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The Crystal Structure of San Miguel Sea Lion Virus Type 5 and the Possible Association of Caliciviruses to Spontaneous Abortions in Terrestrial Animals.

Jeff Lounsbury, Andrew Karplus PhD., Alvin Smith DVM, PhD.

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John B. Hays — BRR Director — 23 Jun 00 Date

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ABSTRACT

Crystallizing a virus can be difficult and not always successful. Forty-eight salts, buffers, and precipitating agents were used in the hanging drop preparations in attempts to crystallize the San Miguel sea lion virus type 5 (SMSV-5). The first attempt resulted in 22 of the 48 solutions forming a precipitate. The second attempt was rerun at 25°C rather than 5°C but crystals were not detected and the project was ended. A second project was an attempt to associate caliciviruses with spontaneous abortions in terrestrial animals. The first of four steps, the fluorescent antibody technique was performed. The fluorescent antibody technique can be used to indicate caliciviral presence in tissue samples. Monoclonal antibodies are prepared by injecting mice with a known calicivirus. The immune system of the mice makes specific antibodies in response to the virus. These antibodies are then extracted and purified. The tissue samples are smeared on a microscope slide and the cells adhere through charge-charge interactions. Fluorescein isothiocyanate is a fluorescent tag that uses a carbon on a carboxyl group to bind to a nitrogen on an amino group of an antibody. Antibodies with tags then attach to viruses in the tissue samples. Fluorescent tags illuminate the monoclonal antibody bound to a specific epitope when viewed under a fluorescent microscope. Particular wavelengths excite the fluorescent tags so they can be seen; a fluorescent green indicates a positive test. Thirty-three batches of tissue samples were prepared and tested for caliciviruses. Sixteen gave a positive fluorescence indicating the presence of the virus.

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INTRODUCTION

This paper focuses on two different experiments. The first experiment was to crystallize a calicivirus, San Miguel sea lion virus type 5 (SMSV-5) in order to more accurately determine the surface structure of the virus. Caliciviruses have never been crystallized, but cryomicroscopy, and computer image-processing gave data to determine the surface structure to a 22Å-resolution [1]. X-ray crystallography has given better data and allowed for higher resolution of the surface structure of different viruses and could further aid in research for vaccines and/or cures. Several steps were involved in crystallizing the SMSV-5 virus. The calicivirus was grown and purified, then the hanging drop method was used in attempting to crystallize the virus.

The second experiment was an attempt to associate caliciviruses with spontaneous abortions in agricultural animals. The fluorescent antibody (FA) technique was used to detect caliciviruses in tissue samples viewed with a fluorescent microscope. Four different biological/biochemical interactions need to be described to understand why the FA technique works. First, how cells stick to microscope slides, second, how do fluorescent tags attach to antibodies, third, why are FA techniques used, and finally, how can the monoclonal antibodies specific for the caliciviruses be generated.

The second experiment follows four main steps. Step 1: Indicating the presence of caliciviruses with a labeled antibody using the FA technique. Step 1 was the only step of the experiment that was performed. Step 2: Tissue samples that showed positive FA results will be sectioned and viewed to pinpoint the exact location of the virus within the tissue. Step 3: Amplification of RNA from virus detected in step 2 using polymerase chain reaction will determine if the calicivirus genome is present in tissue sample. Step

4: Checking amplicons against the cDNA library. The nucleic acid sequence of the amplicon will be checked against recorded calicivirus sequences to determine if the amplicon came from a known calicivirus.

History of Caliciviruses. Caliciviruses can infect a wide range of hosts, as diverse as swine (*Vesicular exanthema* of swine), cattle (*bovine* calicivirus), sea lions, and humans (San Miguel Sea Lion Virus) [2]. Some clinical problems associated with caliciviruses are abortion, diarrhea, persistent respiratory failure, skin lesions, and lameness.

When caliciviruses were first discovered in 1932, they were first classified as Picornaviridae. Subsequently, a single structural capsid protein was discovered, and the classification was changed to a new family, Caliciviridae [1]. From the first outbreak of *Vesicular exanthema* of swine virus (VESV) in 1932 until 1959 when the calicivirus was reported to be eradicated, California's swine herds were the most drastically infected. Nearly one-half the swine herds in the state were found to carry the virus at one time. Additional studies in 1974 estimated that 2 percent of northern fur seals killed during harvest had lesions as a result viral infection [3]. The harvest of seals was good evidence that calicivirus-induced side-effects occurred and also prevalent in marine mammals [2]. Caliciviruses are being studied to find cures and/or vaccines to prevent future outbreaks in livestock that could once again lead to economic disasters.

How Antibodies are Visualized. Antibodies are soluble proteins made in response to foreign proteins, usually a bacterium or virus that has invaded the body. Antibodies can be seen due to a fluorescent dye bound to it. The fluorescent dye emits a fluorescent color after being excited. In 1941, Coons, Creech, and Jones were the first to

develop a FA technique capable of visualizing antibodies using a fluorescent microscope [4].

History of fluorescent Antibody Technique. The study of FA was stopped during World War II. Coons started his research again after the war, and made vast improvements on the technique. FA was shown to be sensitive enough to distinguish among native antigens and contaminant antibodies. Fluorescein isothiocyanate, a green fluorescent dye was used in the experiments by Coons to show conclusively that the FA technique works. In 1954, FAs were shown to be applicable in diagnostic procedures, and different colored dyes were introduced. This discovery helped to pave the way for the different dyes and colors of dyes. Later development and studies produced a more stable form of fluorescein isocyanate which is used today [4].

Preparation of Monoclonal Antibodies. Understanding how FA technique works starts with understanding monoclonal antibodies, and monoclonal antibody preparation using an established protocol [6]. The immune response in mice makes the production of monoclonal antibodies for a particular antigen possible. Inject a mouse with the virus of interest. The mouse's immune system produces B-lymphocytes, which produce antibodies specific for one epitope on the virus. The B-lymphocytes concentrate in the mouse's spleen. The spleen was removed and the cells were isolated.

Myeloma cells can be fused with immune spleen cells using a polyethylene glycol solution to form hybridoma cells. Hybridoma cells are tumor cells that can proliferate as long as there is medium to grow on and will secrete specific antibodies. Hybridoma cells are then diluted and replicated to grow cell cultures from a single cell [6].

The monoclonal antibodies produced from the hybridomas are checked for their specificity using the ELISA against the known caliciviruses. The monoclonal antibody used in this experiment, tested positive for the most calicivirus types.

Impression Smears. Smearing a tissue sample on a microscope slide and fixing it with acetone is known as an impression smear. This works because cells bind to the slide through charge-charge interactions between dipolar cell membranes and the microscope slide surface. The contact between the cells and the slide does not change the conformation of the proteins being tested. Interactions between the slide and the cells are strong enough so cells will not be removed. The smear can then be examined using FA technique. Adding antibodies with attached fluorescence labels that bind to a specific epitope on a virus allows viral presence to be indicated when viewed through a fluorescence microscope.

Fluorescein Isothiocyanates Bind to Antibodies. Fluorescein isothiocyanates are free radicals that contain an unpaired electron. Flourescein interacts with antibodies by binding a carbon from a carboxyl group to nitrogen on an amino group from an antibody [4].

Stopping the reaction between the antibodies and antigens on the slide is very important, and is done with a quenching solution. Quenching solution changes the fluorescent color from green to orange for non-specific binding sites between the antibody and antigen. The quenching also helps heighten the contrast between specific staining and background [4].

Fluorescence. Scientists have known about fluorescence for a long time, but misunderstood how fluorescence worked. Fluorescence was thought to be the diffusion

or scattering of incandescent light. In 1852 G.G. Stokes determined that the true source of fluorescent light was the substance's ability to generate its own light. The color given off is determined by the unique color of light that the compound absorbs and subsequently emits [4].

Fluorescence Microscopes Detect Fluorescent Light. Fluorescence microscopes detect fluorescent light due to the different filters and darkfield condensers they contain. Short spectrum wavelengths must be used to excite the fluorescent tags. The filters in the fluorescent microscope are arranged in a certain order. A heat filter allows most ultraviolet light (UV) to pass through, protecting the other filters and the sample from excess heat. The next filter is blue in color causing the background in the microscope to become darker. The wavelength of light that goes through to excite the fluorescein is between 290 and 577 mμ. Exciter filters remove all visible light, but allows short light waves to pass. The darkfield condensers allow for high contrast, and provide additional protection for the observer from UV light and excess heat. The short wavelengths are absorbed by the fluorescein isothiocyanate, which then emits light visible to the observer [3].

MATERIALS and METHODS I

Purifying the Virus. The first part of the project was to grow and purify the virus. One calicivirus, San Miguel sea lion virus type 5 (SMSV-5), was grown in roller bottles using Vero (African green monkey kidney) cells. The virus was separated from the cell debris by centrifuging at 400Xg for 10 minutes. Then, trichlorotrifluoroethane was used to further purify the virus from the supernatant. The trichlorotrifluoroethane extracted supernatant was centrifuged at 21,000 rpms for 90 minutes to pellet the virus.

Trichlorotrifluoroethane extractions were accomplished by mixing Tris-buffer solution (TBS) with the cell debris pellet. Then, an equal volume of trichlorotrifluoroethane was added, vortexed for 30 seconds, and chilled in ice for 45 seconds. This was repeated 10 times, and finally centrifuged at 2000 rpm for 20 minutes at 4°C. This procedure separates any remaining cell debris from the virus and was repeated until little or no demarkation between the layers. The virus was vortexed, then centrifuged for 20 minutes at 21,000 rpm. The liquid layer was removed. The pellet was suspended in TBS, vortexed, then centrifuged at 2000 rpm for 20 min. The top layer containing the virus was removed, and two parts trichlorotrifluoroethane were added. The mix was vortexed, and centrifuged at 2000 rpm for 20 min; the top layer was removed and centrifuged at 29,000 rpm for 1 h and 30 min. The liquid layer was then discarded. The virus pellet was layered on top of a cesium chloride (CsCl) gradient, then centrifuged at 29,000 rpm for 15-30 h. The visible band of virus was removed from the CsCl, and TBS was added. The solution was centrifuged at 29,000 rpm for 2 h to remove any remaining CsCl [7].

Spectrophotometry. The calicivirus was run through spectrophotometry, using a Warburg program to obtain 3 mg/ml concentration, and a 99.37% purity.

RESULTS and DISCUSSION I

There were 48 different salts, buffers, and precipitants used to attempt crystallization of SMSV-5. The first 47 solutions are the most popular solutions in crystallizing nucleic acids [8]. These solutions have been shown to be good mediums for crystallizing nucleic acids, and also for crystallizing proteins. Solution number 48 was the medium for successfully crystallizing a rhinovirus, which is similar to caliciviruses.

These 48 different solutions at 5°C were not successful conditions for the virus to crystallize. Twenty-two conditions did allow the virus to form precipitate.

Table 1. Reservoir Solutions 1st attempt

Sample #	Salt	Buffer	Precipitant	Conc. Virus	Virus	Temp.
1	0.02 M Ca Chloride	0.1 M Acetate	30% MPD	3 mg/ml	2µl	5°C
2			0.4 M Na, K tartrate	3 mg/ml	2µl	5°C
3			0.4 M NH_4 phosphate	3 mg/ml	2µl	5°C
4		0.1 M Tris	2.0 M NH_4 sulfate	3 mg/ml	2µl	5°C
5	0.2 M Na citrate	0.1 M Hepes	40% MPD	3 mg/ml	2µl	5°C
6	0.2 M Mg chloride	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	5°C
7		0.1 M Cacodylate	1.4 M NA acetate	3 mg/ml	2µl	5°C
8	0.2 M Na citrate	0.1 M Cacodylate	30% 2-Propanol	3 mg/ml	2µl	5°C
9	0.2 M NH_4 acetate	0.1 M Citrate	30% PEG 4 K	3 mg/ml	2µl	5°C
10	0.2 M NH_4 acetate	0.1 M Acetate	30% PEG 4 K	3 mg/ml	2µl	5°C
11		0.1 M Citrate	1.0 M NH_4 phosphate	3 mg/ml	2µl	5°C
12	0.2 M Mg chloride	0.1 M Hepes	30% 2-Propanol	3 mg/ml	2µl	5°C
13	0.2 M Na citrate	0.1 M Tris	30% PEG 400	3 mg/ml	2µl	5°C
14	0.2 M Ca chloride	0.1 M Hepes	28% PEG 400	3 mg/ml	2µl	5°C
15	0.2 M NH_4 sulfate	0.1 M Cacodylate	30% PEG 8 K	3 mg/ml	2µl	5°C
16		0.1 M Hepes	1.5 Li sulfate	3 mg/ml	2µl	5°C
17	0.2 M Li sulfate	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	5°C
18	0.2 M Mg acetate	0.1 M Cacodylate	20% PEG 8 K	3 mg/ml	2µl	5°C
19	0.2 M NH_4 acetate	0.1 M Tris	30% 2-Propanol	3 mg/ml	2µl	5°C
20	0.2 M NH_4 sulfate	0.1 M Acetate	25% PEG 4 K	3 mg/ml	2µl	5°C
21	0.2 M Mg acetate	0.1 M Cacodylate	30% MPD	3 mg/ml	2µl	5°C
22	0.2 M Na acetate	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	5°C
23	0.2 M Ca chloride	0.1 M Acetate	20% 2-Propanol	3 mg/ml	2µl	5°C
24		0.1 Imidazole	1.0 M Na acetate	3 mg/ml	2µl	5°C
25	0.2 M NH_4 acetate	0.1 M Citrate	30% MPD	3 mg/ml	2µl	5°C
26	0.2 M Na citrate	0.1 M Hepes	20% 2-Propanol	3 mg/ml	2µl	5°C
27	0.2 M Na acetate	0.1 M Cacodylate	30% PEG 8 K	3 mg/ml	2µl	5°C
28		0.1 M Hepes	0.8 M Na, K tartrate	3 mg/ml	2µl	5°C
29	0.2 M NH_4 sulfate		30% PEG 8 K	3 mg/ml	2µl	5°C
30			2.0 M NH_4 sulfate	3 mg/ml	2µl	5°C
31		0.1 M Acetate	4.0 M Na formate	3 mg/ml	2µl	5°C
32		0.1 M Hepes	2.0 M Na formate	3 mg/ml	2µl	5°C
33		0.1 M Tris	1.6 M Na, K phosphate	3 mg/ml	2µl	5°C
34		0.1 M Acetate	8% PEG 8 K	3 mg/ml	2µl	5°C
35		0.1 M Hepes	8% PEG 4 K	3 mg/ml	2µl	5°C
36		0.1 M Hepes	1.4 Na citrate	3 mg/ml	2µl	5°C
37			2% PEG 400, 2.0 M NH_4 sulfate	3 mg/ml	2µl	5°C
38		0.1 M Citrate	20% 2-Propanol + 20% PEG 4 K	3 mg/ml	2µl	5°C
39		0.1 M Hepes	10% 2-Propanol + 20% PEG 4 K	3 mg/ml	2µl	5°C
40	0.05 M K phosphate		20% PEG 4 K	3 mg/ml	2µl	5°C
41			30% PEG 1500	3 mg/ml	2µl	5°C
42			0.2 M Mg formate	3 mg/ml	2µl	5°C
43	0.2 M Zn acetate	0.1 M Cacodylate	18% PEG 8 K	3 mg/ml	2µl	5°C
44	0.2 M Ca acetate	0.1 M Cacodylate	18% PEG 8 K	3 mg/ml	2µl	5°C
45		0.1 M Acetate	2.0 M NH_4 sulfate	3 mg/ml	2µl	5°C
46		0.1 M Tris	2.0 M NH_4 sulfate	3 mg/ml	2µl	5°C
47	1.0 M Li sulfate		2% PEG 8 K	3 mg/ml	2µl	5°C
48		10 mM	2% PEG 8000	3 mg/ml	2µl	5°C

Abbreviations: Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid. Buffers: Na acetate buffer pH=4.6, Na citrate buffer pH=5.6, Na Hepes buffer pH =7.5. % is defined as percent by mass [8].

The 22 conditions forming precipitates were used in a second attempted to crystallize the virus. The temperature was the only perimeter changed from 5°C to 25°C. The second attempt to crystallize the virus was also unsuccessful. Though no crystals were formed in these two different attempts this does not mean crystals cannot be obtained by using a different methods or other conditions.

Table 2. Reservoir Solutions 2nd attempt

Sample #	Salt	Buffer	Precipitant	Conc. virus	Virus	Temp.
6	0.2 M Mg chloride	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	25°C
9	0.2 M NH4 acetate	0.1 M Citrate	30% PEG 4 K	3 mg/ml	2µl	25°C
10	0.2 M NH4 acetate	0.1 M Acetate	30% PEG 4 K	3 mg/ml	2µl	25°C
13	0.2 M Na citrate	0.1 M Tris	30% PEG 400	3 mg/ml	2µl	25°C
14	0.2 M Ca chloride	0.1 M Hepes	28% PEG 400	3 mg/ml	2µl	25°C
15	0.2 M NH4 sulfate	0.1 M Cacodylate	30% PEG 8 K	3 mg/ml	2µl	25°C
17	0.2 M Li sulfate	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	25°C
18	0.2 M Mg acetate	0.1 M Cacodylate	20% PEG 8 K	3 mg/ml	2µl	25°C
20	0.2 M NH4 sulfate	0.1 M Acetate	25% PEG 4 K	3 mg/ml	2µl	25°C
21	0.2 M Mg acetate	0.1 M Cacodylate	30% MPD	3 mg/ml	2µl	25°C
22	0.2 M Na acetate	0.1 M Tris	30% PEG 4 K	3 mg/ml	2µl	25°C
28		0.1 M Hepes	0.8 M Na, K tartrate	3 mg/ml	2µl	25°C
30			2.0 M NH4 sulfate	3 mg/ml	2µl	25°C
31		0.1 M Acetate	4.0 M Na formate	3 mg/ml	2µl	25°C
36		0.1 M Hepes	1.4 Na citrate	3 mg/ml	2µl	25°C
38		0.1 M Citrate	20% 2-Propanol + 20% PEG 4 K	3 mg/ml	2µl	25°C
39		0.1 M Hepes	10% 2-Propanol + 20% PEG 4 K	3 mg/ml	2µl	25°C
40	0.05 M K phosphate		20% PEG 4 K	3 mg/ml	2µl	25°C
42			0.2 M Mg formate	3 mg/ml	2µl	25°C
45		0.1 M Acetate	2.0 M NH4 sulfate	3 mg/ml	2µl	25°C
46		0.1 M Tris	2.0 M NH4 sulfate	3 mg/ml	2µl	25°C
48		10 mM	2% PEG 8000	3 mg/ml	2µl	25°C

Abbreviations: Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid. Buffers: Na acetate buffer pH=4.6, Na citrate buffer pH=5.6, Na Hepes buffer pH =7.5, % is defined as percent by mass [8].

MATERIALS and METHODS II

Samples of lung, heart, liver, and other tissues were collected from aborted animal fetuses from around the country. The samples were sliced and smeared on a microscope

slide and allowed to dry. Then the slides were put in acetone for 20 minutes and allowed to dry. When the slides finish drying, they were submerged in a solution of FA buffer for 10 minutes. Then a fluorescein labeled monoclonal antibody in a 1:100 ratio with FA buffer was dripped onto the slides. After the labeled antibodies have been put on the slides the slides were placed in a humidified chamber at 37°C for 20 minutes. The slides were rinsed with FA buffer solution, then washed in FA buffer solution for 10 minutes. The slides were dipped in distilled water and air-dried. A quenching media, is a solution that is added to stop the reaction between the antibody and the antigen, preventing non-specific binding. A bright fluorescent green color indicates a positive test, and a fluorescent orange color indicates non-specific binding [6, 4].

Fluorescence Microscope. The fluorescence microscope is a light microscope with color filters that provide excitation light, and other filters that allow fluorescence to be viewed [3]. Regular microscope slides can be used with the fluorescent microscope. Fluorescent microscopes allow the fluorescence dye, when attached to the specific antibody, and subsequently attached to a specific epotipe to be viewed.

RESULTS and DISCUSSION II

Tissue samples of agricultural animals were tested for the possible presence of caliciviruses. The species of animal tissue samples that the lab received were bovine samples, porcine samples, equine samples, and ovine samples. The tissue age ranged from neo-natal to ten months old. The number of tissue samples per batch ranged from 1 to 7 tissues. Table 3 shows the species, age, and number of tissue samples that were used. Age and species are not found to be indicative of mortality from caliciviruses.

Table 3. Species and Age of Tissue Samples

Sample #	Tissue Batch	Tissue samples of species	Age	# of Tissues	.	Sample #	Tissue Batch	Species	Age	# of Tissues
1	30	Bovine	Fetal	3		17	9	Bovine	Fetus	1
2	33	Porcine	Fetus	5		18	6	Bovine	1 day	2
3	32	Equine	Fetal	4		19	11	Equine	Fetus	2
4	24	Bovine	Fetal	5		20	1	Ovine	Fetus	2
5	22	Bovine	Fetus	4		21	21	Ovine	Fetus	3
6	31	Bovine	Fetal	3		22	17	Ovine	Fetus	3
7	20	Equine	Fetal	7		23	10	Bovine	Fetus	3
8	21	Ovine	Fetus	5		24	29	Equine	Fetus	4
9	13	Bovine	Fetus	2		25	6	Bovine	Fetus	3
10	25	Bovine	Fetus	3		26	7	Equine	Fetus	5
11	27	Bovine	Fetus	3		27	4	Bovine	Fetus	4
12	26	Bovine	Neo-Natal	4		28	3	Porcine	Fetus	4
13	14	Bovine	1 day	4		29	16	Bovine	Fetus	4
14	8	Ovine	Fetus	3		30	28	Ovine	Newborn	4
15	15	Bovine	Fetus	4		31	19	Bovine	10 Mo.	3
16	12	Bovine	Fetus	4		32	18	Bovine	Fetus	5
						33	23	Equine	Fetus	4

Tissue Batch: The order in which tissue samples were tested. Species: Species of the animal tissue samples came from. Age: Age of the animal tissue samples came from. # of Tissues: The number of tissue samples each animal.

The low number of cases tested limits the information to be gained from this table. However, a fairly even number of males and females are displayed. Table 4 shows there is no distinction between the sex of fetuses or if caliciviruses have any part in spontaneous abortions in land animals.

Table 4. Male vs. Female

Sample #	Tissue Batch	Male	Female	Unknown		Sample #	Tissue Batch	Male	Female	Unknown
1	30	X				17	9		X	
2	33	X	X			18	6		X	
3	32		X			19	11	X		
4	24			X		20	1	X		
5	22			X		21	21		X	
6	31			X		22	17	X		
7	20	X				23	10		X	
8	21		X			24	29	X		
9	13			X		25	6	X		
10	25		X			26	7	X		
11	27			X		27	4		X	
12	26			X		28	3	X	3X	
13	14		X			29	16		X	
14	8		X			30	28	X		
15	15		X			31	19		X	
16	12		X			32	18	X		
						33	23		X	

Tissue Batch: The order in which tissue samples were tested. Male, Female: The sex of the animal, the tissue samples came from. Unknown: Sex not provided with sample.

Thirty-three batches of aborted animal fetal tissue samples were used for this experiment. Sixteen of the 33 samples showed a bright fluorescent green color indicating the presence of a calicivirus in the fetal tissue. Table 5 shows that almost half of the batches tested are positive, indicating caliciviruses are present in the tissues of the animals. The 16 samples that showed positive results were sent for sectioning for the second step of the experiment. The remaining samples, however, may still contain caliciviruses and should also undergo further tests. Because this test used one specific monoclonal antibody, the screening for the possible presence of other caliciviruses is incomplete.

Table 5. Positive vs. Negative

Sample #	Tissue Batch	Positive	Negative		Sample #	Tissue Batch	Positive	Negative
1	30		X		17	9		X
2	33		X		18	6	X	
3	32		X		19	11		X
4	24		X		20	1	X	
5	22		X		21	21		X
6	31	X			22	17	X	
7	20		X		23	10		X
8	21	X			24	29		X
9	13		X		25	6		X
10	25		X		26	7	X	
11	27	X			27	4		X
12	26	X			28	3	X	
13	14	X			29	16		X
14	8	X			30	28		X
15	15	X			31	19	X	
16	12	X			32	18	X	
					33	23	X	

Tissue Batch: The order in which tissue samples were tested. Positive: The tissue samples that have indicted a positive result for having caliciviruses present. Negative: The tissue samples that have indicted a negative result for having caliciviruses.

Table 6 gives an overview of all positive results and species of animals. There is no relationship between age and or sex of the animal fetus and positive test results.

Table 6. Positive Vs. Species Vs. Age Vs. Sex Vs. # of Tissues

Sample #	Tissue Batch	Tissue samples of species	Age	Male	Female	Unknown	Positive	# of tissues
1	31	Bovine	Fetal			X	X	3
2	22	Ovine	Fetus		X		X	5
3	27	Bovine	Fetus		X		X	3
4	26	Bovine	Neo-Natal			X	X	4
5	14	Bovine	1 day		X		X	4
6	8	Ovine	Fetus		X		X	3
7	15	Bovine	Fetus		X		X	4
8	12	Bovine	Fetus		X		X	4
9	5	Bovine	1 day		X		X	2
10	1	Ovine	Fetus	X			X	2
11	17	Ovine	Fetus	X			X	3
12	7	Equine	Fetus	X			X	5
13	3	Porcine	Fetus	X	3X		X	4
14	19	Bovine	10 mo		X		X	3
15	18	Bovine	Fetus	X			X	5
16	23	Equine	Fetus		X		X	4

Tissue Batch: The order in which tissue samples were tested. Species: Species of the animal tissue samples came from. Age: Age of the animal tissue samples came from. Male, Female, Unknown: The sex of the animal, the tissue samples came from. Positive: The tissue samples that indicted a positive result for having a calicivirus. # of Tissues: The number of tissue samples each animal.

CONCLUSION

The two different experiments were performed. One gave positive results and the other did not. The attempt to crystallize SMSV-5 was not successful. This negative result does not mean that using different methods, or different salts, buffers, and precipitating agents could not crystallize the virus.

The second experiment gave positive results. The FA technique indicated that caliciviruses were present in sixteen different batch samples of aborted animal tissue. The

next step of the experiment can be conduced to give more evidence that calicivurses are associated with spontaneous abortions in terrestrial animals.

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