Resolution of a cluster of travel-associated *Leptospira santarosai* infection in four adolescents by plasma microbial cell-free DNA detection with the Karius Test®

Hai Nguyen-Tran MD[1], Guliz Erdem MD[2], Marcelo Laufer MD[3], Lori Patterson MD[4], Asim A. Ahmed MD[5], William A. Bower MD[6], Renee Galloway MPH[6], Sara Saporta-Keating MD[1]

[1]Children's Hospital Colorado/University of Colorado, [2]Nationwide Children's Hospital, [3]Nicklaus Children's Hospital, [4]East Tennessee Children's Hospital, [5]Karius, [6]Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention

University of Colorado Anschutz Medical Campus

Children's Hospital Colorado

BACKGROUND

- *Leptospira spp.* are obligate aerobic spirochetes with >300 serovars
- Infections range from subclinical to severe life-threatening illness
- Diagnosis of leptospirosis is challenged by:
  - Non-specific, protean clinical signs and symptoms
  - Fastidious nature of *Leptospira spp*.
  - Long turnaround time and poor sensitivity and specificity of many available tests
- Early diagnosis and prompt antibiotic initiation leads to decreased severity and duration of illness
- Rapid, non-invasive diagnostic techniques such as microbial cell-free DNA (mcfDNA) next-generation sequencing (NGS) may be one technique to overcome the limitations of current available testing of leptospirosis

METHODS

- The Karius Test® (KT) quantifies mcfDNA in molecules/µL (MPM) in plasma
  - Database of >20,000 organisms
  - Reports on >1000 pathogens
  - Performed at a CLIA certified/CAP accredited lab
- Case series of four KT detections of *Leptospira santarosai* at independent U.S. institutions
  - Identified April-August 2021
  - Clinical review performed by pediatric infectious diseases consultants

RESULTS

Case #	1	2	3	4
Gender/Age range (years)	Male/15-18	Male/15-18	Male/15-18	Female/15-18
Presenting symptoms/Additional clinical features	Fevers, chills, malaise, polyarthralgia, myalgias, diarrhea, conjunctivitis/Hepatitis	Fevers, chest pain, myalgias, headache, photophobia, eye burning sensation/Rash developed on torso	Fever, nausea, vomiting, diarrhea, chills, headache, photophobia/Renal and liver injury	Fever, chills, myalgias, emesis, diarrhea, headache, conjunctivitis/Malaise, night sweats, pre-syncope, oliguria and dark urine, photophobia, eye pain
Tmax ºF	102.8	103	104	102
WBC (k/µL) w/ %Neutrophils	7.5 w/77.6%	4.42 w/83.6%	12.8 w/79%	3.88 w/84%
Hgb (gm/dL)/Hct (vol%)	14.7/43	15.3/44.1	14.8/41.9	11.4/35.1
Platelets (k/µL)	170	165	96	143 → 97
PT (seconds)/PTT (seconds)	N/A	N/A	13.1/31	N/A
Na (mmol/L)	137	135	134	141
BUN (mg/dL)/Cr (mg/dL)	16/0.86	16/1.01	21/1.28	7/0.75
ALT (U/L)/AST (U/L)	74/78 → 353/204	11/30 → 46/115	167/143	50/40
ESR (mm/hr)/CRP (mg/dL)/PCT (ng/mL)	N/A/37.2/4.030	17/8/1.46 → N/A/18.1/N/A	42/16.5/N/A	58/26.55/0.25
Blood culture	Negative	Negative	*Pseudomonas oryzihabitans*; Follow up cultures x2: Negative	Negative
Presumptive diagnosis	Sepsis, Leptospirosis, Malaria, Dengue, Chikungunya, Zika, West Nile Virus	Rickettsial Disease, Arboviral Infection, Colorado Tick Fever, Dengue, Chikungunya, Typhoid Fever	Dengue, Leptospirosis, Zika, Chikungunya, Rocky Mountain Spotted Fever, Multisystem Inflammatory Syndrome in Children, Viral Infection	Typhoid/Enteric Fever, Dengue, Leptospirosis
Empiric antibiotic therapy/Antibiotic pretreatment duration prior to the KT (days)	Ceftriaxone/1	Penicillin/0	Ceftriaxone/2	Doxycycline, Ceftriaxone, Ciprofloxacin/5
Karius Test (mcfDNA NGS) result	*Leptospira santarosai* 284 MPM (RI: 0 MPM)	*Leptospira santarosai* 378 MPM (RI: 0 MPM)	*Leptospira santarosai* 28* MPM (RI: 0 MPM)	*Prevotella melaninogenica* 171 MPM (RI: 2.4 MPM) *Leptospira santarosai* 4* MPM (RI: 0 MPM)
Day of illness (DOI)	DOI: 6 days	DOI: 5 days	DOI: 10 days	DOI: 7 days
Turnaround time (TAT)	TAT: 3 days from collection; 2 days from sample receipt	TAT: 2 days from collection; 1 day from sample receipt	TAT: 3 days from collection; 1 day from sample receipt	TAT: 2 days from collection; 1 day from sample receipt
***Leptospira* serology**	*Leptospira* IgM acute: Positive	*Leptospira* IgM acute: Negative	*Leptospira* IgM acute: Equivocal	*Leptospira* IgM acute: Positive
Day of illness (DOI)/Turnaround time (TAT)	DOI: 10 days/TAT: 3 days	DOI: 5 days/TAT: 4 days *Leptospira* IgM convalescent (31 days after symptom onset): Positive	DOI: 12 days/TAT: 2 days	DOI: 14 days/TAT: 6 days
Epidemiological risk or potential exposure	Water rafting in Costa Rica; symptom onset 4 days upon return	Waterfall and parturient mouse exposure in Costa Rica; symptom onset 12 days upon return	Jungle, caves and river water exposure in Belize; symptom onset 6 days upon return	River water exposure in rural Costa Rica; symptom onset 4 days upon return
Targeted therapy after diagnosis	Azithromycin x 2 days	Doxycycline x 10 days	Doxycycline x 14 days	Ciprofloxacin x 2 days
Hospitalization duration	5 days	6 days	5 days	7 days
Outcome	Improved, full recovery	Improved, full recovery	Improved, full recovery	Improved, full recovery

N/A = not applicable; TAT = turnaround time; MPM = molecules/µL; RI = Reference interval which denotes the 97.5%ile MPM of a specific pathogen's mcfDNA in a healthy cohort of 684 subjects; *indicates detection below the Karius commercial threshold disclosed in clinical consultation.

CONCLUSIONS

- KT enabled rapid, non-invasive diagnosis of diverse manifestations of *L. santarosai* in a cluster of four travel associated leptospirosis infections
- *L. santarosai* is commonly found in Latin America in which all cases had recent travel to this region
- Utilization of mcfDNA NGS, such as the KT, has been used to aid in the diagnosis of infections in various scenarios
- Use of mcfDNA NGS may be helpful when:
  - Differential diagnosis is broad
  - Conventional testing is limited
  - Conventional testing is not feasible

IMPLICATIONS

- Potential utility of mcfDNA NGS to quickly diagnose challenging cases and support case definitions
  - Leads to more targeted treatment and can improve patient outcomes
- Use of mcfDNA NGS in public health and epidemiological surveillance may be considered to help identify cluster of cases, particularly notifiable infections such as leptospirosis

REFERENCES

1. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol. 2019; 4(4): 663-74.
2. Chen SM, Andrew A, Weiner B, et al. Fever, Vomiting, and Abdominal Pain in a 9-year-old Boy. *Pediatr Rev.* 2021; 42(10): 566–569.
3. O Equils *et al.* Rapid, Culture-Independent Detection of Leptospirosis with a Plasma-based Microbial Cell-free DNA Next-generation Sequencing Test. Abstract. ASM/WMF 2021.
4. Shapiro ED. Leptospira Species (Leptospirosis). In: Long S, Prober C, Fischer M, editors. Principles and Practice of Pediatric Infectious Diseases. Fifth ed. Philadelphia, PA: Elsevier; 2018. p. 977-80
5. Valverde MLA, Goris MGA, González V, Anchia ME, Díaz P, Ahmed A, et al. New serovars of Leptospira isolated from patients in Costa Rica: implications for public health. J Med Microbiol. 2013; 62(Pt 9): 1263-71.