A Case of Reye's-Like Syndrome in a 68-Day Old Infant: Water Damaged Home, Mold, Bacteria and Aflatoxins

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Abstract: *Introduction*: Reye's Syndrome is characterized by acute encephalopathy, hepatic injury accompanied with elevated serum ammonia, serum fatty acids, amino acids and triglycerides; hypoglycemia, prolonged prothrombin time, fatty infiltration of the liver, and mitochondrial pathology. We present an infant that died at 68 days of age with Reye 's syndrome. The infant and parents were exposed to airborne fungi, bacteria and toxic bioaerosols resulting from water intrusion in the home. The purpose of this study was to investigate and, if possible, to determine the cause of death of the infant with respect to the fungi, bacteria and their toxins present in the families water-damaged home.

Materials and Methods: Health and genetic history was done on the family (father, mother and two siblings). Environmental evaluation was carried out to identify airborne fungi and bacteria in the home. Clinical testing of the baby while in the hospital provided data on blood chemistry and EEG results. Mitochondrial studies on skin fibroblasts and skeletal muscle were carried out testing for functions of Complexes I-IV and mitochondrial DNA mutations. Light and E.M. microscopy were done on liver biopsy material. Immunoaffinity column and fluorometry were used to detect aflatoxins (B1, B2, G1 and G2) in liver autopsy material. The mother's breast milk and urine was tested for mycotoxins: trichothecenes, aflatoxins and ochratoxin.

Results: Medical and genetic history were negative for familial diseases similar to Reye's syndrome and for mitochondrial DNA mutations. Aortic and pulmonic valve abnormalities were observed. Environmental testing revealed the presence of elevated levels of several species of fungi and bacteria in the infant's bedroom and other rooms of the home. Clinical diagnostic tests of the infant revealed metabolic acidosis, elevated serum ammonia, triglycerides, pyruvic and lactic acids, serum alanine, and beta-hydroxybutyrate. Mitochondrial studies showed decreased function of complexes I-IV and the absence of known mutations associated with mitochondrial diseases. Microscopy (light and E.M.) of biopsies demonstrated the accumulation of glycogen in muscle and fatty droplets in the liver. Aflatoxins were detected in the infant's liver (2.1 ppb), and the mother's breast milk (15 ppb), while maternal urine was positive for trichothecenes (4.76 ppb) and ochratoxin (3.4 ppb).

Conclusions: After a review of the peer reviewed literature, we conclude that the infant died of a Reye's-Like Syndrome at the age of 68 days (all 22 cited criteria were met). Clinical and autopsy findings were consistent with this disease process. The valvular abnormalities found are associated with actinomycetes exposure. The medical and genetic histories were negative for any familial diseases of a similar nature. The infant had mitochondrial dysfunction of complexes I-IV, suggesting mitochondrial disease consistent with alflatoxin toxicity. The presence of aflatoxins in the liver supports the causal role of this mycotoxin in the illness of this infant. We have discussed the role of other factors in the indoor environment that may also lead to similar conditions.

Keywords: Aspergillus fumigatus and flavus, aflatoxins, trichothecenes, Reye's syndrome.

INTRODUCTION

Reye's syndrome is an acute encephalopathy with hepatic failure and a variety of abnormal clinical laboratory findings and symptoms These include respiratory distress, encephalopathy, metabolic acidosis, disturbance in consciousness, elevated serum ammonia, fatty acids, amino acids, and triglycerides, hypoglycemia, prolonged prothrombin time, fatty infiltration of liver, mitochondrial pathology, among others [1-5]. Suspected causations are viral (influenza, varicella), aspirin, mitochondrial failure, and toxins (insecticides, herbicides, aflatoxin B1, paint, and hepatotoxic mushrooms) [5-8]. Aflatoxin B1 has been implicated in acute, often fatal, childhood liver disease and encephalopathy New in Zealand [7], Czechoslovakia [8, 9], the USA [10], Thailand [11], and Venezuela [12]. Mitochondrial cytopathology has been postulated as a causative mechanism of the liver damage [13, 14]. Reported mitochondrial enzyme abnormalities include the urea cycle (carbamyl phosphate synthetase, ornithine transcarbamylase, (arginase), glutamate-oxalacetic transaminase, lactic dehydrogenase, glutamate dehydrogenase and isocitrate dehydrogenase [15-18]. Also, familial mutations affecting mitochondrial medium and short-chain 1-3-hydorxyl-acyl-CoA

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dehydrogenase in four patients from three families have been reported [19].

In this communication we describe the parents and a 68 day old female infant exposed to airborne fungi and bacteria in a water damaged home The infant was exposed *in utero* (second and third trimesters) and during 21 of the first 68 days of infant's life. She died at age 2 months and 8 days with clinical symptoms and laboratory findings consistent with Reye's syndrome and aortic and pulmonic valve abnormalities. We present this infant with respect to clinical diagnostic blood chemistry, liver pathology (electron and light microscopy), mitochondrial studies (mutations and enzymatic activity), and the identification of aflatoxins from the liver autopsy specimens, the presence of mycotoxins in maternal breast milk and urine, and family medical/genetic history. The data show the presence of aflatoxin in the liver of the deceased infant as well as decreased activity of mitochondrial enzymes An attempt is made to discuss the these findings with the role of aflatoxins in Reye-Like syndrome. In addition,

we describe two healthy children conceived and delivered after the parents removed themselves from the contaminated environment. It is apparent to us that the mold and bacterial toxins in the water-damaged home were the cause of the health problems of this family as well as the death of the infant girl.

THE INFANT AND FAMILY

The family was referred to Progressive Health Care Group for diagnosis and treatment of symptoms related to exposure to the mold and bacteria present in their water-damaged home.

The Infant

The infant (female) was delivered by Cesarean section at 36 weeks gestation as a result of pregnancy induced hypertension (pre-eclampsia), which was treated with magnesium sulfate. The mother was age 29, primigravida, blood type O, Rh-positive and had been experiencing a lingering flu-like illness for several weeks prior to delivery. Ultrasound revealed intrauterine growth retardation (birth weight = 4lbs, 5 ounces) and cardiac anomalies (tubular coarctation of the aorta, pulmonic valve dysplasia, pulmonary artery stenosis, a patent foramen ovale, and aortic abnormalities. The cardiac anomalies did not require treatment and did not contribute to the demise of the infant. At birth the child was noted to have hepatosplenomegaly, a "blue berry" rash and hemolytic anemia (reticulocytes = 32 to 40 % during the first week post birth), hypotonia and respiratory depression. The respiratory condition was believed to be secondary to hypermagnesemia as result of maternal infusion for preclampsia. The infant was fed mother's breast milk and Neocare24 kcal while hospitalized, stabilized and sent home.

At home the mother had difficulty with feeding, the child taking in only 8 to 10 ounces of infant Similac formula per day. Within a week the infant acted as if it was too painful to eat, had difficulty swallowing, and was observed by the parents drawing knees up to her chest with every effort at feeding. The child did not thrive, appeared ill and irritable and re-entered the hospital at 7 to 8 weeks of age. Clinical signs at the time of the second admission included being edematous, an enlarged liver, hypotonia, respiratory distress requiring intubation secondary to respiratory failure, recurrent hemolytic anemia, encephalopathy, and growth retardation.

Health of the Parents

The parents sought medical attention from one of the authors because of chronic health problems. Both parents were included along with other mold exposed patients in previous published communications that involved immunological alterations and abnormalities in QEEG [20, 21].

Briefly the mother's symptoms included the following: lingering flu-like illness, respiratory distress, chronic symptoms: severe fatigue, cognitive dysfunctions, frequent headaches, and eye irritation. Diagnostics revealed hypersensitivity pneumonitis with small airways obstruction, hyper-proliferation of the nasal mucosa, and rhinosinusitis. Neuropsychological testing revealed neurotoxicity and QEEG showed increased slow wave activity [20]. Immune tests revealed multiple markers of immune activation, and paradoxically, simultaneous features of suppression [21]. After remediation of the mold infested home, and subsequent vacancy, the mother gave birth to two additional children (see siblings below).

The father's symptoms included chronic headaches, distal numbness and tingling of extremities, dizziness with lightheadedness, eye irritation, sinus and throat irritation, skin rash, and wheezing. Diagnostics revealed severe small airway obstruction, and immune

parameters consistent with those found in the spouse [21]. Electrocortical and neuropsychological evaluation by one of the authors showed changes consistent with neurotoxicity [20].

Siblings

Two additional births occurred following death of the infant girl. The two males are presently 5 and 9 years of age with birth weights of 6 lbs 9 oz and 6 lbs 14 oz, respectively. Both had normal head circumference and body length at birth. The eldest was born approximately 4 months following clean-up and then vacancy of the infested home. He was diagnosed *in utero* with unilateral hydronephrosis that resolved the first few weeks following birth. According to the parents he is active and healthy with normal developmental mile stones. This child has asthma. The second boy was never in the contaminated home. He was diagnosed *in utero* with unilateral hydronephrosis that resolved by birth. Both boys have experienced normal develop-mental mile stones and are healthy at the present time.

Genetic Counseling and Familial History

Genetic family counseling was done with respect history of allergies, familial health and birth defects. The family health history did not show evidence of birth defects. Asthma and allergies were present in the maternal and paternal histories.

MATERIALS AND METHODS

Indoor Environmental Assessment

The new home was purchased in November 1998. The deceased infant was conceived prior to move-in and was exposed *in utero* to the microbial conditions of water-damaged indoor environments during the second and third trimesters. The water intrusion resulted from punctured water tubing infiltrating a wall cavity involving the master bath and other regions of the house. The hall bath tub drain was improperly installed, allowing flow under the tub. The microbial assessment involved visual inspection and sampling (wipe, vacuum dust, and bulk samples) and air sampling for bioaerosols (Zefon- Air-O-Cell) to identify airborne mold spores by molds as well as testing for mold and bacteria in dust samples on October 23, 2001. The inspection and sampling was done by Day Break Environmental Group, Phoenix, AZ [22]. Samples were sent to P & K Microbiology Services, Inc., Cherry Hill, N.J. for culture and identification of bacteria and fungi. Viable fungal samples were cultured on Potato Dextrose Agar (PDA) at 25 and 37 ^{o}C. The plates were read by a certified microbiologist between 5 and 10 days after collection, depending on rate of fungal growth. Viable bacterial samples were cultured on Trypticase Soy Agar with 5% sheep blood for aerobic (35 ^{o}C) and thermophylic (50 ^{o}C) bacteria. They were read by a certified microbiologist after 48 hours of incubation.

Neonate Health Status and Clinical Laboratory Testing

The health status of the infant was recorded and extracted from the medical records of the two hospitalizations. They were tabulated and compared to the health status of infants reported in the literature that had Reye's syndrome.

All clinical laboratory testing was performed at Carondelet Saint Joseph's Hospital, Tucson, Arizona. These included *in utero* sonograms and post birth diagnostics as follows: EEG, complete blood chemistry and blood counts, prothrombin and clotting times, urinalysis, TORCH, viral serology, blood enzymes, urine and serum lactic and pyruvic acid, and amino acids. Blood and urine microbial cultures were done over the course of the infant's two hospital stays.

Autopsy, Light and Electron Microscopy (E.M.)

The autopsy and pathology were performed at the Department of Pathology**,** University Medical Center, Tucson, Arizona. Gross autopsy examination was limited to the abdomen. Light and electron microscopy were performed on the liver and skeletal muscle. Sections of the liver were stained for glycogen using toluidine blue and trichrome for muscle microscopy. E.M. was performed to examine for the presence of glycogen and any cytopathology that might be present.

Mitochondrial Studies

Prior to death biopsies were taken from skeletal muscle and subcutaneous (fibroblasts) tissue for mitochondrial respiratory chain studies. Both tissues were sent to the Center for Inherited Disorders of Energy Metabolism (C.I.E.D.M.) at the Clinical Laboratory-Genetics Children's Hospital of Buffalo, Buffalo, N.Y. [23]. The fibroblasts required culturing at the University of Arizona, Health Sciences Center before being sent to C.I.E.D.M. Citrate synthase activity

was used to normalize data obtained for NADH dehydrogenase, succinate dehydrogenase, cytochrome C oxidase, succinate C reductase, and NADH cytochrome C reductase.

Infant and Maternal Mycotoxins

Mycotoxin concentrations in the infant liver and maternal breast milk and urine were determined by RealTime Laboratories, Carrollton, Texas as previously reported [24]. The infant liver was obtained from the University of Arizona, Health Sciences Center, Department of Pathology, Tucson, Arizona. Tissues were received frozen and in paraffin blocks. Samples of breast milk and urine from the mother were collected in sterile containers and sent by overnight FEDEX to Realtime Laboratories and extracted in methanol to test for the presence of aflatoxins, ochratoxin A and trichothecenes.

Briefly, the liver samples were emulsified (25 mg samples) in phosphate buffered saline (PBS, 0.9%) and reagent grade methanol (Sigma-Aldrich) in a 1:1 dilution. The cells and tissues were disrupted using silica beads (Sigma-Aldrich) for 1 minute at the speed of 45 rpm, heated at 65^{o} C for 15 minutes. Samples were centrifuged at 13,000 rpm for 2 minutes. 500 *µl* of cellular extract, placed in a glass tube, and further diluted in PBS prior to testing. At this stage all samples were free of paraffin. Samples were then applied to an AflaTest® column (VICAM, L.P, Watertown, MA) which contains specific monoclonal antibodies directed against Aflatoxins B1, B2, G1, and G2. Columns were washed twice with reagent grade water (Fisher Health Care, Houston, Texas). The samples were eluted from the column to remove the bound aflatoxin with reagent grade methanol. Fluorochrome developer (AFLATEST® Developer, VICAM) was added to the extracted methanol. All samples were read by fluorometry (Sequoia-Turner Fluorometer, Model 450), which was calibrated using standards supplied by VICAM. Spiked standards using known amounts of Aflatoxin B1, B2, G1, and G2 (Trilogy Analytical Laboratory Inc., Washington, Missouri) of human heart tissue were run as validation controls prior to testing (sensitivity of 95% and specificity of 92%). Known control concentrations of aflatoxins (50 ppb, 25 ppb, and 1.25 ppb were run with each test. The eluted solution was then read by fluorometry at 450 Angstroms. The lower and upper limits of detection are 1.0 and 23.0 parts per billion (ppb) respectively. Test results were plotted against the standard curve of the calibrators. Macrocyclic trichothecenes aflatoxins and ochratoxin A were identified as previously reported [24-26] (Figure **1**).

Figure 1: Flow Chart: This chart is designed to assist the readers of this paper in understanding and following the Materials and Methods section. The mold and bacteria in the home had to be identified by airborne spore counts cultures to identify mycotoxin producing species (Tables **1-3**). The evaluation of diagnostic tests performed on the infant (Table **4**) and the comparison of blood and urine chemistry findings to the published literature of Reye's Syndrome (Table **5**) and then extraction and identification of mycotoxins from the mother's breast milk and urine and those detected in necropsy samples of the infant's liver (Table **6**).

1) Indoor Environmental Assessment

A. Visual Inspection
B. Airborne Spores
C. PDA and TSA Cultures

2) Blood and Urine Chemistry of Infant

A. Torch Diagnostics
B. CBC and Blood Chemistry
C. Urine Chemistry

3) Literature Search for Blood Chemistry of Reye's

4) Identification of Mycotoxins

RESULTS

Indoor Environmental Assessment – Bacteria and Molds

The bacteria identified in dust samples from the Baby's Room (BR), Master Bedroom and Living are listed in Table **1**. Thermophilic bacteria (50^{o}C, including *Thermoactinomyces vulgaris*, were cultured revealing concentrations ranging from 3,107 to 7,536 CFU/g of dust. The Gram negative and positive bacteria cultured at 25 ^{o}C ranged from 557,282 to over 2 million CFU/g of dust from the three rooms. The genera included species of Gram positive (*Bacillus, Micrococcus, Rhodococcus*, and *Staphylococcus)* and negative (*Flavobacterium, Pseudomonas, Stenotrophomonas and Methylobacterium*) bacteria. *Streptomyces spp* were also cultured from bioaerosol samples ranging from 27 to 1,117 CFU/m^{3} (data not shown). In addition, cultures of a swab sample of the HVAC duct in the living area detected over loaded concentrations of *Acinetobacter, Flavobacterium*, *Methylobacterium* and *Bacillus* (data not shown), Table **1**.

Table 1: This Table Summarizes the Bacteria Identified in Dust Collected from Various Carpeting in the Home. Bacterial TSA cultures were done at 55 and 25 °C.

Bacteria	Carpet Dust – Baby's Room CFU/g	Carpet Dust – Master Bedroom CFU/g	Carpet Dust – Living Room CFU/g
Bacteria (50 °C)			
Thermoactinomyces vulgaris	1,165	970	870
Thermophilic bacteria	1,942	4,348	6,667
	Total: 3,107	Total: 5,217	Total: 7,536
Bacteria (25 °C)			
Bacillus spp.	79,612	118,841	266,377
Flavobacterium	N.D.	237,681	35,652
Gram Negative and others	47,767	130,725	285,217
Micrococcus luteus	31,845	142,609	439,710
Micrococcus roseus	15,922	N.D.	N.D.
Pseudomonas sp.	127,379[a]	23,768[a]	N.D.
Rhodococcus	143,301	59,420	344,638
Shewanella putrefaciens	N.D.	736,812	511,015
Staphylococcus	79,612	475,362	95,072
Stenotrophomonas maltophilia	31,845	N.D.	130,725
Methylobacterium	N.D.	N.D.	95,072[b]
	Total: 557,282	Total: 1,925,218	Total: 2,103,478

a = non *aeruginosa*; b = coagulase negative.
Bacillus species: potential human pathogens, gram positive.
Streptomyces and other *Actinomycetes* were identified in other samples.
Thermoactinomyces vulgaris – Farmer's Lung Disease
Flavobacterium – gram negative rod, opportunistic pathogen.
Micrococcus luteus: Normal flora of skin, has caused meningitis and pneumonia
Micrococcus roseus: generally believed to be saprophytic and/or commensal; may be opportunistic.
Rhodococcus: sp. equi is pathogenic causing infections in several organs. It belongs to the *Actinobacteria* in the same group as *mycobacterium and cornynebacterium.*
Shewanella putrefaciens: gram negative bacteria. Can infect wounds of lower the extremities. Has cause septicemia.
Staphylococcus: gram positive and can be a human pathogen, particularly *aureus* and *epidermid*
Stenotrophomonas maltophilia: opportunistic causing nosocomial infections.
Methylobacterium- opportunistic pathogens: urinary tract, inflammatory bowel, granulomas.

The molds identified in the interior of the home are summarized in Tables **2** and **3**. *Stachybotrys chartarum* was identified from wall sampling of the infant's bedroom (data not shown). Mold spores to the genus level identified in various wall cavities of the home and in the outdoor air are summarized in Table **2**. The total outdoor count was 773 spores/m^3. Two wall cavities in the master bedroom had counts of 17,078 and 534 spores per m^3. The other wall cavities had counts of 534, 393, 362 and 7,342 spores per m^3. Elevation of spore counts was found with respect to two observations (a) total airborne spore counts exceeded outdoor counts in cavities of the master bedroom and under the kitchen cabinet and (b) Aspergillus/ *Penicillium* spores ranged from 27 to 100 % (average = 58.7 %) of the total spore counts. vs. 26.7 % in the outdoor spore counts and (c) visible mold growth was observed in the interior of the home.

The species of fungi cultured from carpet dust taken from the baby's room (BR), master bedroom (MB) and living room (LR) are listed in Table **3**. At 35 °C the identified fungi included 4 species of *Aspergillus*, including *A. flavus* and *fumigatus*. The cultures done at 25 °C had a different pattern of fungal growth when compared to the 35 °C. *A. flavus* and f*umigatus* were not identified at the lower temperature, while other species of *Aspergillus* were detected. In addition, species of *Cladosporium, Penicillium* along with other fungi grew out at 25 °C. Apparently the optimum temperature for *A. flavus and fumigatus* is 35 °C.

Clinical Laboratory Testing of the Baby

The results of diagnostic testing of the infant are briefly summarized as follows for the first hospital stay: 1) TORCH titers were negative and the urine was negative for CMV three times; 2) The liver function tests were normal, except for an elevated LDH at 1356 µ/L decreasing to 422 µ/L during hospitalization; 3) Serology testing revealed: CMV IgM (negative); Herpes IgG (positive) and IgM (negative); Toxoplasma IgG (negative) and IGM (negative); Rubella IgG (positive) and IgM (negative) and Parvovirus IgG (negative) and IgM (negative), Table **4**.

Table 2: This Table Summarizes the Molds to Genus Level that were Identified from Air Samples taken from various Wall Cavities in the Home

Sample Location	Air Vol (liters)	Spores/m^3	Fungal I.D.	% of Count
South Exterior	292.3	773	Alternaria	5.3
			Asp/Pen Like	**26.7**
			Basidiospores	10.7
			Cladosporium	50
			Hyphal Fragments	1.7
			Unknown	5.7
Master Bedroom South Wall Cavity	29.2	**15,078**	Alternaria	8.7
			Ascospores	5.2
			Asp/Pen like	**27**
			Basidiospores	25.2
			Cladosporium	20.9
			Curvularia	0.9
			Hyphal Fragments	0.9
			Pithomyces	0.87
			Scopulariopsis	0.87
			Unknown	5.2
Master Bedroom North Wall Cavity	29.2	524	**Asp/Pen Like**	**50**
			Basidiospores	25
			Cladosporium	25
Hall Bath East Wall Cavity	29.2	393	**Asp/Pen Like**	**66.7**
			Basidiospores	33.3
Hall Bath West Wall Cavity	29.2	262	**Asp/Pen Like**	**50**
			Basidiospores	50
Kitchen Cabinet Base Cavity	29.2	**7,342**	**Asp/Pen Like**	**100**

Table 3: This Table Summarizes the Mold Identified in Dust Collected from Carpeting in the Home. Mold MEA plates were cultured at 25 and 35 ^{o}C. Note that *Aspergillus flavus* was cultured at 35 and not at 25 ^{o}C. Apparently the optimum temperature for *A. flavus* is 35 ^{o}C.

Fungi	Carpet Dust – Baby's Room CFU/g	Carpet Dust – Master Bedroom CFU/g	Carpet Dust – Living Room CFU/g
Fungi (35 ^{o}C)	N.D.	580	4,638
A. fumigatus	2,330	N.D.	580
A. flavus	2,718	580	290
A. niger	N.D.	N.D.	870
A. terreus	777	580	N.D.
Curv. lunata	388	290	290
Sterile fungi	N.D.	580	N.D.
Penicillium spp.	N.D.	290	N.D.
Rhizopus oryzae	N.D.	290	N.D.
T. harzianum			
Fungi – 25 ^{o}C			
A. alternata	N.D.	2,899	11,884
A. niger	3,883	N.D.	N.D.
A. pullulans	971	725	N.D.
A. sydowii	2,913	3,623	404,058
C. cladosporioides	N.D.	2,174	N.D.
C. sphaerospermum	N.D.	3,623	N.D.
C. lunata	971	N.D.	11,884
P. brevicompactum	1,942	N.D.	N.D.
P. decumbens	971	N.D.	N.D.
Rh. stolonifer	971	725	N.D.
Sterile fungi	3,883	725	11,884
Yeasts	N.D.	725	N.D.
T. harzianum	N.D.	N.D.	N.D.
A. versicolor	N.D.	N.D.	35,652
Rh. Oryzae	N.D.	N.D.	N.D.
D. bispetata	N.D.	N.D.	N.D.
Sp. salmonicolor	N.D.	N.D.	N.D.
A. ustus		N.D.	11,884

Table 4: This Table Summarizes the Results of Diagnostic Tests during the First and Second Hospitalizations. The values for individual blood chemistries are given in the text under clinical laboratory test results.

First Hospital Visit	Second Hospital Visit
TORCH ANTIBODIES[1]	Blood Chemistry[2]
CMV ---------- IgM(-)	Metabolic Acidosis (pyruvate and lactate were elevated
Herpes Simplex – IgG (+)	Ammonia - Elevated
----------IgM (-)	Triglycerides - Elevated
Alanine - elevated	
Rubella ---------- IgG (+)	β-hydroxybutyrate - Elevated
---------- IgM (-)	Prothrombin Time - Increased
PTT - Elevated	
Parvovirus --------- IgG (-)	Fibrinogen - Decreased
---------- IgM (-)	Urine Analysis[3]
Blood Counts	
Toxoplasma ------- IgG (-)	MCH - Decreased
---------- IgM (-)	Hematocrit - Decreased
Neutrophils – 91 %	
Urine CMV ----- Negative	Lymphocytes – 6.2 %
Reticulocytes: Range 2-49.4%	
Serum LDH – 1356 u/L	Hypoglycemia
Decreased to 422 u/L at	Hypoalbuminemia

discharge to home

Enterobacter and Pseudomonas[4]

Repeat TORCH – Negative

EEG – Diffuse slowing & generalized seizure activity

[1]The TORCH panel was considered negative because of negative IgM; [2]Individual values for the blood chemistry are given in the text; [3]Urine tests showed ketouria, (1+), Proteinuria (2+), and occult blood; [4]Broad spectrum antibiotics were given and repeat test were then negative for active bacterial growth.

Laboratory tests during the second hospital stay revealed a variety of abnormalities: Metabolic acidosis with elevated pyruvate and lactate. Other tests showed the following: elevated blood ammonia, triglycerides (843 mg/dL), serum alanine, and beta-hydroxybutyrate (15. 1 mg/dL), hypoglycemia (glucose, 41 mg/dL), hypoalbuminemia (albumin 2.4 mg/dL), and carnitine deficiency. Prothrombin time (PTT) was increased (16.5-18.1), while fibrinogen (75 mg/dL) was decreased. Urinalysis showed ketonuria (1+), proteinuria (2+), the presence of occult blood (3+) and leukocytes (1+). Mean corpuscular hemoglobin (MCH) was low (ranged from 9.3 to 11.0 g/L) with a decreased hematocrit (range from 32.8 to 36.5) and increased reticulocytes (2.5 to 49.4 on different dates) and differential of (Neutrophils 91.1 %, lymphocytes 6.2 %). The EEG showed diffuse slowing and generalized seizure activity. The child's condition deteriorated and she expired at the age of 2 months, 8 days.

Pseudomonas and *Enterobacter* were detected in the blood during the second hospital stay. Treatment with antibiotics cleared the infection.

Comparison of Clinical Findings with the Literature

Clinical findings of the infant in this matter are compared to those reported in the literature regarding Reye's syndrome (Table **5**). All of the findings are in agreement with the literature except for blueberry rash. The reported skin rash in Reye's patients has been maculopapular exanthema.

Autopsy, Electron and Light Microscopic Findings in Liver and Muscle

Liver

The pathologist described the following**:** Marked fatty changes, no mitochondrial hyperplasia with excessive lipid droplet accumulation. Mitochondrial defects characteristic of metabolic or genetic disease were not observed.

Skeletal Muscle

Slight predominance of type 2 fibers on NADH stain. Trichrome stain showed enlarged fibers with increased granular staining without evidence of ragged red fibers. EM findings were consistent with increased glycogen,

which was evident with increased toluidine blue staining under light microscopy. Both EM and Light Microscopy observations demonstrated Infiltration and replacement of muscle fibers with granular material typical of glycogen.

Table 5: This Table Compares the Clinical Findings in the 68 Day Old Infant with those Reported in Reye's Syndrome

	Infant	Reye's Syndrome
Mitochondropathy	Yes	Yes
Acidosis	Yes	Yes
Decreased CO_2	Yes	Yes
Increased triglycerides	Yes	Yes
Increased Ammonia	Yes	Yes
Seizures/convulsions	Yes	Yes
Fatty liver	Yes	Yes
Hypoglycemia	Yes	Yes
Elevated amino acids	Yes	Yes
Elevated pyruvate/lactate	Yes	Yes
Elevated B-hydroxybutyrate	Yes	Yes
Hepatomegaly	Yes	Yes
Hypoprothrombinemia	Yes	Yes
Elevated urine acids	Yes	Yes
Mixed acid/base disturbance	Yes	Yes
Elevated lactic acid	Yes	Yes
Elevated pyruvic acid	Yes	Yes
EEG dysrhythmia	Yes	Yes
Prolonged prothrombin	Yes	Yes
Encephalopathy	Yes	Yes
Hypotonia[1]	Yes	Yes
Blueberry rash[2]	Yes	maculopapular exanthema
Hemolytic anemia[3]	Yes	Yes

[1]Mitochondropathy and encephalopathy have been described in Reye's syndrome. Thus, hypotonia is not inconsistent with Reye's syndrome. [2]Blueberry muffin rash is a descriptive term for purpura lesions reflective of extramedullary hematopoiesis. It is currently thought that the lesions may result from intrauterine infections (e.g. rubella, CMV), malignancies or hematological disorders. [3]Bleeding into to tissues has been reported in case studies associating Aflatoxin B-1 with Reye's syndrome. Hemolysins have been reported for *S. chartarum* and several species of Aspergillus and Penicillium

Mitochondrial Studies

The mitochondrial respiratory chain studies on the subcutaneous fibroblasts were normal. The studies on the striated muscle were abnormal. The citrate synthase and succinate dehydrogenase were reproducibly elevated approximately 200 % above expected normal values. After normalization against observed citrate synthase values, all other enzymes were deficient as per cent of controls as follows: NADH dehydrogenase (21 %); succinate dehydrogenase (55 %); cytochrome C oxidase (26 %); succinate cytochrome c reductase 23 %); NADH cytochrome C reductase (25 %). The results were consistent with a mitochondrial myopathy (data on the mitochondrial tests are not shown)

PCR mitochondrial DNA point mutation assays were negative for the following: MERLAS, MERF, Cardiomyopathy, LHON, Kearns-Sayre, CPEO, Person Marrow/Pancreas, and Maternally Inherited Diabetes/ Hearing Loss. Point mutations or deletions were not detected (results of the mitochondrial studies are not shown).

Genetic Counseling

The familial history revealed maternal allergies and asthma. The mother of the deceased infant had asthma and allergies. The maternal side showed two distant nieces with birth defects: cretinism and autism. A distant relative died in infancy from microcephaly. There was no familial history of either mitochondrial diseases or hemolytic anemia.

Mycotoxins in Liver, Mother's Breast Milk and Urine

Aflatoxin results by immunoaffinity column and fluorometry revealed 2.1 ppb in the liver of the infant, while trichothecenes and ochratoxin A were not detected. Breast milk contained aflatoxins at 15 ppb, while the maternal urine had trichothecenes at 4.8 ppb and ochratoxin at 3.4 ppb. Samples were run in duplicate and all controls and calibrators were within prescribed limits (Table **6**).

Table 6: Mycotoxins Detected in the Fetal Liver and Maternal Breast Milk and Urine. The concentration of Aflatoxins in the infant's skeletal muscle was below the limit of detection (0.58 ppb), therefore, not detected

Mycotoxin	Breast Milk	Urine	Infant's Liver
Trichothecenes	N.P.	4.76 ppb	N.P.
Aflatoxins	15.0 ppb	N.P.	2.1 ppb
Ochratoxin A	N.P.	3.4 ppb	N.P.

Limit of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb); Ochratoxin (2.0 ppb); N.P. – Below limit of detection

DISCUSSION

Water intrusion in buildings and homes is associated with increased growth of bacteria and fungi [27-34]. Bacterial toxins are present in water damaged environments [27, 31-34]. A variety of mycotoxins have been identified in air, carpeting, dust and ventilation systems of water-damaged buildings [35-41]. Bacteria and molds shed nano-particulates at 0.03 – 0.3 microns) that contain potentially toxic by-products [21, 42-45]. The nano- particulates are up to 1000 times or more in concentration than larger particulates (spores and hyphae fragments [33-40]. Trichothecenes, aflatoxins, ochratoxin A and stachylysin have been detected in the body fluids and biopsy materials from individuals residing in water-damaged environments [24-26, 46, 47]. The route of exposure to mold and bacterial by-products most likely results from the inhalation of respirable (diameter ≤5 micron) aerosolized particulates and bioaerosols that are deposited on the nasal mucosa and deep into small airways and alveoli of adults and children where diffusion of toxins into the brain and systemic circulation occurs [38-40 42-45]. Thus, the symptoms and health complaints of the parents in this study are consistent with those reported by others regarding health, moisture and exposure to microbial growth [5, 20, 21, 26, 31, 67-77].

Reye's syndrome is characterized by encephalopathy, fatty infiltration of the liver and viscera, respiratory distress with abnormal clinical chemistry (acidosis, increased ammonia and prothrombin time, and mitochondrial abnormalities, among others) [1-13]. At birth the infant in this case had a purpuric "blueberry rash," hemolytic anemia and respiratory distress that were stabilized in 4 weeks, allowing the child to be discharged to home. Three weeks after discharge, the infant became difficult to feed, flexing its knees and crying out in pain with each attempt at feeding. Upon re-admission, the infant had encephalopathy, hypotonia, unresponsiveness to stimuli, and respiratory distress requiring intubation. The results of the testing of blood elements and chemistry demonstrated abnormalities consistent with Reye's-syndrome. These included, but not limited to, mitochondropathy, hemolytic anemia, hypoglycemia, and elevation of lactic acid, pyruvic acid (metabolic acidosis), triglycerides, ammonia, alanine, beta-hydroxybutyrate with hypoproteinemia (decreased albumin and fibrinogen) and increased prothrombin time (Table **5**). A carnitine deficiency was found in agreement with mitochondrial dysfunction. Urinalysis revealed ketonuria and proteinuria. TORCH and serology for Toxoplasmosis and viral infections were negative. Postmortem histopathology of the liver revealed fatty infiltration. Both EM and Light Microscopy observations demonstrated Infiltration and replacement of muscle fibers with granular material typical of glycogen. In addition, mitochondrial studies on skeletal muscle revealed decreased function in complexes I-IV. Tests for known mitochondrial-DNA mutations were negative. All of these clinical and laboratory findings are compatible with Reye's syndrome (Table **5**) [1-18].

Aflatoxins were detected in the liver biopsy at 2.1 ppb of the infant. Interestingly, Reye's syndrome has been reported in several infants less than 3 months of age in association with exposure to Aflatoxin B_1 [8-12]. Some of the infants were 2-3 days old, suggesting *in utero* exposure to the mycotoxin [9]. Further, a case study published in 1977 reported on a series of 27children ages 2-3 days old to 8 years with Reye's syndrome. Aflatoxin B1 was detected in the livers by chromatography and spectrophotometry [8]. Thus, the aflatoxin detected in the liver in this case suggests a probable intrauterine transfer since the infant was ill from the time of birth. The detection *of Aspergillus flavus* in the home and aflatoxins in the mother's breast milk supports this conclusion (Table **6**).

Aflatoxin B_1 is present in human breast milk, is metabolized and transported by the human placenta and causes infant growth retardation following in utero and post weaning exposure [25, 48-53]. The isolation of the mycotoxin from liver and blood of Reye's cases makes it a likely toxic candidate [7-12, 25, 52-59]. Aflatoxin B_1 obtained from cultures *A. flavus* was given orally to Macaques. The monkeys developed a Reye's-like syndrome in a dose dependent manner [57]. Also, an outbreak of hepatic encephalopathy in Malaysia was attributed to aflatoxin contaminated noodles [58].

Moreover, the toxic effects of Aflatoxin B_1 to mitochondria include: increased apoptosis [59], ultrastructural changes of cristae [60, 61] inhibition of urea cycle enzymes [15], mitochondrial-DNA adduct formation [62, 63], increased turnover of phospho-inositides by activation of phosphatydylinositol cycle [64], and impairment of respiratory function via membrane damage and modification of gene expression [65, 66]. Mycotoxins are present in the food supply, water-damaged indoor environments, maternal milk, as well as human sera, biopsy materials, placenta and neonatal umbilical blood. We believe that more attention should be directed towards fungi, their toxic

metabolites and health effects on humans *in utero*, neo-natal, children and adults. As an example, a recent case study demonstrated Neurofibromatosis Type 1 in an infant that was exposed *in utero* to mycotoxins in a water-damaged home [25]. Moreover, aflatoxin, trichothecenes and ochratoxin A have been reported in the urine of adults with chronic fatigue syndrome exposed to mold in their water-damaged homes [26].

It is appropriate to address a few other issues regarding the complexity of the indoor environment resulting from water intrusion leading to amplification of fungi, bacteria and their biological products [24-46] as follows: 1) Inhalation of 1,3-β-D-glucans from cell walls of mold can lead to inflammation of the lungs and the production of pro-inflammatory cytokines. In addition, sarcoidosis in occupants of water-damaged homes related to exposure to molds has been successfully treated with antifungal therapy [68-71]; 2) Endotoxins released by Gram negative bacteria are associated with acute and chronic inflammatory responses in the lungs of rodents and humans [72-77]; 3) The Actinobacteria (*Streptomyces* and *Mycobacterium spp*) have been identified in damp indoor environments, witch can cause lung infections as well as skin and tissue actinomycetoma [79-84]. Toxic metabolites (valinomycin, monactin, nonactin and staurosporin produced by *Streptomyces spp* in dust co-occurring with fungal secondary metabolites opens the possibility of interactions between bacterial and fungal toxins on the health of occupants of water-damaged homes and buildings [31-41]. With respect to nontuberculin Mycobacterium several species are known to cause Mycobacterium Avium Complex (MAC) and while some species are known to produce the cytopathic toxins, mycolactone [72-84].

In conclusion the Reye's syndrome in this infant was a result of exposure to the complex environment resulting from amplification of bacteria and mold resulting from water-damaged conditions to the families' home. The presence of aflatoxins and fatty changes in the liver and other clinical findings in this case are consistent with case studies previously cited. Infants that develop a Reye's-like syndrome following birth have liver pathology and dysfunction of mitochondria that are associated with exposure to aflatoxin B1. We encourage pediatricians who are confronted with infant cases similar to the one reported herein, to conduct a complete environmental history to determine if exposure to mold and bacteria in a water-damaged indoor environment could be associated with infant morbidity. We are currently investigating the role of water-damage, fungi, bacteria and mycotoxins in an apartment complex, several residential dwellings as well as office complexes where occupants are chronically ill.

CONFLICT OF INTERESTS

Dr. Gray is in private practice. He specializes in injuries resulting from toxins. He has done expert witnessing for defense and plaintiff cases.

Dr. Thrasher: If a consultant in toxic exposure. He has done expert witnessing for both defense and plaintiff cases.

Dr. Hooper: Is owner and director of RealTime Laboratories. RealTime specializes in the identification of mycotoxins in body fluids and tissues. He has done expert witnessing for both defense and plaintiff cases.

Dr. Crago: Is the owner of Neuro Behavioral Health Services. He specializes in neurocognitive testing and QEEG in association with Dr. Gray.

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