PARAMYXOVIRAL AND REOVIRAL INFECTIONS OF IGUANAS ON HONDURAN ISLANDS

Authors: Gravendyck, Marcus, Ammermann, Peter, Marschang, Rachel E., and Kaleta, Erhard F.

Source: Journal of Wildlife Diseases, 34(1) : 33-38

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-34.1.33

Journal of Wildlife Diseases, 34(1), 1998, pp. 33–38

PARAMYXOVIRAL AND REOVIRAL INFECTIONS OF IGUANAS ON HONDURAN ISLANDS

Marcus Gravendyck,[1,2] Peter Ammermann,[1] Rachel E. Marschang,[1] and Erhard F. Kaleta[1]

[1] Institute for Avian and Reptile Medicine, Justus-Liebig-University, Giessen, Germany
[2] Current address: Intervet GmbH, Tackweg 11, D-47918 Tönisvorst, Germany

ABSTRACT: Thirty-five free-ranging healthy spiny-tailed iguanas (31 *Ctenosaura bakeri*, 4 *C. similis*) and 14 green iguanas (*Iguana iguana rhinolopha*) were caught and held in captivity for 2 days. Blood was collected from all animals and their sera were evaluated for antibody titres against reptilian reoviruses, reptilian paramyxoviruses, and avian paramyxovirus-1 (PMV-1). Cloacal and pharyngeal swabs also were collected and examined for viral content by incubation on chicken embryo fibroblasts (CEF) and terrapene heart cells (TH-1). No virus was isolated from the pharyngeal and cloacal swabs on CEF and TH-1. Twenty-three (47%) of 49 sera samples tested positive for reptilian reoviruses by virus neutralization tests. Twenty (41%) of 49 samples had antibodies against one reptilian PMV isolate by virus neutralization tests and 3 (9%) of 34 by hemagglutination inhibition tests. No antibodies were detected against the other PMV isolate of reptilian origin nor against avian PMV-1. This is the first description of serum antibodies against reptilian reoviruses and PMV in wild iguanas.

Key words: Antibody titre, *Ctenosaura bakeri*, *Ctenosaura similis*, green iguana, *Iguana iguana rhinolopha*, paramyxovirus, reovirus, spiny-tailed iguana.

INTRODUCTION

The family Iguanidae belongs to the order Squamata and the Suborder Lacertilia. Both the genera *Iguana* and *Ctenosaura* belong to the Subfamily Iguaninae. Green iguanas (*Iguana iguana*) are found over a wide area of the South American continent, while the subspecies *Iguana iguana rhinolopha* is found north of Nicaragua in Central and North America. Spiny-tailed iguanas (*Ctenosaura* spp.) are found in a more restricted area in Central America and *C. bakeri* has only been described on the island of Utila. The growing awareness of the endangered status of these iguanas has prompted an increased interest in the biology and ecology of wild iguana populations. While the diseases and medical management of captive reptiles is a rapidly expanding specialty in veterinary medicine, diseases of free-ranging reptiles have received little attention. Presently, there are no studies on virus infections in free-ranging iguanas.

The first report of paramyxovirus (PMV) infections in reptiles was provided by Fölsch and Leloup (1976), who described an epidemic in a collection of fer-de-lance snakes (*Bothrops moojeni*) which died of neurological and respiratory disease. Clark et al. (1979) were able to isolate the virus and several reports on PMV infections of snakes followed (Jacobson et al., 1980, 1981, 1992; Ahne et al., 1987a). Serological studies revealed that the PMV isolated from snakes are distinct from mammalian PMV (Ahne and Neubert, 1989). Potgieter et al. (1987) demonstrated similarities between a PMV from an Ottoman viper (*Vipera xanthena xanthena*) and parainfluenza 2-antiserum by hemagglutination inhibition tests (HI). Studies on several PMV isolates by Blahak (1994, 1995) showed that PMV isolates from snakes are endogenous reptile viruses. Most of the isolates described in her studies appeared to be serologically related to avian PMV-7. One isolate, No. 1356/90 from a rattlesnake, showed more similarities with PMV-1 than PMV-7. Richter et al. (1996) also found three PMV isolates from snakes to be endogenous reptile viruses, and none of the isolates in their study showed antigenetic relatedness to known paramyxoviruses. So far, there are no reports of PMV epidemics in iguanas.

Reovirus-like particles, along with other viruses, were first detected by electron microscopy in a papilloma of a green lizard (*Lacerta viridis*) by Raynaud and Adrian

(1976). Further reports of reovirus infections and isolations in snakes followed (Jacobson, 1986; Ahne et al., 1987b; Vieler et al., 1994). Blahak et al. (1995) were the first to isolate two reoviruses from members of the Iguanidae. These isolates (Nos. 1523/93 and 1118/94) were both from green iguanas. Studies on the serological relationship between reptilian reoviruses and other animal reoviruses revealed no homologies to mammalian and avian reoviruses. A comparison of six reptilian reoviruses isolated from different species showed that the two isolates from iguanas belonged to two different serogroups (Blahak et al., 1995).

The objective of this study was to evaluate the occurrence of viruses and antibodies against PMV and reoviruses in wild green and spur-tailed iguanas on the Honduran islands of Utila and Roatán. The samples were collected in conjunction with the Utila spiny-tailed iguana conservation and research project (Köhler, 1995).

MATERIALS AND METHODS

All samples were collected on Utila and Roatán (Islas de la Bahía) between March and April 1996. The islands Utila and Roatán lie on the northern Hondurian coast (16°05′N, 86°51′W; 16°55′N, 86°25′W). Adult green iguanas and spiny-tailed iguanas were caught with specially prepared nooses on the ends of 2 to 4 m long sticks. The nooses were made of synthetic string (similar to shoe laces). The iguanas were caught by slipping the nooses over their heads and pulling the noose tight. The animals were kept in linen sacs for 2 days. Each animal was marked individually after capture to avoid re-sampling and then set free at the same place they were caught.

Up to 1.5 ml of whole blood per animal was collected from the ventral tail vein with a sterile needle (Samour et al., 1984), centrifuged at 1,000 × g for 3 min and serum was removed and frozen on wet ice. The collected serum was kept frozen on wet ice during transportation to the Institute of Avian and Reptile Medicine (Giessen, Germany) where it was stored at −20 C until further testing. Because of the small amounts of serum harvested (50–500 µl per animal) these were prediluted 1:8 with sterile physiological NaCl solution prior to testing. Nevertheless, we were not able to test all sera for antibodies against all viruses.

The pharyngeal and cloacal swabs were put into tissue culture medium (Basal Medium Eagle with Earle's salt solution; Seromed, Biochrom, Berlin, Germany) supplemented with 10% v/v tryptose phosphate broth, 0.1% v/v hepes buffer (Seromed), 0.005% v/v enrofloxacine (Baytril®, Bayer AG, Leverkusen, Germany), and 0.5% v/v nystatin (Moronal®, Heyden GmbH, Munich, Germany) and frozen on wet ice. The swabs were kept frozen on wet ice during transportation to our institute where they were then stored at −20 C until further testing.

After arrival at the Institute for Avian and Reptile Medicine, the swab samples were thawed, sonified (Branson sonifier B-15, Branson Sonic Power, Danbury, Connecticut, USA), and centrifuged at 1,000 × g for 5 min for removal of cell detritus. Chick embryo fibroblasts (CEF, prepared according to Schat and Purchase, 1989) and terrapene heart cells (TH-1, American Type Culture Collection, ATCC, Rockville, Maryland, USA; CCL 50) were infected with the supernatants. All cultures were incubated at 28 C and observed daily for cytopathic effects. After 14 days the cells were harvested, sonified, centrifuged and reinoculated onto CEF and TH-1 for a second passage.

Two reptilian PMV were used for our tests. The first (isolate No. 3319/95) was isolated from a pharyngeal swab of a healthy monitor lizard (*Varanus prasinus*) which was kept together with snakes during a PMV epidemic. This isolate cross reacted with avian PMV-7 serum in hemagglutination inhibition tests (M. Gravendyck, unpublished data). An additional reptilian PMV (isolate 1356/90), which was isolated from a rattlesnake (*Crotalis catalinensis*) was chosen because previous studies had shown that this virus cross-reacted more strongly with avian PMV-1 than with avian PMV-7 serum (Blahak, 1994). An avian PMV-1 also was used because of this cross-reactivity of avian and reptilian PMV. For this purpose strain F (Central Veterinary Laboratory, Weybridge, UK) was chosen, which is usually used as an avian reference strain for the measurement of antibodies against Newcastle disease virus (Asplin, 1952). Both reoviruses from iguanas (Blahak et al., 1995) were used as reference strains in this study. In 1995, a reovirus was isolated from a pool of eight young anoles (*Anolis carolinensis*), which died without any previous symptoms during quarantine (M. Gravendyck, unpublished data). This isolate (No. 1994-01/95) was taken as a third virus isolate for neutralization tests.

Antibody titers were determined by hemagglutination inhibition tests (HI) (β technique) and virus neutralization tests (VNT) (Beard,

TABLE 1. Number, sources, test system used, and references to Reoviruses and Paramyxovirus isolates from reptiles.

Virus	Test system	Isolate No.	Isolated from	Reference
ReoV	VNT[a]	1523/93	*Iguana iguana*	Blahak et al. (1995)
ReoV	VNT	1118/94	*Iguana iguana*	Blahak et al. (1995)
ReoV	VNT	1994-01/95	*Anolis carolinensis*	Unpublished data[d]
PMV-1	HI[b]	strain F	*Gallus domesticus*	Asplin (1952)
rPMV[c]	HI	1356/90	*Crotalis catalinensis*	Blahak (1994)
rPMV	HI/VNT	3319/95	*Varanus prasinus*	Unpublished data

[a] VNT = virus neutralization test.
[b] HI = hemagglutination inhibition test.
[c] rPMV = reptilian PMV.
[d] M. Gravendyck, unpublished data.

1989) (Table 1). Serum was heated to 56 C for 30 min prior to testing in order to inactivate non-specific inhibitors. For HI tests, 25 µl serum were placed in microtiter plate wells (Greiner, 96 U-Bottom Wells, Frickenhausen, Germany) and serial twofold diluted with sterile physiological NaCl solution up to 1:4,096. Antigen (25 µl) with four hemagglutinating units was added to each well and incubated at room temperature (25 C) for 30 min; 50 µl of 1% washed chicken erythrocytes (CRBC) was then added and the result was read after another 30 min incubation at room temperature. The absence of agglutination of CRBC was considered a positive HI response. A titer ≥16 was considered significant. Serum from specific pathogen free (SPF) chicken was used as a negative control in all HI tests. Chicken antiserum against strain F was used as a positive control in both the HI tests with strain F and with reptilian PMV isolate 1356/90. Rabbit antiserum against a PMV isolate from a snake from the same collection as the monitor lizard from which virus Nr. 3319/95 was isolated and which also cross-reacted with avian PMV-7 was used as a positive control for the HI tests with isolate 3319/95 (M. Gravendyck, unpublished data). No iguana sera were available as positive or negative controls. Controls were treated in the same way as the iguana sera prior to testing. For VNT iguana serum samples were again twofold diluted in microtiter plates (Falcon 3072 Microtest tissue culture plate, 96 Flat Bottom Wells, Becton Dickinson Labware Europe, Meylan Cedex, France). Virus suspension, containing 100 $TCID_{50}$ of virus was added to the serum (Table 1). After 2 hr incubation at 28 C freshly resuspended cells (CEF) were dropped into each well. The plates were read microscopically on day 14. A titer ≥16 was again considered significant.

RESULTS

Because the sera were prediluted only titers higher than 8 were detectable. The titers against reptilian reoviruses ranged from 8 to 64. Titers against the PMV from a monitor lizard (isolate No. 3319/95) measured by VNT ranged from 8 to 32 and by HI from 8 to 16. No antibodies were detected against the rattlesnake PMV isolate (1356/90) and the avian PMV-1 (strain F) tested by HI. Titers of 16 and higher were interpreted as virus specific positive reactions in all tests, lower titers were considered nonspecific reactions. Accordingly, five of 14 (36%) *I. iguana rhinovola,* 17 of 31 (55%) *C. bakeri,* and one of four *C. similis* had positive antibody titers against the reovirus 1523/93. Only two of 31 (6%) *C. bakeri* had positive titers against the reovirus 1118/94. No animal had positive antibody titers against the reovirus 1994-01/95 (Table 2). Six of 14 (43%) *I. iguana rhinolopha,* 12 of 31 (39%) *C. bakeri,* and two of four *C. similis* had positive titers against the monitor lizard PMV by virus neutralization tests. Only one serum of 12 (8%) *I. iguana rhinolopha* and two of 18 (11%) sera from *C. bakeri* had positive titers by hemagglutination inhibition tests (Table 2). HI titers were generally lower than the VNT titers, and all animals on which both tests could be carried out with VNT titers ≥16 had HI titers <16. Cytopathogenic virus was

TABLE 2. A serosurvey for reoviruses and paramyxoviruses in free-living *Iguana iguana*, *Ctenosaura similis*, and *Ctenosaura bakeri* from Honduran islands.

Virus	Test used	*I. iguana*	*C. bakeri*	*C. similis*	Total
Reovirus					
1523/93	VNT[a]	5/14 (36)[c]	17/31 (55)	1/4 (25)	23/49 (47)
1118/94	VNT	0/14 (0)	2/31 (6)	0/4 (0)	2/49 (4)
1994-01/95	VNT	0/13 (0)	0/24 (0)	0/4 (0)	0/41 (0)
Avian PMV-1					
Strain F	HI[b]	0/12 (0)	0/18 (0)	0/4 (0)	0/34 (0)
Reptilian PMV					
1356/90	HI	0/12 (0)	0/18 (0)	0/4 (0)	0/34 (0)
3319/95	VNT	6/14 (43)	12/31 (39)	2/4 (50)	20/49 (41)
3319/95	HI	1/12 (8)	2/18 (11)	0/4 (0)	3/34 (9)

[a] VNT = virus neutralization test.
[b] HI = hemagglutination inhibition test.
[c] Number positive (titer 16 and higher)/total tested (percent positive).

not isolated from any of the swabs during two 14 day passages on CEF and TH-1.

DISCUSSION

There are no reports of paramyxoviral infections in iguanas. Therefore, the detection of neutralizing and hemagglutination inhibiting antibodies in wild iguanas was not expected. The antibody titers ranged from 8 to 32; these titers do not indicate an acute paramyxoviral infection. The difference in the titers against the reptilian PMV measured by VNT and HI (isolate No. 3319/95) could result from the difference in the antibody types measured with these tests, or from the presence of nonspecific hemagglutins in the tested sera. These can be removed by treating the sera with washed CRBC, which was not done in this study because of the small amount of serum available from most of the animals tested (50 to 500 μl). This could theoretically have lowered the HI titer measured because of nonspecific hemagglutinating activity of the sera. However, according to Kaleta et al. (1973), nonspecific hemagglutins are seldom in chicken sera and, when present, are only found in very low titers. These can in part be removed by heat inactivation, which was done with the sera tested. Whether this is true for reptile serum as well is unknown.

No controls of confirmed negative and positive iguana sera were available. Chicken and rabbit sera were used as positive and negative controls in the HI tests to determine unknowns. The sera from the animals tested were run together, so that the animals which tested negative were considered negative controls, but no positive control was possible. All of the animals tested were examined clinically on capture and found to be healthy. There was no evidence for an acute viral disease in any of the animals. The determination of a cut-off for what is considered sero-positive for exposure to PMV in reptiles is difficult. Some authors (Jacobson et al., 1991) consider 20 or higher positive for PMV in reptiles while the Council Directive 92/66/EEC (1992) states that 16 should be considered positive for Newcastle disease virus in chicken. Further studies are necessary to study the role nonspecific inhibitors play in reptile sera and to determine specific cut-offs for various viruses.

Antibodies were not detectable against PMV-1 related reptilian PMV or avian PMV-1. However, the small sample size does not allow final conclusions on the presence of PMV-1 related reptilian PMV infections in these populations.

The fact that no virus isolation was possible could be due to the status of the infection in the tested animals. It is possible that the iguanas simply were not shedding virus at the time of sampling.

Although individual reports of reovirus isolations in captive iguanas exist, no reoviruses have yet been isolated from free-ranging iguanas nor are there reports of investigations on the prevalence of reovirus infections in reptiles. So far, it is not possible to connect reovirus infections in reptiles with a specific disease. Raynaud and Adrian (1976) described reovirus-like particles in a papilloma, but this is the only report of skin lesions combined with reovirus infections. Vieler et al. (1994) reported a reovirus infected snake with neurological dysfunctions. In addition, one reovirus was isolated during a PMV-epizootic in a snake collection (Blahak et al., 1995). Reoviruses have been isolated after the sudden death of animals which were introduced into a reptile collection (Blahak et al., 1995), but no typical pathological findings have been reported for reptiles with reovirus infections. It seems that these viruses, parallel to reovirus infections in birds, may be facultative pathogens, such as in stress situations (Heffels-Redmann and Kaleta, 1992). Chicks are often susceptible to reovirus infections; the *I. iguana* (1523/93) and the *A. carolinensis* (1994-01/95) reoviruses were both isolated from young animals. Transmission studies are needed in order to clarify the role reoviruses play in disease etiology in reptiles. In contrast to the fact that there are only three serotypes of mammalian reoviruses (Joklik, 1981) there is a great variety of different serotypes in avian reoviruses when tested with polyclonal antibodies (Heffels-Redmann and Kaleta, 1992). Studies with monoclonal antibodies against avian reoviruses showed that only one of three tested neutralizing antibodies was type-specific, the others were only group-specific (Meanger et al., 1995). The differing titers against the various reoviruses used in this study may be due to serological differences between iguana reoviruses.

LITERATURE CITED

AHNE, W., W. J. NEUBERT, AND I. THOMSEN. 1987a. Reptilian viruses: Isolation of myxovirus-like particles from the snake *Elaphe oxycephala*. Journal of Veterinary Medicine B 34: 607–612.

———, I. THOMSEN, AND J. WINTON. 1987b. Isolation of a reovirus from the snake, *Python regius*. Archives of Virology 94: 135–139.

———, AND W. J. NEUBERT. 1989. Antigenic relationship between three pleomorphic RNA viruses isolated from different snakes. Herpetopathologia I: 65–72.

ASPLIN, F. D. 1952. Immunization against ND with a virus of low virulence (strain F) and observations on subclinical infection in partially resistant fowls. The Veterinary Record 64: 245–249.

BEARD, C. W. 1989. Serologic procedures. *In* A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth and J. E. Pearson (eds.). American Association of Avian Pathologists, Kendall/Hunt, Dubuque, Iowa, pp. 192–200.

BLAHAK, S. 1994. Untersuchung zum Vorkommen von Paramyxoviren bei Schlangen und Charakterisierung ausgewählter Isolate. Veterinary Medical Dissertation, Justus Liebig University, Giessen, Germany, 147 pp.

———. 1995. Isolation and characterization of paramyxoviruses from snakes and their relationship to avian paramyxoviruses. Journal of Veterinary Medicine B 42: 216–224.

———, I. OTT, AND E. VIELER. 1995. Comparison of six different reoviruses of various reptiles. Veterinary Research 26: 470–476.

CLARK, H. F., F. S. LIEF, P. D. LUNGER, D. WATERS, P. LELOUP, D. W. FÖLSCH, AND R. W. WYLER. 1979. Fer de Lance virus (FDLV): A probable paramyxovirus isolated from a reptile. Journal of General Virology 44: 405–418.

COUNCIL DIRECTIVE 92/66/EEC OF 14 JULY 1992. Introducing community measures for the control of Newcastle disease. Official Journal of the European Communities No. L 260/1.

FÖLSCH, D. W., AND P. LELOUP. 1976. Fatale endemische Infektion in einem Serpentarium. Tierärztliche Praxis 4: 527–536.

HEFFELS-REDMANN, U., AND E. F. KALETA. 1992. Reovirusinfektionen. *In* Krankheiten des Wirtschaftsgeflügels, Vol. 1, B. Heider and G. Monreal (eds.). Gustav Fischer Verlag, Jena, Stuttgart, Germany, pp. 525–551.

JACOBSON, E. R., J. M. GASKIN, C. F. SIMPSON, AND T. G. TERRELL. 1980. Paramyxo-like virus infection in a rock rattlesnake. Journal of the American Veterinary Medical Association 177: 796–799.

———, ———, D. PAGE, W. O. IVERSON, AND J. W. JOHNSON. 1981. Illness associated with para-

myxo-like virus infection in a zoologic collection of snakes. Journal of the American Veterinary Medical Association 179: 1227–1230.

———. 1986. Viruses and viral associated diseases of reptiles. Acta Zoologica et Pathologica Antverpiensia 79: 73–90.

———, G. M. GASKIN, J. P. FLANAGAN, AND R. A. ODUM. 1991. Antibody responses of Western diamondback rattlesnakes (*Crotalus atrox*) to inactivated ophidian paramyxovirus vaccines. Journal of Zoo and Wildlife Medicine 22: 184–190.

———, ———, S. WELLS, K. BOWLER, AND J. SCHUMACHER. 1992. Epizootic of ophidian paramyxovirus in a zoological collection: Pathological, microbiological, and serological findings. Journal of Zoo and Wildlife Medicine 23: 318–327.

JOKLIK, W. K. 1981. Structure and function of the reovirus genome. Microbiological Reviews 45: 483–501.

KALETA, E. F., O. SIEGMANN, H. LÜDERS, AND W. JANSSEN. 1973. Kinetik NDV-spezifischer Antikörper in Hühnern II. Nachweis, Bedeutung und Elimination von nicht-spezifischen Inhibitoren und Agglutininen in Hühnerseren. Avian Pathology 2: 153–167.

KÖHLER, G. 1995. Schutz- und Forschungsprojekt Utila-Schwarzleguan. Elaphe 3: 73–78.

MEANGER, J., R. WICKRAMASINGHE, C. E. ENRIQUEZ, M. D. ROBERTSON, AND G. E. WILCOX. 1995. Type-specific antigenicity of avian reoviruses. Avian Pathology 24: 121–134.

POTGIETER, L., R. E. SIGLER, AND R. G. RUSSELL. 1987. Pneumonia in Ottoman vipers (*Vipera xanthena xanthena*) associated with a parainfluenza-2-like virus. Journal of Wildlife Diseases 23: 355–360.

RAYNAUD, A., AND M. ADRIAN. 1976. Leisons cutanees a structure papillomateuse associees a des virus chez le Lezard vert (*Lazerta viridis*). Comptes Rendues Academie des Sciences Paris 283: 845–847.

RICHTER, G. A., B. L. HOMER, S. A. MOYER, D. S. WILLIAMS, G. SCHERBA, S. J. TUCKER, B. J. HALL, J. C. PEDERSEN, AND E. R. JACOBSON. 1996. Characterization of paramyxoviruses isolated from three snakes. Virus Research. 43: 77–83.

SAMOUR, H. J., D. RISLEY, T. MARCH, B. SAVAGE, O. NIEVA, AND M. JONES. 1984. Blood sampling techniques in reptiles. The Veterinary Record 114: 472–476.

SCHAT, K. A., AND H. G. PURCHASE. 1989. Preparation of cell cultures. *In* A laboratory manual for the isolation and identification of avian pathogens, 3rd ed., H. G. Purchase, L. H. Arp, C. H. Domermuth and J. E. Pearson (eds.), American Association of Avian Pathologists, Dubuque, Iowa, pp. 171–172.

VIELER, E., W. BAUMGÄRTNER, W. HERBST, AND G. KÖHLER. 1994. Characterization of a reovirus from a rattlesnake, *Crotalus viridis*, with neurological dysfunction. Archives of Virology 138: 341–344.

Received for publication 16 January 1997.