. 6). Only one individual has had evidence of serologic titer to Hantaan virus greater than 1:32, and of all positive individuals, none appear to have developed higher titers to Prospect Hill virus than to the prototype strain. Peromyscus is more closely related to Microtus than to the murid rodents, however infection with the murid strain viruses appears to be more likely in the Peromyscus populations being examined, despite potential exposure to the agent circulating within the Microtus population.

No significant differences were observed in the distribution of seropositive animals according to sex in this species. Females were three times as likely to be seropositive as males however, i.e. 7% for males vs. 21% for females.

CATS:

We have screened 649 domestic cats for antibody to HTNV by IFAT. The majority of these animals (98%) were collected in Baltimore during 1980-1981; ten additional animals were obtained in 1984-1985 from a particular alley. We have obtained the majority of our cat sera through the cooperation of the Baltimore City Municipal Animal Shelter (M.A.S.). All trapping of cats was conducted by official M.A.S. wardens, and trapped animals were maintained at the shelter for the proscribed period (5 days) prior to being euthanatized by M.A.S. personnel. The M.A.S. received no compensation for these services.

The origins, numbers and prevalence rates of antibody in Baltimore cats are summarized in Table 6. Overall antibody prevalence rate was low (6.5%) and there was no significant difference between cats classified as 'stray' or 'not wanted' by the M.A.S. These classifications are somewhat arbitrary and signify only whether the individual relinquishing the cat claims prior ownership.

Cat sera was collected from two veterinary clinics in Baltimore. Vet cats had intermediate levels of antibody compared to M.A.S. and alley cats. These cats represent an older (X age 5.6$\pm$3.5 yrs) population of animals compared to those from the M.A.S. (X age 2.1$\pm$1.9 yrs).

Alley cats or feral cats trapped from the Reservoir Hill section of Baltimore in 1981 had a significantly higher antibody prevalence rate compared to cats from the other two sources. Unfortunately, when M.A.S. retrapped this alley in 1984-1985 to obtain sera and tissues from cats, we found only a single seropositive (titer = 32) animal out of ten trapped. This particular alley had an extremely large rat population in 1980 but has since been the target of extensive and ongoing rat control efforts. In 1980-1981 over 50% of the rats trapped from Reservoir Hill had evidence of antibody (Table 2), and several of the seropositive cats were known to have eaten rats. Although we have not systematically trapped this alley in recent years, we have observed a few rats, and HTNV continues to be present as a single trapped rat was seropositive (titer > 2048).

Table 6.

Prevalence of IFA antibodies to HTNV in Baltimore cats of different origin

Origin	N	N $\geq$ 32	% Positive
M.A.S. 'not wanted'	382	18	4.7
M.A.S. 'stray'	216	14	6.5
Vet hospital	27	3	11.1
Alley 1980-1981*	14	6	42.9
Alley 1984-1985*	10	1	10.0
TOTAL	649	42	6.5

* Feral cats from Reservoir Hill

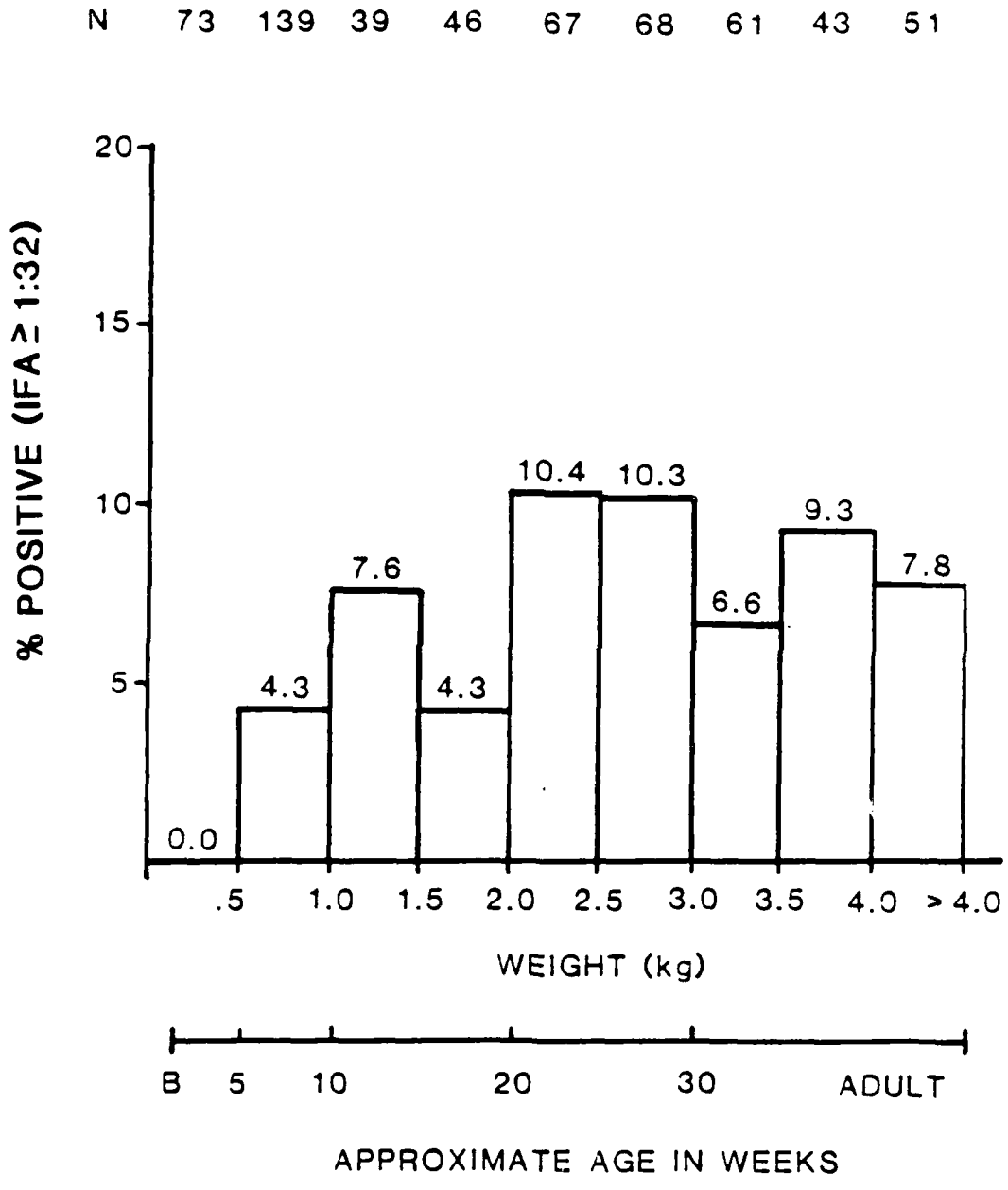

Fig. 7. Prevalence of antibody to HTNV in Baltimore cats based on .5 kg weight classes.

Analysis of weight-specific antibody prevalence rates in cats from the M.A.S. show a trend for increasing prevalence with increasing weight Fig. 7. Cats normally reach adult weights (2.5 kg for females; 3.5 kg for males) at approximately six months of age. Few kittens under 1.0 kg had antibody, while prevalence rates peaked at over 10% in young adults. The association of antibody prevalence with weight and age may be due to exposure of adolescent cats. Eruption of permanent dentition and development of efficient hunting skills have usually occurred by six months of age.

The distribution of seropositive cat IFA titers are shown in Fig. 8e. The GMT is 83.3, and the distribution is similar to that of other species tested, with the exception of rats. We believe cats are only infrequently infected with HTNV and are probably not significant reservoirs for this virus in Baltimore. We plan to continue sampling cats in particular locales where rats are numerous and prevalence rates in these rodents are high.

DOGS:

We screened 164 dog sera collected during 1980-1981 from the M.A.S.. All dogs were over six months of age. IFA antibody to HTNV was found in two (1.2%). In both case titers were = 32.

The lack of significant antibody prevalence and titers in dogs indicates that this species is rarely infected by HTNV and is unlikely to play a role in the maintenance or transmission of this agent between species of mammals in Baltimore.

HUMANS:

We have collected approximately 450 human sera from the Eastern Health District of Baltimore, of which 300 have been screened by IFA for antibodies to HTNV. These sera originate from a sexually transmitted disease clinic, and many are patients residing in locations within study areas from which rats and other mammals are being collected. Human sera are being collected with the cooperation of Dr. David Glasser, Maryland Department of Health and Mental Hygiene, and the age, sex, and address to block number is available for the majority. We have intentionally refrained from collecting data that can identify individual sera with patient name or exact address.

Overall 37 (12.3%) of the 300 human sera have antibody titers $\geq$ 32. The majority of these (N = 28) have titers in the range of 1:32, but nine have titers > 128 to prototype HTNV (Fig. 8f). The distribution of patient ages, sex, and associated highest titers is shown in Table 7. Although the numbers are too small to allow analysis of age or sex specific prevalence rates, they do suggest that humans within Baltimore are infected with HTNV. Human titers $\geq$ 128 have only rarely been reported from North America, and these preliminary findings suggest that the widespread infection of rodents in Baltimore may be mirrored in the human population.

At the present time it is impossible to state that the presence of antibody is related to infection acquired within Baltimore, or even within the United States, or that infection results in any clinical illness. However, the ages of some individuals with high titers make it unlikely their infections were acquired outside the United States. We cannot provide histories of any seropositive individual and detailed epidemiological studies are needed. We are attempting to elicit the aid of physicians in the nephrology and cardiology clinics at Johns Hopkins Hospital in the collection of sera from patients

Table 7.

Age, sex and prevalence of antibodies to HTNV in humans tested from Baltimore

Sex	Age	N	% ≥ 32	Highest titer
Male	< 19	25	8.0	128
	20-24	54	14.8	256
	25-29	48	10.4	128
	30-34	26	11.5	32
	35-39	18	16.7	32
	> 40	8	25.0	64
Female	< 19	38	13.2	512
	24-24	34	8.8	128
	25-29	26	7.7	64
	30-34	10	20.0	32
	35-39	6	33.3	2048
	< 40	5	0.0	32
Total		298*	12.3	2048

* Two individuals with age or sex unknown

presenting with symptoms compatible with mild forms of HFRS. We plan to continue our collection of sera from the EHD and will report an analysis of the geographical distribution of patients at a future time. These results are based on IFA serology alone and attempts to confirm them by PRN against various isolates of HTNV, are in progress.

DISCUSSION

The urban rat is the most significant reservoir of HTNV in Baltimore. Although we have not sampled all mammalian species to the same extent, we believe that the common occurrence, high rate of infection, and peridomestic habits of this animal make it the major reservoir species in the maintenance of HTNV in Baltimore. Infected rats have been found at each location sampled in the city, demonstrating the potential for infection to occur in any habitat that supports this species. We further suggest that the widespread distribution of infected rats, taken in conjunction with the limited extent of rat movement in urban environments, indicates that HTNV has been enzootic in urban rats in Baltimore for a long period of time. Our findings are not consistent with the hypothesis proposing recent introduction of HTNV via international shipping.

Other species of mammals (Mus, Felis, and Peromyscus) show some evidence of infection with HTNV but both prevalence rates and IFA antibody titers are significantly lower than rates observed in rats (Table 3; Fig 3). We cannot be certain that low IFA titers in mice are specific to HTNV, because frequently low titered sera fail to neutralize prototype Hantaan virus. Limited evidence obtained with cross IFA tests suggests that these species are infected with virus more closely related to rat strains of HTNV than mice strains (PH virus).

The exception to other small mammals is Microtus pennsylvanicus. This species had an antibody prevalence rate over 20% and end-point titers were most frequently greater when tested against PH virus than prototype Hantaan. It is likely that Microtus carries a virus in Baltimore that is distinct from that found in urban rats. The possibility that other small mammals are infected with a virus similar to PH is being examined.

In urban parklands we have found rats which are smaller and have lower antibody prevalence rates and titers compared with animals from residential urban locations. We have already mentioned that these findings may be in part due to differences in age structure and survivorship in these two populations. A second alternative is the possibility that these rats are infected with a related virus that is distinct from that of urban rats and more closely related to (Prospect Hill). Park rats share habitat with Microtus, Peromyscus, and Mus and it is possible that PH or similar virus is being passed between rats and mice. A third alternative, based on habitat differences, is that parklands provide fewer and poorer resources and therefore support a smaller and less closely packed rat population. These conditions might decrease rat-rat contact and transmission of infectious agents, although in residential locations we have seen no clear indication that prevalence of antibody is positively correlated with increasing rat population density as measured by rat trap success. These differences in results were unexpected and have not been adequately explained and clearly require more attention.

Our observations on the association of increasing antibody prevalence and titer with rat weight may be of value in gaining insight into patterns of HTNV transmission in rat populations. The implications of these findings are discussed in detail in the attached reprint. We hope that additional serum obtained

Table 3

Summary of species tested for antibodies to HTNV from Baltimore

Species	N	N ≥ 32	% ≥ 32*	Highest Titer
Carnivora				
Canis familiaris	164	2	1.2	32
Felis catus	649	42	6.5	2048
Rodentia				
Rattus norvegicus	414	204	49.3	32,768
Mus musculus	341	45	13.2	512
Microtus pennsyl.	67	13	26.0	512
Peromyscus leuc.	31	3	12.5	128
Tamias striatus	9	0	-	< 32
Sciurus carolin.	25	0	-	< 32
Primates				
Homo sapiens	300	37	12.3	2048

* Denominator does not include recaptured individuals

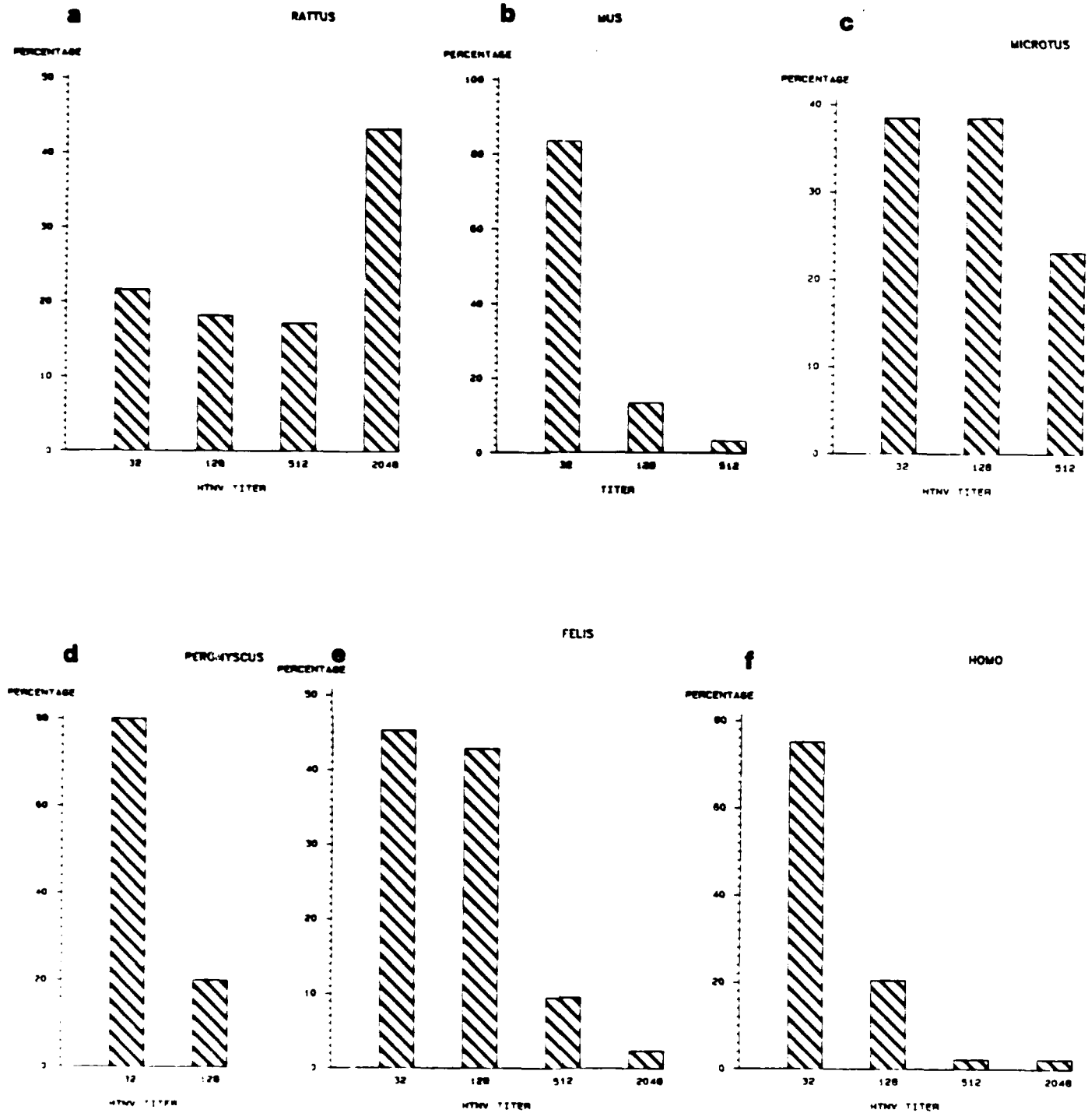

Fig. 8. Distribution of IFA titers to HTNV in seropositive individuals by species. a) Rattus. b) Mus. c) Microtus. d) Peromyscus. e) Felis. f) Homo.

from marked rats, will allow us to establish rat age at seroconversion. Laboratory experiments to examine the possibility of congenital transmission of HTNV in rats are planned and should both clarify the ontogeny of neonatal antibody responses to this agent, as well as help in the interpretation of the field data.

The finding that humans have significant levels of antibody against HTNV in Baltimore is not unexpected considering the wide range of species with evidence of infection. However, the relatively high antibody prevalence rate in the absence of clearly identifiable disease is surprising. Some of the critical questions that must be answered include: 1) is the antibody specific for HTNV, and if so, against what strain of virus? 2) if specific, are infections acquired in Baltimore? 3) are infections causing any clinical disease?. Detailed epidemiological studies will obviously be required to answer the majority of these questions, although we hope to answer question 1 during the course of the next year. If infection of humans with HTNV is occuring in Baltimore, the data we are currently collecting should prove invaluable in determining routes of transmission in this environment.

PROBLEMS AND RESOLUTIONS:

Two problems arose during the course of investigation over this first year. Both are now resolved. One area of difficulty entailed legal license to collect small mammals in urban parkland within the City of Baltimore. The second development concerned temporary cessation of Vero E-6 cell culturing activities regularly carried out by personnel at USAMRIID, as well as temporary reduction in available laboratory facilities at USAMRIID due to routine decontamination.

Collection of small mammals from parkland was temporarily curtailed during much of the spring. Despite permission granted by the Maryland State Department of Natural Resources to each member of this research effort to sample the mammal fauna of Baltimore City and surrounding counties, authorities within Baltimore City requested that additional conditions be met to assure public safety during our trapping efforts. Pursuant to these requests, the Johns Hopkins University has indemnified the City of Baltimore against all claims made during or due to our collection procedures, and has procured adequate insurance to cover any legal and/or medical costs incurred as a result of legal action taken by a third party against the Johns Hopkins University. A memorandum of agreement was drawn up by counsel for the University as well as the Office of the City Solicitor, and has been approved by all parties, allowing resumption of trapping efforts.

Problems with maintenance of the E-6 Vero cell line have been circumvented and virus isolation, as well as plaque reduction neutralisation assay of collected sera are being initiated. Facilities are now up to full operation, although a one month curtailment of laboratory use in the second of the two dedicated facilities is expected in late August.

CURRENT RESEARCH AND PLANNED RESEARCH:

The following items are still being researched or will be studied during the next year.

1. Continuation of MRR sampling of small rodents.

2. Characterization of infections occurring in small mammals from different habitats - IFA and PRN serology.

3. Isolation of viruses from small rodents - commencing Fall, 1985.

4. Continue collection of human sera. PRN serology.

5. Initiation of laboratory study of potential for transplacental transmission of HTNV. Includes description of antigen distribution in embryonic and fetal tissues of rats.

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