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Case report

Respiratory disease associated with migrating Ascaris larvae in a beef calf

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ABSTRACT

A group of 4-month-old beef calves were examined for clinical respiratory disease with labored breathing, coughing, and fevers of over 104 °F. Necropsy of one of the calves revealed lungs that were not collapsed but had red mottled appearance on cut surface. Assessment of lung tissue by bacterial culture and PCR did not reveal bovine bacterial or viral respiratory pathogens. Histopathology of affected tissues and lymph nodes revealed larval ascarid nematodes. In combination with phylogenetic analysis, amplification and sequencing of ITS1 was used to identify the larvae as Ascaris.

1. Introduction

Differential diagnoses for parasitic pneumonia in cattle in North America are infection with the trichostrongyle lungworm Dictyocaulus vivipurus (Panuska, 2006) and larvae of the ascarids - Toxocara vitulorum and Ascaris spp. (Bowman and Georgi 2017). Dictyocaulosis can be diagnosed by (a) demonstrating the slender, white adult worms in the bronchi of the caudal lobes in gross specimens at necropsy, (b) visualizing prominently alate adult worms with coelomyarian musculature and embryonated egg in the uterus in bronchi by histopathological techniques, or (c) through the recovery of larva using the Baermann technique (Al-Qudah et al., 1995). However, confirmatory diagnosis of ascarid larval infections by classical pathological and parasitological techniques is difficult, without additional molecular techniques because (a) complete sets of microanatomical measurements and descriptions of only a few ascarid larvae in mice tissue are available (Bowman, 1987), and it is unknown if the larvae measure the same in cattle lungs (b) ascarid larvae undergo changes in length and width to different extents during the migratory phase (Nichols, 1956a).

Cattle are the natural definitive hosts for the ascarid Toxocara vitulorum, which follows a typical hepato-pulmonary-tracheal migratory route and therefore larvae of this species can be demonstrated in the liver, lung, and intestine of an infected bovine (Roberts, 1990). Past reports of pulmonary pneumonia associated with migration of Ascaris spp. in cattle have been documented in experimental infections and situations where cattle had a known history of being housed in buildings/paddocks contaminated with pig feces (McCraw and Greenway, 1970; McCraw and Lautenslager, 1971; Morrow, 1968).

While migrating T. vitulorum larvae in cattle lungs in non-endemic areas may represent an outbreak in the herd (Chelladurai et al., 2015), cattle are only accidental hosts for Ascaris and worms do not mature to the adult stage. Given the different approaches for managing each disease at the herd level, it is important to differentiate between the larval ascarids using molecular techniques such as PCR. In this report, we demonstrate the utility of combining histopathology and PCR to identify ascarid larvae associated with respiratory disease in beef calves.

2. Materials and methods

2.1. Necropsy and histopathology

A field necropsy was conducted on an approximately 4-month-old calf. The referring veterinarian submitted fresh heart, lung, intestine, ear notch, and lymph node to Iowa State Veterinary Diagnostic Laboratory. Upon arrival, sections of lung, heart, lymph node, intestine, and ear notch were fixed in 10% neutral-buffered formalin, processed for histopathology using routine methods, and embedded in paraffin. Four-micron thick sections were stained with haematoxylin and eosin and the slides were examined by a board-certified veterinary pathologist (RD). All methods were performed in accordance with the University Institutional Animal Use and Care committee as well as local laws.

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https://doi.org/10.1016/j.vprsr.2017.12.005
Received 8 November 2017; Accepted 24 December 2017
Available online 27 December 2017
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2.2. Molecular studies

Total genomic DNA was extracted from the formalin-fixed, paraffin-embedded lung tissue, using MagMAX FFPE DNA Isolation Kit (Life Technologies, Grand Island, NY) according to the manufacturer’s protocol. DNA was eluted into 100 μl of nuclease free water and stored at −20°C.

PCR Amplification of the partial Internal Transcribed Spacer 1 gene was carried out in 24 μl reactions, with 1 μl of genomic DNA, 1 × PCR buffer, 3 mM MgCl₂, 0.25 μM of each dNTP, 0.5 μM of each primer (5’-CCG GGC AAA AGT CGT AAC AA-3’ and 5’– TAG TGC TCA ATG TGT CTG CA-3’) and 2 units of Taq polymerase per reaction (Promega, Madison, WI) (Arizono et al., 2010). PCR products were sequenced twice in both directions on an Applied Biosystems 3730xl DNA analyzer at the Iowa State University DNA Facility, and assembled with the CAP3 sequence assembly program (Huang and Madan, 1999). Sequences were compared with those in the NCBI GenBank database using nucleotide BLAST.

Multiple alignments of nucleotide sequence data were made using ClustalW and trimmed to 360 nt on MEGA7 (Kumar et al., 2016). Best model fit for nucleotide substitutions was selected using jModeltest 2.1.10 (Darriba et al., 2012). Corrected Akaike Information Criterion (AICc) calculations were used to select the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with a discrete gamma distribution (HKY + G) as the best fit model for Maximum Likelihood tree construction using MEGA7.

3. Results

3.1. Necropsy and histopathology

A necropsy was performed by the referring veterinarian who submitted tissues to the Iowa State University Veterinary Diagnostic Lab for evaluation. Grossly, the lungs had a slightly non-collapsed appearance and mild red mottling on cut surface. Formalin-fixed tissues were embedded in paraffin and processed.

Within the lung, bronchi and bronchioles were frequently ectatic and contained fibrin admixed with degenerate neutrophils and had many 50-90 μm diameter nematode larvae enmeshed within the exudate (Figs. 1, 2). Epithelium of bronchi and bronchioles was variably attenuated or hyperplastic with loss of cilia. Airways were cuffed by lymphocytes and plasma cells. Surrounding lobules were partially atelectatic and alveoli were filled by high-protein edema and scant fibrin.

The interstitium was variably, mildly expanded by lymphocytes and fibrin. Larvae had coelomyarian, polynymarian musculature, and intestine(*) lined by a single layer of cuboidal epithelium. (400 × magnification).

3.2. Ancillary test results

Lung tissue had no growth on routine bacterial culture and PCR testing for Mycoplasma bovis, Mannheimia haemolytica, Histophilus somni, and Pasteurella multocida were negative. Both PCR and immunohistochemistry were negative on lung tissue for bovine coronavirus, bovine respiratory syncytial virus, and infectious bovine rhinotracheitis virus (Bovine Herpesvirus-1). Bovine viral diarrhea virus PCR of lung tissue was negative, as was immunohistochemistry on ear notch. No parasites were observed on fecal flotation.
3.3. Sequencing results

PCR amplification of the partial Internal Transcribed Spacer 1 gene was carried out on DNA extracted from formalin fixed paraffin embedded lung tissue, sequenced on an ABI 3730xl DNA Analyzer at the Iowa State University DNA facility, and assembled using CAP3 (available at http://doua.prabi.fr/software/cap3). Assembled sequences were compared to sequences on GenBank using nucleotide BLAST. A 368 nt fragment of the partial ITS1 gene was obtained after sequencing. Nucleotide BLAST revealed that the sequence had 100% identity with several isolates of Ascaris spp. (KY576142, KY576143, KU522455, AB576588, AB571295, EF153619-EF153623) and 82–84% identity with Toxocara vitulorum (KT737382, KJ777180-KJ777182). PCR amplification of the mitochondrial cytochrome oxidase I (cox1) and nuclear SSU (18S) gene using the FFPE DNA template failed.

3.4. Phylogenetic analysis

To demonstrate the phylogenetic relationship between the migrating ascarid larvae and other ascarids, a Maximum Likelihood tree was constructed (Fig. 4). A pig isolate from a previous study that had a 3 band PCR-RFLP pattern (ITS PCR amplicons digested with HaeIII) from the state of Iowa, USA was included in the analysis (Jesudoss Chelladurai et al., 2017).

Partial Ascaris ITS1 gene sequenced in this study was found to cluster with the partial ITS1 gene from other Ascaris isolates. In contrast, Toxocara vitulorum, Toxocara cati and Toxocara canis occur in a distinctly separated cluster.

4. Discussion

Pneumonia associated with migrating Ascaris spp. has been described in cattle (McCraw and Greenway, 1970; McCraw and Lautenslager, 1971; Morrow, 1968), based solely on larval morphology in tissue sections, and larval recovery from the lung using the Baermann method. However, it is difficult to differentiate the larvae of ascarids in histological sections. This report details the use of molecular techniques for the identification of Ascaris in formalin fixed paraffin embedded (FFPE) tissue from the lung of a calf, to support gross and histopathological diagnoses.

Compared with mild gross lesions, histopathology revealed more serious inflammation associated with larvae migrating in the lung. Characteristics of the larvae included alae, coelomyarian musculature, prominent lateral chords, and an intestine lined by uninucleate cells. Morphological features allowed for preliminary identification of the larvae as an ascarid larvae (Nichols, 1956b). An acute to subacute bronchiolitis and interstitial pattern was observed similar to the lesions observed by McCraw (McCraw and Lautenslager, 1971). Contrary to the findings described by McCraw and Lautenslager, gross lesions were fairly subtle in this case. Follow-up on the outcome of the disease in the remaining cohort of calves was unavailable.

Larval migratory patterns following the ingestion of infective egg and hatching in the alimentary tract are similar among members of the ascarids. A hepatic-pulmonary-tracheal route is followed by Ascaris in its definitive (Douvres et al., 1969) and accidental hosts including humans and cattle (Kennedy, 1954; Nichols, 1956b). Toxocara vitulorum, the cattle ascarid, must be considered in the differential diagnosis, because it follows a similar hepatic-pulmonary-tracheal route (Warren, 1971), infects calves, and has been recently reported in North America (Chelladurai et al., 2015; Davila et al., 2010; Woodbury et al., 2012). Therefore, molecular techniques are essential for obtaining a definitive diagnosis. Adults of the Dictyocaulus viviparus which reside in the bronchi are also capable of causing parasitic bronchitis (Panuska, 2006).

The use of polymerase chain reaction (PCR) to amplify the multi-copy, nuclear internal transcribed spacer region (ITS) that occurs between the SSU and LSU ribosomal DNA for the identification of ascarid larvae has been described (Ishiwata et al., 2004). Although this approach is specific and sensitive enough to differentiate between Dictyocaulus viviparus, Toxocara vitulorum and Ascaris spp., neither this method nor PCR-RFLP of the ITS gene can be used to differentiate between pig and human isolates of Ascaris (Nejsum et al., 2017). But, this approach is still useful to draw comparisons and establish a preliminary population epidemiology between isolates from a given area and from across the world (Chelladurai and Brewer, 2017). Given the propensity of Ascaris for cross transmission between animals and humans (Anderson, 1995), and the very high identity of amino acid sequences encoded by the mitochondrial genome in human and pig isolates (Liu et al., 2012), the debate on the existence of one or two species continues. The limitations of the use of the ITS gene and difficulty in amplifying other genes from FFPE tissue precludes identification of the Ascaris larvae in this study as being from a pig or human source.

In this case, we characterized a case of pneumonia in beef calves that was solely associated with Ascaris larvae, while no other viral or bacterial agents were detected. There are no products labeled for the treatment of Ascaris larvae in cattle, but removing cattle from bedding or soil contaminated with swine feces may decrease future clinical signs. In this case, there was no known exposure to swine feces. Because ascarid ova can live for years in soil, the incriminated soil or bedding should be removed from cattle lots to prevent future cases.

Ethics

The authors certify that we complied with all local laws and Iowa State University IACUC rules while conducting this research.

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