HUMAN ENTEROVIRUSES IN ANIMALS AND ARTHROPODS IN THE CENTRAL PHILIPPINES

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INTRODUCTION

Enteroviruses are known to be widely distributed in human beings (Beran *et al.*, 1967), lower animals (Kalter, 1958) and birds (Plummer, 1965), especially in the tropics. Incomplete classifications of the thousands of strains which have been isolated worldwide have indicated that these viruses are quite specific in host species. However, on several occasions, human types, ie, polioviruses, coxsackieviruses and echoviruses, have been reported from lower animals. In all except a few reports of coxsackievirus infections and one possible clinical infection in swine by echovirus type 8, these recoveries have been associated with inapparent infections. Further studies on the possible roles of lower animals in the transmission of enteroviral infections to man are definitely needed.

In this laboratory, human enterovirus strains were not found in rectal swabs from 184 immature swine, but neutralizing substances were found in low to moderate titer (1 : 16 - 1 : 256 *vs* 100 $TCID_{50}$) in normal porcine sera against 17 of 18 human enterovirus types tested. Piglets experimentally fed echovirus types 1, 6, 7, 9, 11 and 13 were found to shed viable virus for 1 or more days in the case of types 6 and 13 (Gregorio, 1969).

Polioviruses have rarely been recovered from animals. In 1958, Koprowski reported the isolation of type 1 virus from a calf, accompanied by specific antibody rise. In 1959, Sommerville reported several isolations of type 1 virus from droppings and intestinal contents of a budgerigar. Experimental attempts to infect cattle with polioviruses have been unsuccessful (Sabin and Fieldsteel, 1955), and Sommerville (1959) was unable to elicit experimental infection or antibody production in healthy budgerigars.

Coxsackieviruses appear to infect a variety of animals much more readily than do polioviruses, and frequently with greater pathogenicity. Lundgren *et al.*, (1968) recovered 136 strains of coxsackieviruses types B1-B3 and B5 from 38 Beagle dogs sampled several times over 1 year. These dogs, maintained in experimental animal kennels, remained clinically normal throughout the study. Later, Lundgren *et al.*, (1970) isolated coxsackieviruses type B1 from other healthy dogs in the same and other kennels. Verlinde *et al.*, (1958) in the Netherlands, reported 18 cases of mild febrile illness in children in 6 families associated with contact with sick coughing pigs in a swine show. Coxsackievirus type A5 was recovered from the lungs of 2 of the sick pigs and serological responses to this virus but no recoveries of virus were reported in the affected children. In experimental studies, the disease was reproduced in healthy swine, and viral recovery and serologicalr esponse were obtained in the laboratory cases. Makower and Skurska (1957) have reported the isolation of coxsackievirus type A5 from the brain of a fox in Poland.

Echoviruses have occasionally been recovered from animals. In 1956, Moscovici *et al.*,(1959) in Italy recovered an enterovirus

from a young pig suffering from diarrhoea; the agent was later shown to be neutralized by echovirus type 8 antisera. Pindak and Clapper (1964) recovered 29 strains of echovirus type 6 from clinically healthy Beagles in an experimental animal kennel and later Lundgren *et al.*, (1968) recovered 22 strains of the same virus from healthy dogs in the same colony. Lundgren *et al.*, found no serological response to the echovirus infections in these dogs. O'Connor and Morris (1955) recovered Coxsackievirus type A4 from the blood of one of 18 live trapped cottontail rabbits.

Numerous experimental animal studies have been performed using human enteroviruses. Successful infections have been reported with polioviruses in monkeys (Landsteiner and Popper, 1909), chimpanzees (Howe and Bodian, 1941), gorillas (Guilloud, 1966), orangutans (Guilloud, 1966), cotton rats (Armstrong, 1939), mice (Koprowski *et al.*, 1954), hamsters (Mayer *et al.*, 1952), and bats (Reagan *et al.*, 1952); with coxsackieviruses in suckling (Dalldorf and Sickles, 1948) and cortisone treated adult mice (Kilbourne and Horsfall, 1951), monkeys (Wenner *et al.*, 1960), chimpanzees (Melnick and Kaplan, 1953), and possibly Beagle dogs (Lundgren *et al.*, 1971); and with echoviruses in monkeys (Lou and Wenner, 1962), chimpanzees (Itoh and Melnick, 1957), rabbits (O'Connor and Morris, 1955), and dogs (Pindak and Clapper, 1966). Pathogenicity varied widely with types of viruses and routes of inoculation. Contact transmission of polioviruses from experimentally inoculated to healthy monkeys has been reported to be very limited (Craig and Francis, 1958).

Neutralizing antibodies or substances against human enteroviruses have been found frequently in a variety of animal sera. In tests of several hundred sera reported in the paper of Bartell and Klein (1955) and the summary paper of Gelfand, (1961) positive findings have been most frequent in cattle, in which passive transfer through colostrum to calves has been shown. Positive findings have also been reported occasionally in swine, rarely in horses, not in sheep, rarely in chickens, rarely in dogs, not in cats, rarely in rats, in one naturally infected cottontail rabbit, in one fox, in one chipmunk, in one marmoset but not in a variety of other small mammals and birds. Mc Ferran *et al.*, (1968) have shown evidence that the neutralizing substances against polioviruses in cattle are not specific antibody.

Human enteroviruses have been recovered repeatedly from several arthropods. Both flies and cockroaches have yielded polioviruses and evidence has been presented that polioviruses may replicate in at least one species of flies. Coxsackieviruses and echoviruses have been recovered from flies but have not been shown to replicate in them (Horsfall and Tamm, 1965).

The most comprehensive epidemiological study of human enteroviruses in animals appears to be that of Grew *et al.*, (1970) in Costa Rica in 1968. In a longitudinal study of 9 households, 56 persons and 46 domesticated animals were sampled repeatedly. Poliovirus type 1 and coxsackievirus A20 were recovered from both human and associated animal stools, and coxsackievirus A9 was found in animals but not human beings during the study.

MATERIALS AND METHODS

Animals and arthropods tested

Dogs: Dog carcasses submitted to the rabies diagnostic section of this laboratory during 1967-1969 were sampled for enteroviral studies if the intestines were intact and in fresh condition. Approximately 1 gram sections of caecum and, in most cases, also

ileum and rectum with their contents were removed and stored in separate vials at –70°C until tested. When available, sera extracted from clotted heart blood were collected. A total of 149 carcasses of dogs originating from both rural and urban communities in the central Philippines were sampled, with caeca from all, ileum from 109 and rectum from 105 animals.

Bats: Bats netted in free flight were exsanguinated by cardiac puncture. Caeca with their contents were collected at necropsy; both sera and caecal samples were stored at –70°C. A total of 458 bats collected from 5 towns in Negros Oriental were examined during 1965-1969.

Rats and shrews: Live trapped rats and shrews were exsanguinated by cardiac puncture. Sera and caecal samples were collected and stored as for bats. A total of 402 rats and 9 shrews collected from 3 communities on Negros Island were examined during 1967-1969.

Houseflies: *Musca domestica* houseflies were collected on each of 2 days from urban homes, a slaughterhouse, a public market and a farm by attracting them to food and then stunning lightly. A total of 2,117 flies were collected with forceps, classified by date and source into chilled test tubes, and then stored at –70°C. At the time of laboratory assay, they were separated into pools of 25 each by date and source of collection.

Cockroaches: *Periplaneta americana* cockroaches were hand caught with gloves from houses in 3 urban sites, placed into a large bottle, and frozen at –70°C. A total of 131 cockroaches were live captured. Prior to laboratory study, the heads, wing sheaths and legs were severed and the frozen bodies were used for assay.

Skinks: Household skinks (scincidae) of 2 species, *Cosymbotus platyurus* and *Hemidactylus frenatus* were hand caught, with or without stunning as needed, from houses in 3 urban sites. The live skinks were placed into a large bottle, and euthanized and preserved by placing at –70°C. Prior to assay, the carcasses were thawed, cut open and the intestines removed for study.

Specimen preparation

At the time of laboratory assay of specimens, these were triturated in chilled mortars with pestles to prepare 20% suspensions in Hanks basic salt solution (BSS) (Melnick, 1955) with antibiotics. Excised intestinal portions were ground with their contents. Entire houseflies were ground in pools; the bodies of cockroaches were prepared singly. The suspensions of specimens were lightly centrifuged and supernatant fluids were inoculated into cell cultures or stored at –70°C until tested.

Cell cultures

Primary monkey *(Macaque cynomolgus)* renal cell cultures prepared in roller tubes essentially by the method of Youngner (1954) were used in all viral isolations and serological tests. Lactalbumin enzymatic hydrolysate medium with 5% calf serum was used during the preinoculation growth phase; just prior to inoculation, cultures were changed to serum free Eagles medium with added cystine (Dubes and Wenner, 1957).

Virological and serological studies

Cytopathic agents were identified using intersecting pools of standard typing sera* against 100 $TCID_{50}$ of the test viruses. Serum antibody levels from animals yielding viruses were measured by neutralization tests in cell cultures using 4 fold serial serum dilutions

* Microbiological Associates, Inc., Baltimore, Maryland, U.S.A.

against 100 $TCID_{50}$ of the homologous viruses.

RESULTS

Fourteen strains of human enteroviruses were recovered from 11 animals and one pool of houseflies tested. The species collected and those yielding viruses are shown in Table 1. Poliovirus type 1 was the only type recovered from dogs; the 5 positive animals came from 2 cities and 3 towns. One dog yielded virus in caecum, ileum and rectum; in all other cases, only a single portion of the intestine was positive. Poliovirus type 1 was recovered from one rat trapped in a hospital compound.

Coxsackievirus type B3 was encountered in one *Cynopterus brachyotis bat*. This colonial bat commonly inhabits trees near areas of human habitation and utilizes fruits and flowers for food. This virus was also recovered from 2 *Rattus norvegicus* rats. These rats commonly live in small groups in or near areas of human habitation. The 2 positive rats were trapped in a hospital compound. Coxsackievirus type B3 was also recovered from one of 55 pools of 25 houseflies each, caught in a public market in a lower class urban area and Coxsackievirus type B6 was recovered from one *Rattus norvegicus* trapped at a residence in the same area. A single echovirus, a strain of type 1 was recovered from an *Emballonera* sp. bat. This colonial

Table 1
Animals assayed for enteroviruses and number yielding viruses.

Animal	Species	No. of Animals	No. Positive	% Positive
Dogs	*Canis familiaris*	149	5	3.4
Bats	*Cynopterus brachyotis*	187	1	0.5
	Rousettus amplexicaudatus	64	0	0
	Macroglossus lagochilus	61	0	0
	Emballonera spp.	42	1	2.4
	Scotophilus temminckii	40	0	0
	Ptenochyrus jagori	36	0	0
	Mineopteros spp.	10	0	0
	Haplonycteris spp.	8	0	0
	Hipposideros diadema	5	0	0
	Harpyonycteris whiteheadi	3	0	0
	Nyctemine spp.	2	0	0
Rats	*Rattus norvegicus*	156	4	2.6
	Rattus mindanensis	65	0	0
	Unidentified *Rattus* spp.	190	0	0
Shrews	*Suncus occultidens*	9	0	0
Flies	*Musca domestica*	2117 (84 pools)	1	1.2% of pools
Cockroaches	*Periplaneta americana*	131	0	0
Skinks	*Cosymbotus platyurus* and *Hemidactylus frenatus*	72	0	0

Table 2
Results of laboratory assays for enteroviruses in animals.

Animals Yielding Enteroviruses	Species	Age	Place of Collection	Positive Specimens	Virus Type	Homologous Antibody Titer*
Dog A-2	*Canis familiaris*	Adult	Rural town	Caecum	Polio type 1	1 : 8
Dog Du-44	*Canis familiaris*	Adult	City	Ileum	Polio type 1	1 : 32
Dog B-7	*Canis familiaris*	Juvenile	City	Rectum	Polio type 1	-
Dog Z-2	*Canis familiaris*	Adult	Rural town	Ileum, caecum, rectum	Polio type 1	-
Dog SJ-2	*Canis familiaris*	Adult	Rural town	Caecum	Polio type 1	-
Bat A-32	*Cynopterus brachyotis*	Adult	Rural town	Caecum	Coxsackie B-3	-
Bat A-183	*Emballonera* sp.	Adult	Rural town	Caecum	Echo type 1	-
Rat R-156	*Rattus norvegicus*	Adult	Residence, City	Caecum	Coxsackie B-6	1 : 24
Rat R-197	*Rattus norvegicus*	Adult	Hospital, City	Caecum	Polio type 1	-
Rat SDR-17	*Rattus norvegicus*	Adult	Hospital, City	Caecum	Coxsackie B-3	-
Rat SDR-55	*Rattus norvegicus*	Adult	Hospital, City	Caecum	Coxsackie B-3	-
Flies, Pool DF-5	*Musca domestica*	Adult	Market, City	Whole flies	Coxsackie B-3	-

* Where titers not listed, determinations were not made.

cave dwelling bat is insectivorous. Enteroviruses were not recovered from cockroaches or skinks captured in lower and middle class urban residences.

Only 3 sera were available for titration against the homologous viruses. All were positive in low titer for neutralizing substances. The types of enteroviruses recovered in relation to age and source of the hosts, tissues yielding viruses, and antibody levels determined are shown in Table 2.

DISCUSSION

The relative frequency of recovery of polioviruses from dogs in this tropical area is quite different from findings reported in temperate countries. Four of the five positive dogs yielded virus in only one of the 3 tissues examined. In only one animal were caecum, ileum and rectum all positive; 2 yielded virus in caecum only and one each in ileum and rectum only. This limited presence of the viruses within the digestive tracts of most dogs, coupled with low serum neutralization titers indicated a low infectivity of the virus for this host.

The recovery of human enteroviruses from bats and rats represent the first reported findings in these hosts. The finding of coxsackievirus in one of 111 *Cynopterus brachiotis* frugivorous bats netted in one town during a one month period, with serological evidence of active infection in this animal, was considered to be an interesting but apparently uncommon infection.

SUMMARY

Fourteen strains of human enteroviruses were recovered from specimens of the digestive tracts of 149 dogs, 458 bats, 402 rats, and

84 pools of houseflies. Included were 8 strains of poliovirus type 1, 4 strains of coxsackievirus type B3, and one strain each of coxsackievirus type B6 and echovirus type 1. The dog specimens were obtained from animals submitted for rabies diagnosis; the bats, rats and houseflies were all live captured. Serological tests against the homotypic virus on 2 dogs and one rat provided evidence that the presence of the human enteroviruses in these animals represented true infections. Enteroviruses were not recovered from any of 9 shrews, 131 cockroaches or 72 household skinks.

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