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Apollo Rising: Acute Conjunctivitis Outbreak in India, 2022

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Abstract

Purpose: To identify pathogens associated with the 2022 conjunctivitis outbreak in Tamil Nadu, India.

Methods: This prospective study was conducted in November of 2022. Patients with presumed acute infectious conjunctivitis presenting to the Aravind Eye Clinic in Madurai, India were

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eligible. Anterior nares and conjunctival samples from participants were obtained and processed for metagenomic RNA deep sequencing (RNA-seq).

Results: Samples from 29 patients were sequenced. A pathogen was identified in 28/29 (97%) patients. Coxsackievirus A24v, a highly infectious RNA virus, was the predominant pathogen and detected in 23/29 patients. Human adenovirus D (HAdV-D), a DNA virus commonly associated with conjunctivitis outbreaks, was detected in the remaining patients (5/29). Hemorrhagic conjunctiva was documented in both HAdV-D and coxsackievirus A24v affected patients but was not the predominant clinical presentation. Phylogenetic analysis of coxsackievirus A24v revealed a recent divergence from the 2015 outbreak.

Conclusions: Coxsackievirus A24v and HAdV-D were co-circulating during the 2022 conjunctivitis outbreak in Tamil Nadu, India. Clinical findings were similar between patients with HAD-V and coxsackievirus A24v associated conjunctivitis. As high-throughput technologies become more readily accessible and cost-effective, unbiased pathogen surveillance may prove useful for outbreak surveillance and control.

INTRODUCTION

Infectious conjunctivitis outbreaks are of major public health importance as they may be the harbinger of a worldwide pandemic.[1] Often presumed to be viral in etiology[2], the exact pathogen in infectious conjunctivitis is rarely microbiologically confirmed. Traditional "gold-standard" microbiology techniques carry a high false negative rate and may not be ideal for the diagnosis of unexpected pathogens.[3] In this era of global spread of emerging pathogens, unbiased diagnostic techniques, such as metagenomic RNA deep sequencing analysis (RNA-seq), allow for detection of unanticipated pathogens including both DNA and RNA viruses as well as bacteria, fungi, and parasites. While conjunctivitis outbreaks are uncommon in the United States, in other parts of the world, more frequently in Asia and Africa, conjunctivitis outbreaks occur more frequently and often seasonally.[4,5] In November 2022, over 100,000 people sought treatment for conjunctivitis in Tamil Nadu, India.[6] The outbreak coincided with the start of the northeast, or winter, monsoon season. Here, we describe the clinical presentation and pathogens identified in the anterior nares and conjunctiva of patients presenting to outpatient eye clinics in Madurai, India during this time.

METHODS

Design, Setting, and Participants:

SCORPIO is an international research consortium focused on identifying pathogens responsible for infectious conjunctivitis worldwide.[7] The Aravind Eye Center is a participating site. This study adhered to the tenets of the Declaration of Helsinki. The Institutional Review Board of the University of California, San Francisco (UCSF), and Aravind Eye Hospital in India approved the study. Informed written consent was obtained from all patients. Inclusion criteria required symptoms of acute infectious conjunctivitis for less than 14 days. Signs and symptoms of infectious conjunctival include conjunctival erythema and ocular discharge that includes tearing and/or purulence[8]. For each patient, swabs from both anterior nares and each conjunctival fornix were obtained and placed in

DNA/RNA-Shield media (Zymo Research, Irvine, CA) to inactive infectious agents and to preserve nucleic acids for storage and ease of handling. All samples were de-identified and laboratory personnel were masked.

Laboratory Methods:

Samples were processed for RNA-seq as previously described.[7] Extracted RNA was converted to cDNA and sequencing libraries were prepared using the NEBNext ULTRA II RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) and then pooled and sequenced on the NovaSeq system (NovaSeq 6000, Illumina, San Diego, CA) using 150-nucleotide paired-end sequencing. For pathogen identification, human reads were first removed by alignment of all paired-end reads to the human reference genome 38 (hg38) and the *Pantroglodytes* genome (panTro4, 2011, UCSC) and then quality filtered. Reads passing the quality filtering step were then processed for duplicate removal and complexity and then host filtered again. Remaining non-host reads were then aligned to the entire NCBI non-redundant reference database. A sample is determined to be positive for pathogen based on the pre-specified criteria: 1) it is known to be a human pathogen and represent the most abundant reads after water background subtraction or 2) two or more unique reads covering separate regions in DNA virus genomes or 3) 1 or more unique reads matching RNA virus genomes.[9,10]

Statistical Methods:

Phylogenetic analysis was performed by first aligning the matched reads against the D90457.1 coxsackievirus A24 reference genome using bowtie2 [bowtie2]. “Samtools” and “Bcftools mpileup” commands were then used to build the consensus sequence for regions with a read depth greater than 5 [samtools+bcftools]. Consensus sequences with a genome coverage >99% were then compared to various published enterovirus references in order to create a time tree by running the “augur” toolkit [augur].[11] 95% confidence intervals were calculated using the Adjusted Wald method.

RESULTS

In the 10 months prior to November 2022, the Aravind outpatient eye centers treated approximately1,000 patients for conjunctivitis each month (Figure 1). In November alone, a total of 4,109 patients presented with conjunctivitis. Case numbers quickly decreased by December 2022. Samples from 29 patients were obtained during a 5-day period from November 10 to November 14, 2022. The mean age was 38 years old (standard deviation: +/− 15 year). 45% of the patients were male. Bilateral conjunctivitis was reported in 67% (95% CI: 51% to 83%). The mean duration of eye symptoms prior to presentation to the eye clinic was 2 days (95% CI: 1 to 3 days). 42% of the patients (95% CI: 25% to 59%) reported similarly sick contacts. All patients reported symptoms of tearing and purulent discharge (95% CI: 86% to 100%). Itching was not a reported symptom in any case (95% CI: 0% to 14%).

Subconjunctival hemorrhages were present in 17% (95% CI: 7% to 35%) of the patients. Representative clinical photos are shown in Figure 2. No patients had pseudomembranes/

membranes or subepithelial infiltrates (corneal involvement) on slit lamp examination. No patient had preauricular adenopathy on physical exam (95% CI: 0% to 14%). The most common comorbid systemic symptom was rhinorrhea (18%, 95% CI: 5% to 31%). No other systemic symptoms were reported. Unlike prior SCORPIO conjunctivitis cohorts, no patients presented on topical medications.[7,12]

Unbiased RNA-seq analysis identified Coxsackievirus A24v (CV-A24v, human enterovirus species C) in 23/29 (79%) patients and human adenovirus D (HAdV-D) in 5/29 (17%) patients. No pathogen was identified in 1/29 (3%) patients. Both CV-A24v and HAdV-D viruses are known causes of conjunctivitis outbreaks. CV-A24v is a nonenveloped, positive-sense single-stranded RNA virus and has been associated with acute hemorrhagic conjunctivitis.[13–15] Phylogenetic analysis was performed on CV-A24v genomes isolated from 10 patients. The isolates from this outbreak aligned most closely with the variants detected from the Réunion Island outbreak in 2015 and grouped within Genotype IV (Figure 3).[16]

DISCUSSION

Acute hemorrhagic conjunctivitis (AHC) was initially called Apollo 11 disease when an outbreak in Ghana and Nigeria in 1969 coincided with the timing of the American spaceflight which landed humans on the moon.[13] Known pathogens for AHC include enterovirus type 70 and CV-A24v. CV-A24v is classified as an *Enterovirus C* species within the *Picornaviridae* family. It was first identified in a conjunctivitis outbreak in Singapore in 1970.[14,17] CV-A24v initially circulated only in Southeast Asia but was later identified worldwide as the pathogen responsible for two AHC pandemics that started in 1985 and 2002.[15,18] The last documented epidemic of CV-A24v associated conjunctivitis was in 2017, with thousands of cases reported in the Caribbean and South America.[19,20] In that epidemic, the initial cases were documented in Mexico and then spread to include Cuba, the French West-Indies, the Bahamas, the Turks and Caicos Islands, Panama, the Dominican Republic, Grenada, Nicaragua, and Belize.[19] Genomic analysis based on the partial VP1 (structural) and C3 (non-structural) nucleotide sequences of CV-A24v identified from patients in the region of Cayenne in French Guinea showed close alignment to the isolates identified from a small outbreak in Uganda that started in December 2016.[19]

Infections from most enteroviruses occur year-round with peaks in the spring and fall. Infections from *Enterovirus C*, however, generally have a peak in the winter months where people are frequently indoors.[21] Thus, the timing of the current CV-A24v-associated conjunctivitis outbreak in India is not surprising. The incubation period of CV-A24v, is typically 24–48 hours. While it can spread via respiratory droplets, the common route of transmission is through fomites or direct contact with inoculation of the ocular surface from the fingertips.[22] Enteroviruses can survive on surfaces for hours, which allows for easy human transmission, particularly among those in close living quarters. CV-A24v has evolved to have affinity for sialic acids which are extensively expressed on the outer cell membranes of the human respiratory tract and ocular surface.[15] Bulbar conjunctival petechiae or hemorrhages are characteristic although do not appear to be pathognomonic. Indeed, the majority (87%) of the patients positive for CV-A24v in this study did not present

with hemorrhagic conjunctivitis and conversely, 1/5 (20%) patients who was positive for HAdV-D had petechial conjunctival hemorrhages. Unlike HAdV, corneal involvement is thought to be less frequent with enterovirus-associated conjunctivitis. While both HAdV-D and CV-A24v were co-circulating during this report's outbreak, none of the enrolled patients had co-infections. This finding is in contrast with a prior observation during the Delta surge in Madurai in 2021, where co-infections with HAdV-D and SARS-CoV-2 did occur in conjunctivitis patients who presented to the outpatient clinics.[9]

Prior studies, including studies from SCORPIO, showed that human adenoviruses are the predominant pathogens associated with conjunctivitis in India.[7,23,24] Limitations of this study include that not all patients who presented for care during this outbreak were swabbed and recruitment at this single site was restricted by study-approved doctors' availability, participants elective consent, and the surveillance nature of the study. However, given that the majority of the patients swabbed during this November 2022 outbreak were positive for CV-A24v and only 17% of the patients were positive for HAdV-D, these results suggested that this outbreak was driven by CV-A24v but that HAdV-D remained in circulation.

Whole genome analysis of the CV-A24v isolated from this recent India outbreak demonstrated that these isolates clustered more closely to the genomes detected in the Réunion Island outbreak that occurred in 2015 and not to those detected in the Mexico and French Guinea outbreaks in 2017.[16] Réunion Island is a French territory that is located in the Indian Ocean and is included in the national health insurance program of France. From January to April 2015, the Réunion Island had over 100,000 consultations for conjunctivitis. The Réunion Island outbreak resulted in over 180,000 ocular drop kits sold, 231 emergency department visits, at a total cost of over 3 million Euros.[16] These data illustrate the immense societal and public health burden that can occur with just a single conjunctivitis outbreak.

In addition to the societal costs and ocular morbidity, viral-associated conjunctivitis outbreaks can result in epidemics and pandemics. CV-A24v is known to cause epidemics and pandemics of AHC. One of the first cases of SARS-CoV-2 was noted by an astute ophthalmologist in China.[25] While it was reported that the patient did not have conjunctivitis at that time, subsequent studies showed that SARS-CoV-2 can be associated with conjunctivitis.[7,26,27] Ocular findings among viruses may be non-distinguishable and systemic symptoms may not be present. In this patient cohort, the only respiratory complaint was rhinorrhea in 1/5 of the participants. None of the patients with SARS-CoV-2 associated conjunctivitis in our prior study reported systemic symptoms.[9] Thus, ophthalmologists may be frontline in the detection of future epidemics as non-complicated appearing diseases like conjunctivitis may have major public health implications.

This outbreak highlights that DNA and RNA viruses can cause infections in the same outbreak and have no differentiating clinical features. While the local Department of Public Health was able to identify both HAdV and enteroviruses with pathogen-directed nucleic amplification tests (NAATs)[6], the implementation of technologies that can detect broad genome types (DNA and RNA pathogens) for respiratory pathogens with ocular manifestations may be necessary for future outbreaks.

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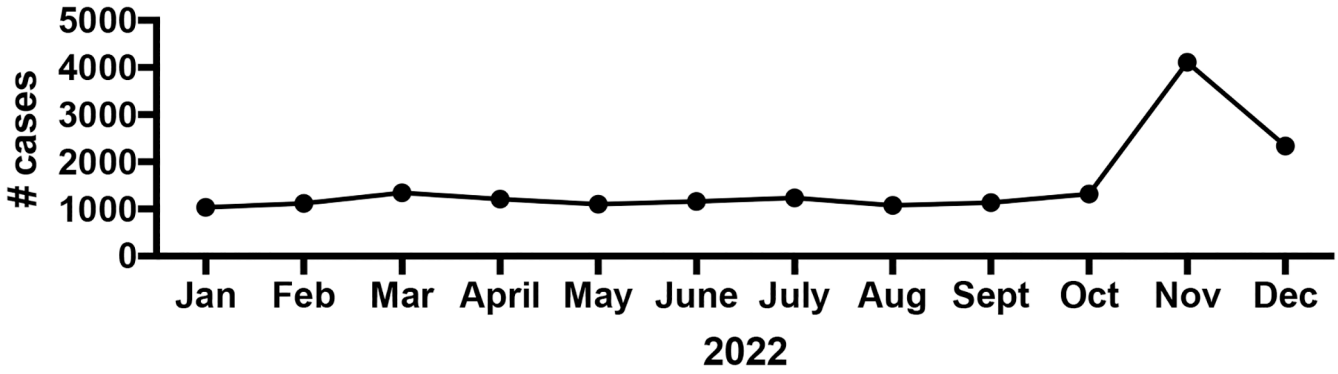

Figure 1:
The number of conjunctivitis cases per month at the Aravind Eye Center in 2022. A November 2022 outbreak is demonstrated.

Figure 2:

A montage of representative eye photos of Coxsackievirus A24v-associated (top 4 rows) and human adenovirus-associated (bottom row) conjunctivitis taken during the 2022 conjunctivitis outbreak in India.

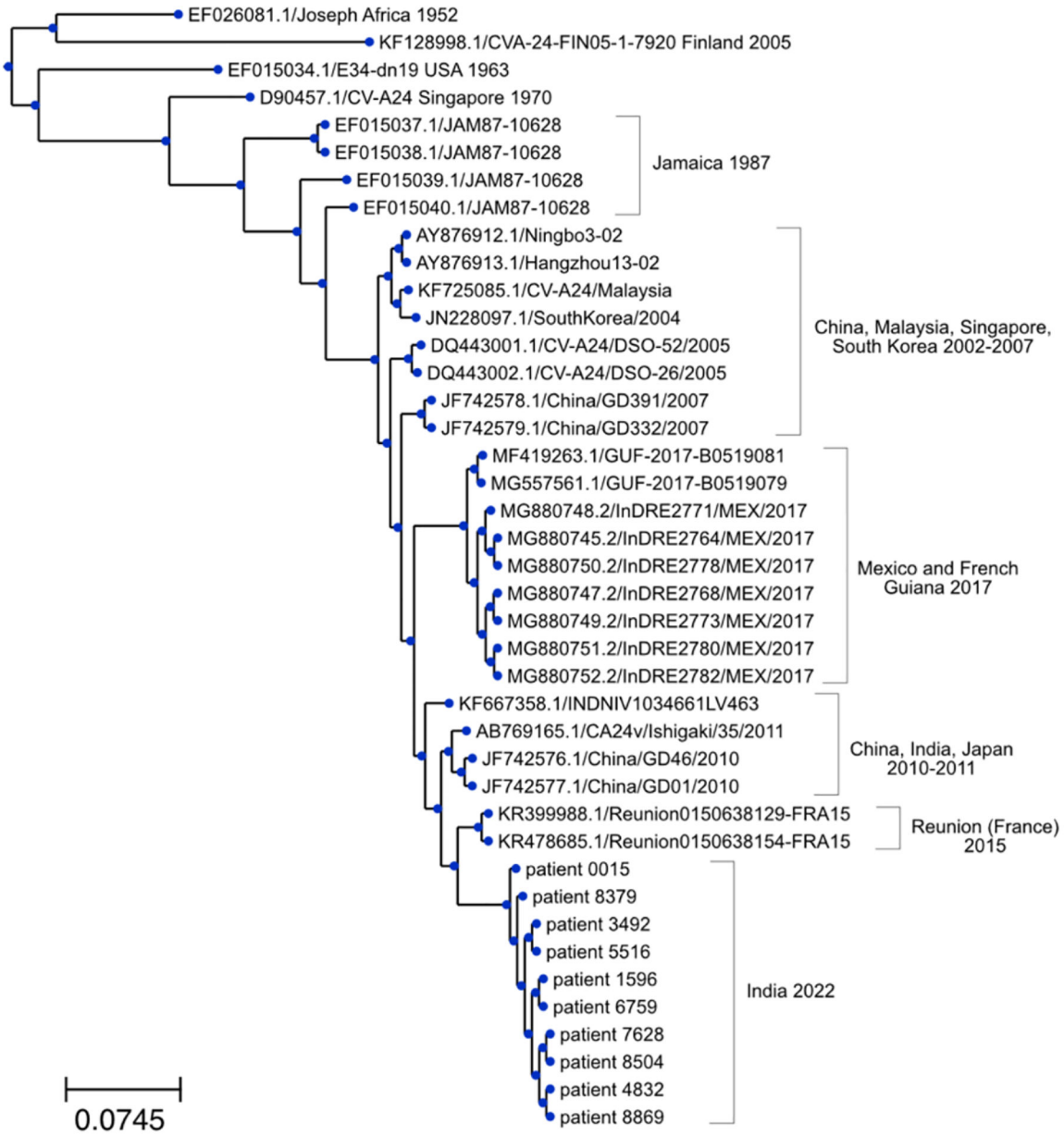

Figure 3:

Maximum-likelihood phylogenetic tree based on the genome sequences of CV-A24v isolated from this 2022 outbreak compared to prior outbreaks.