Received: 18 October 2021 | Accepted: 18 October 2021

DOI: 10.1111/jvim.16304

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

LETTER TO THE EDITOR

Response to letter regarding "Utility of the combined use of 3 serologic markers in the diagnosis and monitoring of chronic enteropathies in dogs"

Thank you for your letter, which raises some valid questions regarding our manuscript. With this response, we provide answers to each point raised as well as additional clarification where appropriate. It should be noted that the manuscript underwent the standard JVIM review process and all questions raised were adequately addressed for acceptance. Many of the issues noted in the letter were not raised by either reviewers or editors, and would have been readily addressable.

First, we would like to state clearly that the assays evaluated in this manuscript are neither screening tests for chronic enteropathy (CE)/inflammatory bowel disease (IBD) nor a replacement for a full diagnostic workup. They could, however, be used as aid in the distinction between chronic diarrhea caused by extragastrointestinal vs gastrointestinal (GI) causes which can be important in private practice, as this could help determine if additional investigations are required. If extra-GI disease is likely, additional tests to rule in/out exocrine pancreatic insuffiency, Addison's disease or chronic pancreatitis should be performed. If GI-related disease is suspected, additional testing including abdominal ultrasound and GI biopsies should be considered. Second, the manuscript is based on a large field study where all dogs enrolled and all the collected data were prospective in nature, with the markers used in the study selected from a published assay development study.[1] In that prior study, all dogs diagnosed with CE had gastrointestinal biopsies performed (see Table 1). The two studies were designed in combination to reflect common private practices of 16 veterinary centers and over 30 veterinarians. Detailed protocols were provided with inclusion/exclusion criteria based on current standards[2] for CE workup to achieve consistency across centers. Age and sex distribution in the study cohorts were not statistically different as determined by Kruskal-Wallis and Fisher exact tests. More than 30 breeds were represented, with the top 5 being shared among cohorts. Diagnosis was based on samples taken at enrollment before any treatment was initiated. Treatment decisions made after diagnosis were not protocolized and left to the individual veterinarians.

With regards to the CE/IBD cohort recruited, every effort was made to perform a comprehensive diagnostic workup and to include information on follow up over several months. As stated in the article, there were 36/157 cases of CE/IBD that were confirmed by GI biopsy. This subset of enrolled dogs was considered representative because they were not preselected (ie, biopsy was offered and fully subsidized for all clients) showing high accuracy of correctly identifying CE/IBD using the marker profile in 36 out of 38 cases. Furthermore, the letter correctly noted that the non-IBD cohort in the field study was represented by 24 dogs. The non-IBD cohort was comprised of dogs displaying chronic extra-GI signs and showed marker profiles distinctive from the CE/IBD group in a statistically significant manner, as receiver operating characteristics curves and 3-dimensional plot demonstrate. In fact, the markers included in the assay were selected based on their discrimination between cohorts exhibiting GI vs extra-GI signs in the first study published.[1] Additional studies are being pursued to further expand dog cohorts with chronic diarrhea of various causes to better define the clinical utility of these assays. To be clear, we do not outline any suggested sequence of clinical tests in the manuscript. We emphasize that veterinarians should interpret diagnostic results with all other clinical data available.

Regarding the use of IBD serology in human medicine, we respectfully disagree with the authors of the letter. Gastroenterologists have been using it since at least 1994 to distinguish ulcerative colitis (UC) from Crohn's disease (CD) in patients with suspected IBD.[3] These patients are often unable (pediatric) or unwilling to do invasive procedures or present with inconclusive results on biopsies. These tests are not included in current gastroenterology guidelines primarily because they are regulated under clinical laboratory improvements amendments (CLIA) and not Food and Drug Administration, therefore making insurance reimbursement difficult. Despite this, Prometheus, for example runs hundreds of thousands of tests per year with new testing modalities that include genetic markers. Other large commercial laboratories (Quest, LabCorp, ARUP) also provide IBD sero-diagnostics.

Serology based on IgA markers is well established in existing tests.[3] IgA is produced in the gastrointestinal associated lymphoid tissue (GALT) with serum titers resulting from B-cell responses induced by antigens in the intestinal lumen.[4] Over the last 7 years, we optimized an IgA-based serology platform to be used in dogs. IgA were selected from all other Ig isotypes because they were determined to be the best discriminatory markers for extra-GI vs GI disease.[1] Comprehensive analytical validation was performed for all markers

TABLE 1 Study cohorts

	Healthy	CE/IBD	Extraintestinal diarrhea
Assay development study[1]	58	70 All confirmed by biopsy	23 Chronic (11) and acute (12) mix
Field study	33	157 38 biopsied, 36 confirmed	24 All chronic
Total	91	227	47

Abbreviations: CE, chronic enteropathy; IBD, inflammatory bowel disease.

included in this manuscript following standard International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines[5] including range, linearity, precision, robustness, and stability. We would like to highlight that precision/reproducibility for all three assays is between 95.8% and 98.6% in our laboratory; and that, in the presence of interfering agents in the serum, there was no or minimal effect on the recovery signal readouts (0%-5% for lipids and bilirubin, and 0%-15% for markedly hemolyzed samples). The assays use as reference standards high-quality, well-titrated cell lines expressing dog-IgA specific for all three biomarkers. Furthermore, a comprehensive external validation of both performance and quality control of all assays was performed by Antech Laboratories (unpublished).

Regarding antigliadin antibodies (AGA) and their potential role in dogs, we offer the following thoughts. Increased AGA concentrations in humans are associated with celiac disease. However, they have also been detected in nonceliac gluten sensitivity (NGCS),[6] a diagnosis which includes patients who do not have celiac disease but still suffer from chronic abdominal pain and diarrhea. The clinical relevance of AGA has been ascertained in humans, but its comparative importance in dogs is just emerging. As noted by the authors' letter, gluten intolerance and high AGA serum titers have been clinically linked to dogs suffering from epileptoid cramping.[7] More recently, another study found that 30 dogs with chronic enteropathy and 18 dogs with intestinal T-cell lymphoma also had increased serum AGA concentrations.[8] With regards to our study, we have found that dogs with chronic GI disease have increased AGA in 54% of dogs with extraintestinal chronic diarrhea and in 75% for dogs diagnosed with CE/IBD, compared to just 6% of healthy dogs. These data clearly indicate a role for these antibodies as potential biomarkers for dogs with chronic diarrhea, although the exact clinical usefulness will need to be elucidated in future studies. The article did not mention, much less advocate for, the use of gluten free diets in dogs with high AGA titers. Additional studies that include the use of hydrolyzed diets in AGA seropositive dogs are currently underway to evaluate their potential clinical importance.

Regarding author affiliations, the authorship page clearly acknowledges all institutions involved in the study. The lead author, Dr Estruch, is the CEO of Vetica Labs. In order to conduct a study of this scope, the dogs were enrolled, diagnosed and managed by 3rd party veterinarians some of which are associated with the VCA Clinic group, and others with independent veterinary clinics under fee-for-services arrangements with Vetica Labs. All assays were performed by Vetica Labs in a blinded fashion. We hope that our article will act as catalyst for future discussions and studies on complementary approaches to diagnose and manage chronic enteropathies in dogs.

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