UNITED STATES DEPARTMENT OF AGRICULTURE

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NATIONAL ADVISORY COMMITTEE ON

MICROBIOLOGICAL CRITERIA FOR FOODS

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PLENARY SESSION

+ + + + +

August 30, 2023
11:00 a.m.

Virtual/WebEx

CHAIR: MR. J. EMILIO ESTEBAN
Under Secretary for Food Safety, USDA
NACMCF Chair

VICE CHAIR: DR. DONALD PRATER
Acting Director, Center for Food Safety
And Applied Nutrition
NACMCF Vice-chair

MODERATOR: MS. KRISTAL SOUTHERN
Designated Federal Officer

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DR. DE ANN DAVIS
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DR. JAMES DICKSON
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MS. MICHELLE LOCKEY, Event Producer
MS. SHANTEL WILLIAMS

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(1:00 p.m.)

MS. LOCKEY: Welcome. And thank you for joining today's conference, National Advisory Committee on Microbiological Criteria for Foods Conference. All audio connections are muted at this time. I will give you instructions at the time of public comment on how to enter the queue.

Should you need closed captions, please click on the CC icon at the lower right of the webinar screen. There is also an interpreter present on video, and you may pin that video or change the layout to view those screens as needed using the layout button. If you require technical assistance, please open the chat icon at the bottom of your screen and send a message to the event producer. And with that, I'll turn the conference over to Kristal Southern. Please go ahead.

DR. SOUTHERN: Thank you, and good morning, everyone. Welcome to the Plenary Meeting of the National Advisory Committee on Microbiological Criteria for Foods, commonly referred to as NACMCF. I now call this meeting to order.

The purpose of the committee is to provide impartial scientific advice and/or peer reviews to

federal food safety agencies for use in the development of an integrated national food safety systems approach that assures the safety of domestic, imported, and exported foods.

My name, again, is Dr. Kristal Southern. I'm with the USDA Food Safety and Inspection Service. And I serve as the designated federal officer for NACMCF, and as the director of the NACMCF secretariat. Before we get started, I wanted to give you a little bit of background on NACMCF membership and the work of this 2021 to 2023 term committee.

NACMCF members are appointed by the secretary of agriculture. They go through a rigorous process, and that helps to ensure that membership is fairly balanced in terms of points of view represented and the functions to be performed. Committee members are chosen based on their expertise in microbiology, public health, food science, and other relevant disciplines, and this is in order to obtain the scientific perspective, experience, and point of view of all stakeholders.

The activities of the NACMCF are carried out in part by subcommittees that are focused on specific areas being considered by the full committee. It is an honor to be appointed to NACMCF, and we are

incredibly thankful to the members that provide the scientific advice to our federal agencies involved in food safety.

NACMCF has made important contributions to a broad range of critical food safety issues. This 2021 to 2023 NACMCF committee has worked over the last two years on three charges. The USDA FSIS charge on enhancing salmonella control in poultry products was completed and the report was adopted in November 2022.

After adopting that report, the committee began addressing the first question of the Food and Drug Administration's cronobacter in powdered infant formula charge for which they will provide updates at this meeting today. Also today, the committee will discuss and vote on adopting a report they prepared over the last two years in response to questions posed by the Food and Drug Administration on Cyclospora cayetanensis in produce.

Before we dive in, I want to provide a few housekeeping items to keep in mind as we move forward, and just some reminders that the event producer provided as well at the start of the meeting. But first, please note that this plenary meeting is being recorded. FSIS will post the meeting and transcripts when they become available on the FSIS website at

www.fsis.usda.gov.

This is a virtual meeting, and with exception of our committee members and designated speakers, your microphones are automatically muted when you logged in, and you will not have the ability to use your camera during the meeting. As you can see, a sign language interpreter will be present for the duration of the meeting. And in addition, closed captions can be enabled by clicking the closed caption or CC button, or bubble, excuse me, in the bottom left of your screen.

There will be two comment periods today for members of the public. The first will be to receive public comments on the Cyclospora cayetanensis in produce charge, and the second will be to receive public comments on the cronobacter species in powdered infant formula charge.

If during registration, you indicated that you wish to provide oral comments and confirmed your intent to do so via a follow-up email with the NACMCF secretariat, I will call on you during the respective comment period. The event producer will unmute you when it is your turn to speak, at which time a pop-up message will appear, and you'll need to accept this message in order to unmute yourself.

As time allows, we will open up the comment period to those who wish to comment but did not preregister and confirm via email. If this applies to you, feel free to place yourself in the queue during the public comment period by utilizing the raise hand feature. For our phone line, audio-only attendees, you will need to press pound 2 to enter the queue during the public comment period.

We request that all attendees please introduce yourself by providing your name and affiliation before providing comment. Each person will be provided three minutes to make their comments, and then the event producer will move on to the next person in the queue. Again, the event producer will remind us of these instructions at the appropriate time.

And lastly, the chat feature is available for our virtual attendees. Any comments made in the chat will be shared with the committee after today's meeting.

I'll now proceed to taking roll of the NACMCF executive committee and members of the NACMCF committee. When your name is called, please unmute and announce yourself by stating here or present. And we'll start with the executive committee. For the

USDA Department of Agriculture's Undersecretary for Food Safety and NACMCF chair, Dr. Emilio Esteban.

MR. ESTEBAN: Present.

DR. SOUTHERN: Food and Drug Administration's acting director of the Centers for Food Safety and Applied Nutrition and NACMCF vice chair, Dr. Donald Prater

MR. PRATER: Present.

DR. SOUTHERN: Food Safety and Inspection Service liaison Dr. Denise Eblen.

DR. EBLEN: Hi, thanks.

DR. SOUTHERN: Food and Drug Administration liaison, Dr. Eric Olson.

DR. OLSON: Present.

DR. SOUTHERN: Centers for Disease Control and Prevention liaison Dr. Arthur Liang. Okay. Department of Commerce liaison, Dr. Jon Bell. And our Department of Defense liaison, Colonel Alisa Wilma. Okay.

So we'll now move on to NACMCF committee members, and these are the members that will help to establish the -- the count of today's members will establish our quorum for today's meeting. Again, when your name is called, please unmute and announce your presence by stating here or present. Dr. Stan Bailey.

Dr. Peggy Cook.

DR. COOK: Present.

DR. SOUTHERN: Dr. DeAnn Davis.

DR. DAVIS: Present.

DR. SOUTHERN: Dr. Francisco Diez-Gonzalez.

DR. DIEZ-GONZALEZ: Present.

DR. SOUTHERN: Dr. James Dickson. Dr. Joseph Eifert.

DR. EIFERT: Present.

DR. SOUTHERN: Dr. Philip Elliott.

DR. ELLIOTT: Present.

DR. SOUTHERN: Dr. Betty Feng.

DR. FENG: Present.

DR. SOUTHERN: Dr. Kathleen Glass.

DR. GLASS: Present.

DR. SOUTHERN: Ms. Janell Kause. Dr. Mahipal Kunduru. Dr. Elisabetta Lambertini.

DR. LAMBERTINI: Present.

DR. SOUTHERN: Ms. Shannara Lynn.

MS. LYNN: Present.

DR. SOUTHERN: Dr. Wendy McMahon.

DR. MCMAHON: Present.

DR. SOUTHERN: Lieutenant Colonel Audrey McMillan-Cole. Dr. Angela Melton-Celsa.

DR. MELTON-CELSA: Present.

DR. SOUTHERN: Dr. Joelle Mosso.

DR. MOSSO: Present.

DR. SOUTHERN: Dr. Haley Oliver. Dr. Omar Oyarzabal. Dr. Tanya Roberts. Dr. Scott Stillwell.

DR. STILLWELL: Present.

DR. SOUTHERN: Dr. Robert Tauxe. Dr. Max Teplitski.

DR. TEPLITSKI: Present.

DR. SOUTHERN: Dr. Valentina Trinetta.

DR. TRINETTA: Present.

DR. SOUTHERN: Dr. Bing Wang.

DR. WANG: Present.

DR. SOUTHERN: Dr. Benjamin Warren.

DR. WARREN: Present.

DR. SOUTHERN: Dr. Wandy -- excuse me, Randy Worobo. Dr. Teshome Yehualaeshet.

DR. YEHUALAESHET: Present.

DR. SOUTHERN: Dr. Francisco Zagmutt.

DR. ZAGMUTT: Present.

DR. SOUTHERN: Okay. Thank you. So, Event Producer, can we check to see if there are any committee members that may be on the attendee line. If you are, can you raise your hand because we had some technical challenges this morning, so we want to make sure that everyone is on the right line?

MS. LOCKEY: Yeah. Also going through the rest of the names on the list, but if you are on the attendee line, please just send me a chat and I will move you over.

DR. SOUTHERN: Is there anyone on the line?

MS. LOCKEY: Not that I see.

DR. SOUTHERN: Okay. So we'll move forward. We have 20 of 29 members present, which meets quorum for today's meeting. Next, we'll proceed with opening remarks by the undersecretary for Food Safety and NACMCF chair, Dr. Emilio Esteban. That will be followed by the Food and Drug Administration's acting director for the Centers for Food Safety and Applied Nutrition and NACMCF vice chair, Dr. Donald Prater. Welcome, Dr. Esteban.

DR. ESTEBAN: Thank you, Kristal. And good morning to all. I apologize for the delay in starting, also for the fact that I'm joining you via phone at this point. I will continue to try to connect through the application.

We have a very good meeting today, it's a very full meeting. As Kristal stated, there are at least two things we're going to be talking about. One is the adoption of the Cyclospora report which I believe the committee's been working on for a couple

years. And then we're going to hear an update on the charge that the committee received for a question on -- the first question on Cronobacter.

There's been a lot of work on both of the subcommittees, so look forward to the discussion, and I -- from the Office of the Secretary, I really want to thank you all, committee members, for the tremendous contributions that you give to NACMCF, to public health, and for food safety. So thank you, and I will try -- again, I continue to try to join you via the application. Back to you, Kristal. Thank you.

DR. SOUTHERN: Thank you. We'll now go to introductory remarks from Dr. Donald Prater.

DR. PRATER: Yes. Thanks, Dr. Southern. It's a real pleasure to be with you today. I'll keep my remarks short recognizing the time that we have. Just my sincere thanks to all the committee members and to the expert subcommittee members for the work you continue to do to support this committee.

The work is really relevant to what we do for public health. I'm looking forward to hearing more about this. This is my first meeting as vice chair, so while I'm new to this group, not new to food safety. I've been here at FDA for over 20 years, but really looking forward to hearing the work of this

committee to see how it can further help us to protect public health.

So happy to be on. Sincere thanks to everyone. And also thinking about colleagues that may be in Florida or in the path of the hurricane down there. So thanks for all the things that you're doing to continue to support this work. Back over to you, Dr. Southern.

DR. SOUTHERN: Thank you, Drs. Esteban and Prater. The committee will now proceed to a discussion on the report and recommendations regarding the FDA charge Cyclospora cayetanensis in produce.

This subcommittee is led by our members Dr. Max Teplitski and Dr. Peggy Cook. Committee members, to participate in the discussion, please raise your hand to be recognized by the subcommittee co-chairs. You all should have complete control of your mute button, so if we run into any issues, feel free to jump in and we'll see how the discussion flows.

We do, however, request that you mute yourself when you're not speaking. And when speaking, if possible and if your bandwidth allows, we request that you turn on your camera.

I'll now turn it over to you Dr. Teplitski.

DR. TEPLITSKI: Thank you, Dr. Southern.

I'd like to share my screen please.

MS. LOCKEY: Give me one moment, I'll transfer rights. Okay. Go ahead.

DR. TEPLITSKI: Thank you, and good morning. The subcommittee worked for almost two years during which the committee examined reports published in peer-reviewed literature, reports of completed research projects funded by USDA agencies via the CRIS database. And in databases --

MS. LOCKEY: So someone is unmuted or connected twice. One moment please. Okay. Yep, someone was connected twice. You may go -- please go ahead.

DR. TEPLITSKI: Good morning, again. The subcommittee worked for almost two years during which the committee examined reports published in peer-reviewed literature, reports of completed research projects funded by USDA agencies via the CRIS database, and in databases of the Center for Produce Safety.

Authors of key publications representing federal and academic labs were invited to present their discoveries and answer questions from the subcommittee. The subcommittee also interviewed representatives of laboratories who conduct sampling

or testing as well as companies that develop testing tools.

Since the draft report was published in the Federal Register, we received two written comments which were incorporated into the revised report. Specifically, number one, the role of farm laborers in the transmission was challenged in public comments and by the members of the committee.

The subcommittee recognized that the published data on carriage of Cyclospora cayetanensis by the farm laborers is scarce, and the link between carriage by the laborers and contamination of fresh produce has not been rigorously tested and documented. Therefore, the revised language still highlights concerns that symptomatic or asymptomic but infected laborers may be the source of the parasite in a production environment or in other settings including household. But this is framed now as a hypothesis to be tested.

Number two, submitters of public comments also highlighted the need for a comprehensive set of public health measures to understand cases not associated with foreign travel or with consumption of foods obtained through traditional routes of commerce. This concern is now highlighted, especially in the

context of an earlier report, that two states were seemingly responsible for over 35 percent of documented cases of Cyclosporiasis.

Number three, the individual submitting public comments also highlighted the need for robust tools for identifying Cyclospora cayetanensis in the environment, and the subcommittee agrees with these comments.

Number four, in addition, we have obtained clarification from the authors of the broad study in which American genotypes/species of Cyclospora was suggested. The clarification was that the word American in this study is meant all the North American continent and not of the United States of America.

This clarification was helpful, therefore, the discussion on this topic was truncated, but did not change the conclusion reached by the subcommittee that the hypothesis that Cyclospora cayetanensis has become established endemically in the United States needs to be rigorously supported with data.

Number five, a reference to the study of new 2022, a report of an environmental survey for Cyclospora cayetanensis using 18S ribosomal RNA genes as a target for the sequencing of the amplicons was also added to this section on methods in which

molecular detection was discussed.

Number six, there were other relatively minor edits that improved cohesiveness of the narrative. We removed some adjectives, but did not change the conclusions, nor veiled data from completed published but not yet peer-reviewed studies results of which are already well known to the scientific community. With this, I'd like to proceed to the report which is now presented on your screen.

Executive summary of the findings. Cyclospora cayetanensis is a coccidian protozoan parasite belonging to the phylum Apicomplexa. Order, Eucoccidiorida, Family, Eimeriidae described between 1993 to 1994 as a newly identified human gastrointestinal pathogen.

Within the genus Cyclospora, only Cyclospora cayetanensis is known to infect humans. However, recent advances in genomics separated Cyclospora cayetanensis into three proposed species with the two new proposed species also considered parasitic to humans. Cyclospora ashfordi species nov, and Cyclospora henanensis species nov.

For the purpose of this document and to reflect the proposed status of the new nomenclature, Cyclospora cayetanensis refers to all three species of

Cyclospora parasitic in humans.

The parasite produces oocysts that are resistant to harsh environmental conditions, and many chemical treatments commonly used to reduce the presence of bacterial pathogens in the specialty crop environmental, and in agricultural inputs.

Cyclospora cayetanensis is the etiological agent of cyclosporiasis. Its host range is limited to humans. Detected in association with human illness in many parts of the world, Cyclospora cayetanensis previously was considered to be a pathogen acquired during childhood in developing --

(Loss of audio)

(off the record)

(On the record)

DR. TEPLITSKI: -- is key to developing infective prevention and management strategy. I will now read the recommendations. I will pause, and I will proceed with the wrap.

To facilitate research, for example validation of surrogates, studies on environmental systems and attachment, and identification and validation or control strategies, the committee urges development of practical methods to propagate Cyclospora cayetanensis oocysts under laboratory

settings.

Recommendation number two, because of the limited availability of Cyclospora cayetanensis oocysts, research with surrogates, and specifically with the close relative Eimeria, can be informative for identifying control strategies and learn about the persistence in the production environment.

Recommendation number 3, method development for the detection of Cyclospora cayetanensis in food and environmental samples should include the evaluation of multiple genetic targets representing different regions of the genome. Modifications to current molecular methods for the detection of Cyclospora cayetanensis should be thoroughly validated for impacts on specificity before using modified methods on food or environmental samples.

Conversely, detection methods should be designed to be robust, reproducible, and tolerant of minor modifications in methodologies. For example, brand of equipment or reagents, minor deviations in PCR conditions, et cetera, without sacrificing specificity or sensitivity.

Recommendation number four. Given that the hypothesized likeliest source of the parasite in the food production environment, individuals with a

history of recent travel to areas where infections with Cyclospora cayetanensis are common or other exposures to the parasite, preventative measures should center around clear sanitation guidelines, ensuring onsite capacity for implementing sanitation protocols, i.e. readily available handwashing stations with soap, et cetera, and periodic training of the employees. I'm going to pause now.

I will now read the charge from the FDA to the NACMCF verbatim. Background Cyclospora species of protozoan parasites in the phylum Apicomplexa that can parasitize different species of mammals with remarkable host specificity. Cyclospora has a complex lifecycle and can only multiply within an infected host.

Among the Cyclospora species, only Cyclospora cayetanensis is known to infect humans. All other species are associated with infections of other animals. This parasite is characterized by environmentally hardy oocysts that are shed in stools of infected persons. These oocysts are shed unsporulated and are not infectious.

Once released into the environment, unsporulated oocysts require approximately seven to 14 days under certain environmental conditions to

sporulate and become infectious. The oocysts are thought to be transferred to the surface of foods through environmental routes, e.g. through human fecal pollution carried by agricultural water, and subsequently infect the hosts of the produce when consumed.

Once consumed, a sporulated oocyst replicates in the human gastrointestinal track and continue the infection cycle as unsporulated oocysts are shed in stool. This cycle continues as human fecal pollution, again, contaminants the environment.

A limitation to widespread Cyclospora cayetanensis research is the inability to directly culture or propagate the organism. Researchers rely solely on acquired oocysts to conduct research. Some work has been done to use surrogate organisms to mimic the lifestyle of Cyclospora cayetanensis, however, with limited positive results. A positive Cyclospora cayetanensis finding is indicative of the presence of human fecal contamination as humans are the only known reservoir.

Cyclosporiasis is characterized by symptoms such as explosive diarrhea, vomiting, fatigue, and weight loss. Cyclospora cayetanensis has become a major public health and food safety concern during the

last few years. Outbreaks of Cyclosporiasis infect thousands of individuals in the United States annually with a steady increase in reported cases over the recent years.

In 2020, CDC reported 1,221 laboratory confirmed cases of cyclosporiasis in people who had no history of international travel. In 2019 and 2018, there were 2,408 and 2,299 cases reported each year respectively. Comparatively, between 2000 and 2017, the total number of cases reported for cyclosporiasis in the United States was 1,730.

Additionally, Cyclosporiasis typically results in symptomatic illness in the general population regardless of age in the United States. Whereas, in the endemic areas, young children and immunocompromised individuals are most at risk for severe illness.

Outbreaks of cyclosporiasis generally occur during the warmer months of May through September for the northern hemisphere, and November through March for the southern hemisphere. Historically, outbreaks have been linked to the ingestion of contaminated berries, fresh cilantro, basil, and more recently, ready-to-eat bagged salads.

Several efforts have been implemented to

develop molecular detection methods of Cyclospora cayetanensis in food and environmental samples. These methods have been used to assist epidemiological investigations and surveys to estimate the prevalence of Cyclospora cayetanensis in commodities in growing regions. Despite these scientific efforts, there are still several significant knowledge and data gaps that hamper the implementation for effective measures to prevent the contamination of produce with oocysts of this parasite.

The subcommittee obtained 16 questions from FDA. These are the questions. What is known about the prevalence, incidence, and burden of disease of cyclosporiasis in the United States and internationally?

Are there specific segments of the U.S. population that may be at higher risk for infection? What is the geographic distribution of cases in the United States? What is the diversity of Cyclospora cayetanensis genotypes in the United States and internationally? What factors may contribute to contamination with Cyclospora cayetanensis? Are certain factors more significant than others?

How does the seasonality, incidence and prevalence of Cyclospora cayetanensis compare

throughout the United States and internationally, and what factors may contribute? Extrinsic factors that may influence sporulation and survival? Environmental factors, influence in movement, others? What environmental data exists for Cyclospora cayetanensis in food products in environmental samples domestically and internationally? What trends have been observed, and what methods of detection were used?

What types of foods have been attributed to outbreaks of cyclosporiasis domestically and internationally? And what, if any, contributing factors, sources, or routes of contamination have been identified? Is monitoring for Cyclospora cayetanensis by testing food products, agricultural environment, and agriculture inputs being applied as a management strategy currently?

Are there best practices for monitoring the presence of Cyclospora in agricultural production? Has monitoring led to the development and implementation of effective preventive measures? What are the available approaches for characterizing the relatedness of different strains of Cyclospora?

What are currently available test methods? What type of validation has the method undergone? What are the matrices for which the methods have been

validated? What information exists on accessing viability of oocysts? What preventative measures exist for the control of Cyclospora? How effective have then been? What impediments to the development of effective preventative measures in the Cyclospora and how have they been implemented?

What is known about Cyclospora persistence, survival in food such as produce in the environment. What is known about transfer and attachment of Cyclospora cayetanensis from environmental samples to produce? What other coccidian parasites could serve as a surrogate?

Are there indicator organisms that can be used to determine the likely presence or absence of Cyclospora in various matrices? What is known about the role of vectors in the transmission of Cyclospora? What role do farm workers play in the transfer of Cyclospora cayetanensis contamination? How can farm workers serve as both sources and routes of contamination and what strategies have been utilized to mitigate the contamination from farm workers?

Are there practices for maintenance and conveyance of wastewater, septage or human waste that may increase the incidence of Cyclospora cayetanensis contamination? Which waste water septage and human

waste treatments in the United States are effective against Cyclospora cayetanensis? Which treatments may not be effective against Cyclospora cayetanensis?

Does municipal water treatment adequately reduce, control, or eliminate Cyclospora? Can effective municipal water treatment systems be scaled to treat agricultural water used in production? How do practices compare for domestic growers versus international growers?

What elements or points in the parasite lifecycle are potential targets of strategies to disrupt this progression, eliminate or destroy oocysts, stop dissemination into the environment, and prevent food contamination? What are the control measures that should be evaluated for effectiveness against Cyclospora?

What is a recommended protocol for evaluating the effectiveness of control measures? And what are the relevant factors, available data, and data gaps needed to develop an informative quantitative risk assessment model for Cyclospora cayetanensis contamination and risk of illness?

I will highlight the approach by the committee, and after that, we will look at blocks of answers instead of me reading the entire document.

So approach by the subcommittee. A number of comprehensive reviews for both peer-reviewed literature on Cyclospora have been published recently and consulted by this committee. However, in this rapidly involving field, a reliance on only peer-reviewed publications was deemed limiting by the subcommittee.

Therefore, in addition to the peer-reviewed studies accessible via PubMed, the committee consulted scientific reports such as those found in the databases of completed or ongoing projects found in the United States Department of Agriculture Current Research Information System, USDA CRIS database, and the database is maintained by the Center for Produce Safety.

The committee accessed documents released by federal agencies into the public domain, and heard semi-structured testimonies from academic, federal, and industry researchers working with Cyclospora cayetanensis and other parasites. Results of these findings are presented in this report.

The subcommittee notes an ongoing conversation about the nomenclature of Cyclospora and a proposal to separate Cyclospora cayetanensis into three species with the addition OF Cyclospora ashfordi

and Cyclospora henanensis. All three of these species are parasitic to humans. However, because all prior research functionally defines Cyclospora cayetanensis as the only member of the genus responsible for the human Cyclosporiasis, and to reflect that the nomenclature is not widely accepted, and all prior publications refer to this parasite as Cyclospora cayetanensis or C. cayetanensis, the rest of this report will continue to refer to these organisms as Cyclospora cayetanensis or C. cayetanensis.

Finally, the committee notes recent peer-reviewed and non-peer-reviewed studies from academic and federal laboratories that demonstrated limitations of the detection of Cyclospora cayetanensis relying solely on the PCR primers designed to amplify 18S regions of the organism ribosomal RNA genes, and/or internal transcribed spacer ITS.

When PCR products from environmental samples amplified with primers targeted regions of the 18S ribosomal RNA gene were sequenced, the majority of them, over 90 percent, were identified as low-side (ph.) of the Eimeria species, parasitic in various animals, but not humans, or failed to result in a sequenced PCR product matching a sequence from Cyclospora cayetanensis at least under some

conditions.

Sequencing of the low-side amplified using primers targeting the ITS region resulted in three out of 16 confirmations by sequencing. Therefore, throughout this report, when discussing environmental and food samples, the detection of amplicons in a PCR reaction, unless a secondary positive identification step was performed, does not confirm the presence of Cyclospora cayetanensis, nor a presumptive presence of the parasite regardless of the conclusion drawn by the authors of the original publication at the time of the original publication.

The committee organized the charge questions into five groups, sources and routes, questions four, 11, 14, and 15. Group two, prevalence, persistence, and indicators, questions 1, 2, 10, and 13. Analytical methods, question 3, 6, 7, and 8. Group four, control strategies and surrogates, questions 5, 9, 12, 15(b), 16, and 17. And five, relevant factors and data gap, question 18.

I will now highlight groups of answers by the subcommittee, and I will invite any discussion or any comments. I see a hand raised from Dr. Warren.

DR. WARREN: Yes. Thanks, Max. Seeing if I can get my video started here as well. Let's see.

There we go. My comment is back on line 354. If you scroll for the whole paragraph there, I guess 352 to the bottom.

This section is the approach by the committee, and I think this is an area where we've raised some issues in the language in the way that the report talks about some of the studies that were considered. For this particular section being the overall approach taken by the committee, I would think that we would be a little bit summative in nature. That seems odd that we go into citing five different studies at this point in the document.

But in terms of the language and some of the concerns that we've raised through the drafting and development of this report, these studies all used different forms of methods, different types of methods. They all target areas of the 18S portion of the genome, but they use at times different primers. They use different PCR conditions. In short, they're different methods.

And I think one of the things that the report is not clear on is when we discuss the results from some of these studies, it's at times taken to represent all 18S methodology, the results from one or two of these studies, which is an over-generalization

where you wouldn't expect the same performance from one method into a different method.

So that's just an ongoing concern here, and it shows up in two other locations in the report. But I think that is a comment that others have provided as well. And I think the language needs to be modified so it doesn't over-generalize the results of one study to represent a multitude of methods.

DR. TEPLITSKI: Thank you, Dr. Warren. Are there any other comments? All right. In the absence of further comments, we will proceed to the sources and routes.

I understand that the members of the committee were presented with the draft of this report. At this point, I will invite any comments from the committee. In the absence of comments from the committee, we're moving on to the next section of the report.

MS. LOCKEY: Looks like we do have a comment from Emilio.

DR. TEPLITSKI: I'm sorry. Emilio, go ahead please.

MS. LOCKEY: You're unmuted. Please go ahead.

DR. ESTEBAN: Made a comment, nobody reacted

to it, and I'm curious as to whether we can get some reaction by the committee. Otherwise, I don't know what the conclusion of that discussion was. So specifically, I think at line 352 as well before you scroll there, so what is it that the committee wants to do with Dr. Warren's comment? Acknowledge it? Acknowledge it and modify the text? Modify the text? Or simply write it in as a comment from one of the members? Would you like to sort of wrap up that discussion before we move on to sources and routes?

DR. TEPLITSKI: Mr. Secretary, I appreciate your comment. The committee had an in-depth discussion on this topic. The reason this verbiage is included on the front end is to highlight that over the last decades there were a number of studies that surveyed for the presence of Cyclospora in the environmental samples.

These studies came from a diversity of labs. Federal labs, academic labs. They came from the lab domestically, the labs overseas. Those methods used a diversity of targets, many of them targeted 18S reagents or the ITS reagents.

Over the last two decades, the methodology on the detection of Cyclospora has evolved. And the reason we -- the reason this verbiage was provided on

the front end to highlight the fact that as the report discusses, other published studies that conducted various environmental surveys in different areas in the United States and internationally.

Sometimes authors reached a conclusion about the prevalence of Cyclospora in certain samples based solely on the results of the PCR results at the time based on the tools, and the data, and the approaches that were available.

Here, we're highlighting the fact that throughout the report, unless there was a secondary identification steps, instead of stating that Cyclospora was detected in the environmental sample, the report will refer to the detection of amplicons which is an accurate summary of those studies.

If an amplicon was subjected to either sequencing, or if there was a secondary confirmation step throughout the report, you will see the use of the amplicon or the use of the term low-side amplified. It may make for a clunky narrative, but we attempted to make that narrative technically accurate. So that's why there is this introduction on the front end to explain why certain words were chosen throughout the report, if that makes sense, Mr. Undersecretary.

DR. ESTEBAN: No, no. Thank you, Max. And you can call me Emilio. It's cool. I think the --

MS. LOCKEY: We lost Emilio for a second.

DR. ESTEBAN: Are we still on?

DR. TEPLITSKI: Yes.

MS. LOCKEY: Yes, you're on.

DR. ESTEBAN: Okay. So, Max, thank you very much for that clarification. So if I interpret what you're saying is that we're not criticizing the method. There was a plethora of methods used, and so because of that and because of the type of a consistency and the variety of methods, you are suggesting that the report simply makes a distinction by highlighting either low-side or amplicons rather than 18S approach.

So I appreciate your explanation, it makes the rest of the report more understandable to me. And since I'm not an expert, I'll just remain quiet and let you guys continue. Thank you.

DR. TEPLITSKI: Yes, sir. And that's not a comment on the 18S methodology because in some areas of the world, the ITS was used as -- internal transcribed spacer, my apologies -- was used as a target for detection. But again, the science today is better than the science yesterday, and certainly

better than the science half a decade ago.

And because the committee went back to retroactively analyze some of the published reports that were published, we wanted to provide clarity and consistency in the verbiage. And this paragraph explains why this report landed on the use of the word amplicon or amplified low-side unless there was a secondary confirmatory step.

DR. ESTEBAN: Yeah. Perfectly clear to me. Thank you, sir.

DR. TEPLITSKI: Thank you. I'm sorry, if there are no other comments on the source or routes, we'll move on to the next cluster of questions. The next cluster of questions focuses on prevalence, persistence, and indicators.

All right. We'll move on to the next cluster of questions. The next cluster of questions focuses on analytical methods, isolation, concentration, detection, and confirmation. Are there any comments from the members of the committee? Dr. Warren, I see your hand.

DR. WARREN: Yeah. So I apologize for that, I was clicking the wrong button there for a minute. I do have a comment on I believe it is line 1070. Okay.

DR. TEPLITSKI: 1017?

DR. WARREN: Oh, and this version of the report is a little than the one I was looking at. Okay. Hold on one second. The line numbers are different on this one than the one that I was looking at comments on. Hold on one second. Let's see.

So if you go to the start of the analytical methods section, it's the second paragraph. And this is, as I mentioned before in my opening comment, there are three sections in the document where similar language is presented.

This is a second one where some of these studies that used some methods are represented as indicating a common method targeting 18S ribosomal RNA genes. Whereas, there are other methods, different methods that you would expect different results. And again, this language is over-generalizing the results from these studies to mean this would be the expected result for all methods targeting 18S.

DR. TEPLITSKI: Thank you, Dr. Warren. Are there any other comments from the committee? All right. Dr. Warren, do you have any other comments on this section?

DR. WARREN: I believe I do. Hold on one second, because our line numbers are different, I'm trying to find which -- so on Question 3, it's just

the same -- it would be the third occurrence of similar language. Sorry, didn't start my video here. It would be the third occurrence of similar language in the second paragraph under the response to Question 3(a). So just note the -- that additional location within the report where the same language is presented.

DR. TEPLITSKI: Noted. Thank you, sir. Are there --

DR. WARREN: And then --

DR. TEPLITSKI: -- any other comments?

DR. WARREN: -- I had one more comment. I want to make sure -- I think it's in the same section. It's just on a question. Okay. This one would be down under Question -- the response to Question 7. And on mine it's -- okay, let's see. Under Question 7, there's a subheading PCR methods targeting the 18S ribosomal RNA genes. Two, three. This would be at the ending of the fourth paragraph.

DR. TEPLITSKI: I'm sorry. How does the paragraph begin please?

DR. WARREN: It begins, "The current FDA method for detection of Cyclospora cayetanensis in agricultural water." So it's the -- or the paragraph that talks about Chapter 19(c). Yes, that one.

Okay. So in this paragraph, the report is discussing the multi-lab validation study that was used to validate the FDA BAM for use with agricultural water. Down at Line 1347, scroll down. So as part of this publication, after the work was done for the validation study, there were six samples that were taken from open water sources in Maryland, and they were analyzed using the BAM methodology. And from those, there were three of six samples, as the report indicates here, that were found to be positive by the 18S method for Cyclospora cayetanensis.

In that report, we also talk about other work that is going on about other methods. And, you know, certainly 18S methodology is, you know, and it has its issues with specificity given that there's not a lot of, you know, there's parasites that are out there for which there's not full length 18S sequences available. So developing methods for these types of organisms is very difficult.

For that reason, we've acknowledged there's work on other methodology underway. And we talked about using one of those methods as a secondary confirmation for these amplicons. A line there that says 1351 -- or line number 1351 where it says, "Amplicons resulting from these environmental samples

were not sequenced with questions about specificity of detection." That line is not entirely correct.

The report of Durigan 2020 talks about one of these tools that targets the mitochondrial genome as being used as a secondary confirmation. It was cited in that study as unpublished data. We provided that second reference where that data was then subsequently published in a '22 publication where these three samples -- actually, all six of the water samples were analyzed by the mitochondrial method, which is later discussed in this report. And those amplicons were also sequenced confirming the detection by the primary 18S methodology for all three of these samples. So this is an incorrect statement within the report.

DR. TEPLITSKI: So, Dr. Warren, I acknowledge that you have made a comment on the previous version of the draft referencing a Durigan 2023 study.

DR. WARREN: It's 2022.

DR. TEPLITSKI: What?

DR. WARREN: It's Durigan and 2022. Max, I think the line in the version you're looking at would be 1411.

DR. TEPLITSKI: So this is the study that

you're referencing, Durigan 2022, development of a molecular marker based on the mitochondrial genome for detection of Cyclospora cayetanensis in food and water samples?

DR. WARREN: Correct.

DR. TEPLITSKI: Correct?

DR. WARREN: Yes.

DR. TEPLITSKI: So this study, the Durigan 2022 is not the study that is discussed in this paragraph. The study focuses on 18S targets, and this study I believe focuses on the mitochondrial targets. And what is your concern about the sequencing? Is there an unpublished data showing that studies -- that the samples from the previous study are referenced in another study?

DR. WARREN: No, Max. I think the way that the approach to this report was made was you said in the beginning, you're going -- we were going to refer to result as amplicons unless a secondary confirmation was done. In this case, the primary PCR was an 18S qPCR.

And then subsequent to that a secondary detection and confirmation method was used to confirm the results of that PCR. And in this particular case, that secondary method was a mitochondrial-based method

where it amplified and then sequenced segments of that part of the genome, and that successfully confirmed the initial findings of the 18S.

So the language here is raising questions whether or not the detection from the 18S primary qPCR was specific. However, that was accomplished, that was confirmed through the secondary mitochondrial method. That's not dissimilar to some of the other studies that are discussed in this paper where a primary qPCR for 18S was used, and then a secondary method in the case of one of them of full genotyping method that uses eight different locations on the genome was attempted to confirm.

So the way that the report reads here, it infers that the initial 18S detection in the water samples was never confirmed, and it raises questions about its specificity. What we're saying is it was confirmed by a secondary assay which not only included detection of a different portion of the genome, but also sequencing of that amplicon.

DR. TEPLITSKI: May I ask a clarifying question?

DR. WARREN: Mm-hmm. Yes.

DR. TEPLITSKI: So in the Durigan 2022, which is currently not cited, was it the 18S amplicon

that was sequenced, or was the mitochondrial amplicon that was sequenced?

DR. WARREN: No, it was the mitochondrial amplicon.

DR. TEPLITSKI: So just clarifying, and this paragraph refers to the amplicons of the 18S ribosomal RNA which were not sequenced as you're asserting. Is that correct?

DR. WARREN: That's right. The, you know, the way that the 18S in this particular method, sequencing the 18S amplicon is not informative. So a way that the targeted segment of the genome for the 18S method is designed and sequencing that amplicon is not informative for confirmation purposes.

So that's why we have worked on other methodology, other targets which include sequencing of those targets to confirm detection. And in this case, that's what was done, and it confirmed the initial qPCR 18S detection.

DR. TEPLITSKI: May I ask another clarifying question?

DR. WARREN: Again, Max, that's not different than the way other studies that are discussed in this report have approached attempts to confirm initial results from an 18S qPCR.

DR. TEPLITSKI: May I ask a clarifying question?

DR. WARREN: Yes.

DR. TEPLITSKI: Is this statement that the 18S amplicons resulting from the environmental samples were not sequenced, is that the correct statement?

DR. WARREN: It is true that the 18S amplicons were not sequenced. Yes.

DR. TEPLITSKI: Okay. So this statement is accurate to the Durigan 2022, and in light of the Durigan -- sorry. Is accurate per Durigan 2020 and is accurate per Durigan 2022?

DR. WARREN: Well, it's accurate per 2020. In that paper, we also discussed the results as unpublished data at that time. It was just part of a subsequent publication two years later. But it's discussed in both papers.

DR. TEPLITSKI: May I ask another clarifying question?

DR. WARREN: Mm-hmm.

DR. TEPLITSKI: So your assertion is that even though the 18S amplicons in the study were not sequenced as stated, there were other targets, mitochondrial genes that were sequenced in a subsequent study. Is that your assertion?

DR. WARREN: Max, what I'm saying is in this paper, it discusses that the initial detection of 18S through the 18S qPCR, those samples were further analyzed by the method developed for mitochondrial targets. The mitochondrial amplicon was sequenced, and that sequencing confirmed via secondary detection and sequencing method the 18S result.

There are no questions about the specificity of the 18S results for the three water samples because they were further confirmed by a secondary detection and amplicon sequencing method.

DR. TEPLITSKI: The reason I'm asking these questions is that I'm trying to find the verbiage that will be accurate to represent what experiments were conducted in these studies, and what results were obtained. So is that your assertion that -- well, do you agree with the first half of the sentence?

DR. WARREN: If it is -- if you add in to specify that the amplicons from the 18S qPCR were not sequenced, that is a true and accurate statement.

DR. COOK: But, Dr. Warren and Max, a lot of this topic I believe we duly note this and address this, and we can move on to other context of the paper if acceptable.

DR. WARREN: It is to me. We can work

offline on agreeable language. But just noting that this, you know, this was a statement that was not correct in the report, and we need to modify it before final -- the report is finalized.

DR. COOK: Okay. Okay.

DR. SOUTHERN: So --

DR. COOK: Thank you, Dr. Warren.

DR. SOUTHERN: So, yes, we can. In the interest of time, it will be good to move on. There was a lot of committee discussion about some of this in the lead up. However, the intent is to vote on adopting this report today, which is why we're going through it. So if there is language that needs to be addressed, it would not be that we're going to add additional language after this meeting. But the co-chairs will -- the co-chairs, Max and Peggy, will make the decision on to handle each of the comments as they come in.

DR. ESTEBAN: So --

DR. SOUTHERN: So I just want to make that clear.

DR. ESTEBAN: Kristal, Ben, everybody, may I suggest right now an insertion that addresses 50 percent of Ben's comments, and the proposal is simply to insert the word on Line 1351 to say, however, the

18S amplicons were not sequenced, which I think Ben has agreed is a correct statement. So let's at least insert that language that specifies that the 18S amplicons were not sequenced, which is I believe by everybody a true statement. Is that correct?

DR. WARREN: Yes, that's correct.

DR. ESTEBAN: So if we are -- if we specify that part, then that part of the question (indiscernible). Then the next part is should we add text at, you know, the paragraph says, however, additional work conducted with this application mitochondrial amplicons, we solved the issue by whatever. I mean that's -- so just add one sentence (indiscernible) additional work, and then you can answer -- reference to Durigan in 2022 that refers to the mitochondrial DNA, and then the whole paragraph is resolved.

DR. WARREN: Well --

DR. SOUTHERN: Thank you. Thank you, Dr. Esteban. Are there others from the committee who would like to provide additional comments on this discussion? We'd like to hear from other members of the committee on this discussion as well as the potential suggested language that has been offered by Dr. Esteban.

Okay. Hearing none, and if you are trying to speak and your audio isn't working, please try and raise your hand. I'll leave that then to Max and Peggy as the co-chairs, is it your decision to move forward with adding that language as suggested by Dr. Esteban so that we can move to the next section?

DR. TEPLITSKI: So I have a bit of a technical issue which I'm trying to undo restricted editing. But for some reason -- so I'm going to read the suggested verbiage. "However, 18S amplicons resulting from the environmental samples were not sequenced." We'll put a period after it.

And then we'll acknowledge that a secondary step using MITC targets resulted in sequencing of Cyclospora cayetanensis products, something along those lines. Is that acceptable to Dr. Warren and to the rest of the committee? Are there any objections to including that?

DR. WARREN: No, I agree with that. I think we need to just make sure you reference the correct mitochondrial target because there's multiple studies with different mitochondrial targets. So as long as you go back, it's the --

DR. TEPLITSKI: (Indiscernible) -- is that correct?

DR. WARREN: It's the MIT3 was used in that publication.

DR. TEPLITSKI: All right. MIT3 is noted.

DR. WARREN: Yep. Thank you.

DR. TEPLITSKI: Thank you for that comment. Dr. Warren, are there any other comments on the analytical methods?

DR. WARREN: No, that was my final comment on that. Thank you.

DR. TEPLITSKI: Thank you, sir. Are there any other comments from the committee on the analytical methods section?

DR. MCMAHON: This is Wendy. I just wanted to add -- I guess Ben's comment about the reference. Like which -- is it the 2022 reference that would follow that statement then?

DR. TEPLITSKI: Yes, ma'am. It'll be followed by --

DR. MCMAHON: Okay.

DR. TEPLITSKI: -- a Durigan 2022.

DR. MCMAHON: Okay. Thank you.

DR. TEPLITSKI: Thank you, ma'am. All right. We're moving to the next cluster of questions. Control strategies and surrogates. Are there comments from the committee? Hearing none, we're moving to the

next cluster of questions.

The next cluster focuses on relevant factors and data gaps, what we know and what we don't know. Are there any comments from the committee? Hearing none, moving forward.

Dr. Southern, I believe that concludes the discussion of the report by the committee.

DR. SOUTHERN: Okay. So thank you. Thank you, Dr. Teplitski, for that, and also Dr. Warren and others who participated in that discussion. Thank you very much. And then also thank you, Dr. Teplitski and Dr. Cook, for being -- serving as the co-chairs on this subcommittee.

Before we move to public comments, are there any additional questions or comments from the executive committee or members of the committee on the Cyclospora report and recommendations? If so, you can raise your hand or if you are -- most of you should be on the speaker line, you can unmute yourself.

DR. ESTEBAN: Kristal, this is Emilio. I just wanted to thank all the committee for working on this. It's been over two years of work, and to get to a point where we can actually have consensus on the report is very admirable. So thank you, all, for your flexibility and willingness to agree on this advice

for the secretary.

DR. PRATER: Kristal, it's Don Prater here, just adding my thanks to the committee and to the subcommittee. So thank you for your work on this report.

DR. SOUTHERN: Thank you, both. So the Event Producer, are there any hands for the panelists? I don't see any.

MS. LOCKEY: Not that I can see, no.

DR. SOUTHERN: Okay. All right. So we'll keep it moving because we are a little behind, but that's okay. We want to make sure that we allow time for that discussion.

We'll now move to public comment. We had one person that registered to -- that preregistered to provide comment and sent confirmation through a follow up email from the NACMCFP secretariat. So we'll now move to Jennifer McEntire with Food Safety Strategy LLC.

DR. MCENTIRE: Hi. Are you able to hear me?

DR. SOUTHERN: Yes.

DR. MCENTIRE: Wonderful. Well, thank you for giving me the opportunity to comment on this important issue. I am Dr. Jennifer McEntire with Food Safety Strategy, previously with IFPA and United

Fresh.

There are challenges in trying to detect this organism with confidence, as we've heard the robust discussion, in both product as well as the environment. And yet, there are large outbreaks, and even more sporadic cases that clearly need to be managed. So I would like to commend the subcommittee for an outstanding job on the report. It really presents a thorough review of what is known about this organism, which is not much, compared to bacterial foodborne pathogens.

I'd also like to commend FDA for charging NACMCF with this topic and asking great questions, questions that the industry has been asking for years. There's been a lot of discussion earlier about the subcommittee view that positives in many of the research studies are presumptive, that 90 percent false positive rate for environmental samples when using 18S RRNA methods.

While I heard Dr. Warren's concerns and understand that, these are peer-reviewed publications that purport to have found Cyclospora cayetanensis. These are the things we lean on, and it's clear that things we thought we knew might not actually be right and requires the use of methods, newer methods using

forward to have that confidence in the studies which would lead to confidence in mitigations and preventative measures.

I strongly support the subcommittee's recommendation that additional research is needed with respect to survival times and persistence of the organism. I would add persistence in viability in the various stages of its lifecycle and the committee also recommends additional works on sporulation rates with which I absolutely concur.

Given the difficulties in conducting such research when you don't have oocysts because they can't be propagated, and in light of the number of confirmed illnesses in the U.S. each year, I urge FDA to consider how to work with public health officials to collect and harvest more oocysts from those who are infected and share them around with the research community so that we can do more work on this pathogen.

I'd also like to comment on the committee response to question 9 on preventative measures, and specifically on washing. Here the report cites one study that showed it was difficult to remove Cyclospora from raspberries, but it's in conflict with a later statement in response to question 17 that

suggests that washing dislodges the pathogen.

The report also notes in response to question 9 that antimicrobials commonly used in produce wash water are generally ineffective. Only chlorine is mentioned, but PAA is in that header. If the committee was not able to find research on PAA, it would be good to call out that data gap. I did have an inquiry last week from a colleague who was approached by a PAA vendor who assured her, and I quote from her message, "Was sure that PAA would kill Cyclospora." So I think having some clarity around that could be helpful if there's an opportunity to make a quick edit.

Finally, I want to close by supporting a statement in the report in response to question 18, advocating for a risk-based and not hazard-based approach. Given that detection methodology is still evolving, it seems that with advances in genotyping, we'll be able to learn so much more about outbreaks and their vehicles, and from there be able to identify risk factors.

For now, we think that humans are the sole hosts, and I support the committee's recommendation in response to question 5 that monitoring for fecal pollution broadly may be more useful than trying to

find Cyclospora cayetanensis and figure out what that means even if we do think we found it. So I appreciate the committee's time, and I'm happy to follow up on any of these topics.

DR. SOUTHERN: Thank you, Dr. McEntire. I'll go to the co-chairs, Max and Peggy. Did you all want to respond to that, or do we want to acknowledge the comment and move on?

DR. TEPLITSKI: I certainly want to thank my former colleague, Dr. McEntire, for providing the comments. The question 9 and 17 and the PAA in the heading are certainly important considerations. So let's think of a way that we can move forward with the vote this afternoon now, but also acknowledge that minor edits restricted to these two questions may need to be considered.

DR. COOK: Agreed.

DR. SOUTHERN: Okay.

DR. COOK: Yes. Yes, I agree, Max. Thank you.

DR. SOUTHERN: Okay. So we did not have others pre-register to comment. We are quite behind in our agenda, but I will open it up because, again, this is for the purpose of adopting -- voting on the report to see if there are additional persons,

attendees would like to comment. If so, please raise your hand. And you'll be acknowledged by the event producer.

MS. LOCKEY: Yes. If you'd like to make a comment, you can click the raise hand icon located at the bottom of your screen. If you are on the phone only, you can press pound two on your telephone keypad. There is one comment in the chat if you'd like me to read it.

DR. SOUTHERN: Yes, please.

MS. LOCKEY: Looks like from Bianca Preedo (ph.). And they ask, hello, was the update on the cronobacter in powdered infant formula charge already given?

DR. SOUTHERN: Nope. We are going to be moving to that after the vote. We are a little behind on our schedule. That happens at times. But we do complete the meeting. So we'll be moving to that shortly. In the interest of time, we'll go ahead and move forward if there are no additional comments from the audience.

And so now I want to -- thank you to the committee members and commenters who are participating in today's discussion. We'll now proceed with a roll call vote on adopting the report. Is there any

opposition to move forward with the vote, not voting on but to move forward with the vote on adopting the report titled response to questions posed by the Food and Drug Administration Cyclospora cayetanensis in produce?

Okay. Hearing no opposition to moving forward with the vote, we'll now do a roll call vote. Those that are in favor of adopting the report titled response to questions posed by the Food and Drug Administration Cyclospora cayetanensis in produce will as their names are called respond aye, yes, or yay. Those opposed will respond no or nay. To abstain, a member may say present or abstain. If a member is not ready to vote when called upon, you may also say pass and request to be called on to vote again after the roll call is complete.

As the designated federal officer for NACMCF, I'll call -- I will call the role and the assistant committee specialist, Mrs. Shantel Williams, will repeat and record the vote. Just want to confirm, Shantel, are you on the line? Okay.

MS. LOCKEY: Shantel is on the panelist list, however, I'm not sure if she's able to unmute at the moment.

DR. SOUTHERN: Okay. Let me confirm is

Kristi Akers, are you on the line?

MS. WILLIAMS: Are you guys able to hear me?

DR. SOUTHERN: Yes, we can hear you now. Shantel, can you hear us?

MS. WILLIAMS: Yes. I can hear you loud and clear.

DR. SOUTHERN: Okay. Great. So we'll go ahead and move forward with the vote. Again, I will call your name in alphabetical order. Please respond in the affirmative, negative, abstain, or pass. And Shantel, they'll be -- immediately after you give your vote, Shantel will give your name and your vote to ensure that we're -- she will repeat, and that -- make sure we have -- we're recording the correct vote. Okay. So we'll start with Dr. Peggy Cook.

DR. COOK: Yes.

MS. WILLIAMS: Peggy Cook, yes.

DR. SOUTHERN: DeAnn Davis.

DR. DAVIS: Yes.

MS. WILLIAMS: DeAnn Davis, yes.

DR. SOUTHERN: Francisco Diez-Gonzalez.

DR. DIEZ-GONZALEZ: Yes.

MS. WILLIAMS: Francisco Diez-Gonzalez, yes.

DR. SOUTHERN: Joseph Eifert.

DR. EIFERT: Yes.

MS. WILLIAMS: Joseph Eifert, yes.
DR. SOUTHERN: Betty Feng.
DR. FENG: Yes.
MS. WILLIAMS: Betty Feng, yes.
DR. SOUTHERN: Kathleen Glass.
DR. GLASS: Yes.
MS. WILLIAMS: Kathleen Glass, yes.
DR. SOUTHERN: Mahipal Kunduru.
MR. KUNDURU: Yes.
MS. WILLIAMS: Mahipal Kunduru, yes.
DR. SOUTHERN: Shannara Lynn.
MS. LYNN: Yes.
MS. WILLIAMS: Shannara Lynn, yes.
DR. SOUTHERN: Wendy McMahon.
DR. MCMAHON: Yes.
MS. WILLIAMS: Wendy McMahon, yes.
DR. SOUTHERN: Angela Melton-Celsa?
DR. MELTON-CELSA: Yes.
MS. WILLIAMS: Angela Melton-Celsa, yes.
DR. SOUTHERN: Joelle Mosso?
DR. MOSSO: Yes.
MS. WILLIAMS: Joelle Mosso, yes.
DR. SOUTHERN: Omar Oyarzabal?
DR. OYARZABAL: Yes.
MS. WILLIAMS: Omar Oyarzabal, yes.

DR. SOUTHERN: Scott Stillwell? And I believe Scott may be having issues with his audio. My understanding is that he is present. So if you're able to, can you put your response in the chat? Okay. We'll mark it -- we'll just leave it blank for now. We'll move on and then, of course, Scott if you're able to, you can put your vote in the chat to the Event Producer or directly to me?

Robert Tauxe? And I believe Rob may also be having audio issues. So if either of you, Robert or Scott, can hear me, please enter your vote in the chat so that we can record your vote. We can't -- if you are trying to speak, we can't hear you. I'll keep moving. Max Teplitski?

DR. TEPLITSKI: Yes.

MS. WILLIAMS: Max Teplitski, yes.

DR. SOUTHERN: Valentina Trinetta? Okay. I also see that you are on the line. If you are having trouble unmuting, can you please put your response in the chat? Bing Wang?

DR. WANG: Yes.

MS. WILLIAMS: Bing Wang, yes.

DR. SOUTHERN: Benjamin Warren?

DR. WARREN: Yes.

MS. WILLIAMS: Benjamin Warren, yes.

DR. SOUTHERN: Teshome Yehualaeshet?

DR. YEHUALAESHET: Teshome Yehualaeshet, yes.

MS. WILLIAMS: Teshome Yehualaeshet, yes.

DR. YEHUALAESHET: Yes.

DR. SOUTHERN: And Francisco Zagmutt?

DR. ZAGMUTT: Yes. Okay. Thank you, everyone, for the vote. I want to follow up on Scott Stillwell, are you able to unmute so that we can record your vote?

MS. LOCKEY: Scott said yes in the chat.

DR. SOUTHERN: Okay. Thank you. And Robert Tauxe?

MS. LOCKEY: Robert, if you can put your response in the chat please.

DR. SOUTHERN: Okay. And we'll just do one check back on Valentina Trinetta. Okay. That concludes our voting. Shantel, I just want to confirm that Francisco Zagmutt voted yes?

MS. WILLIAMS: Okay. Francisco Zagmutt, yes.

DR. ELLIOTT: Hi. This is Phil Elliott. You didn't call my name.

DR. SOUTHERN: My apologies. Phil Elliott?

DR. ELLIOTT: Yes.

MS. WILLIAMS: Phil Elliott, yes.

DR. SOUTHERN: My apologies for that.

DR. LAMBERTINI: You have my name, apologies if I missed it. My vote is yes.

DR. SOUTHERN: Okay. That was Elisabetta Lambertini. Okay.

MS. WILLIAMS: Elisabetta Lambertini, yes.

DR. SOUTHERN: Okay. And my apologies for missing that, going down the list and trying to make sure I'm only calling on the folks that are present, and I ended up missing some of you, so my apologies. Is there anyone else that is a committee member that I did not call your name for the vote?

And then one last check, Robert Tauxe or Valentina Trinetta, are you able to enter your vote into the chat? Okay. We will consider those two votes are not voting. Shantel, can you please provide the results of the vote?

MS. LOCKEY: Valentina also --

MS. WILLIAMS: We have --

MS. LOCKEY: -- said yes in the chat.

DR. SOUTHERN: Oh, sorry, sorry. Let's go back. Can you --

MS. LOCKEY: Valentina said yes in the chat.

MS. WILLIAMS: Valentina Trinetta, yes.

MS. LOCKEY: And if Joseph Doncore (ph.) a committee member?

DR. SOUTHERN: Who was that?

MS. LOCKEY: Joseph --

MS. WILLIAMS: No, he's --

MS. LOCKEY: Okay.

DR. SOUTHERN: No. Okay. Shantel, so that concludes the vote. Shantel, can you please tell us the results of the vote?

MS. WILLIAMS: We have 21 for yes, zero no, zero abstains.

DR. SOUTHERN: Thank you very much. The yays -- yeah, and if you could, everybody, mute. Now everyone can go back to muting. Okay. The yays have it, and the report title Response to Questions Posed by the Food and Drug Administration Cyclospora cayetanensis in produce is adopted.

Undersecretary Dr. Esteban, as the chair of NACMCF and acting director Dr. Prater as the vice chair of NACMCF, the report has now been adopted officially by the NACMCF committee.

For those who there was information put into the chat, that link will take you to the report that was posted for public comment. Public comment period ended last Friday, August 25th. That is not the same

version as the report that we went over today. That is the first version, and then the report that they went over today is the version that has been updated to account for additional committee discussions as well as written comments that we received, and then of course, as you saw today, some of the comments that happened during the meeting. Once this report that was discussed today has been finalized, that will be posted on the FSIS website for your reading pleasure.

Okay. So, again, thank you, everyone. We had a lot of discussion. Yes, we're behind in the agenda, but we are going to move forward, and we will complete the agenda so I hope you can stay on a little longer with us because now we'll have some updates on the cronobacter species in powdered infant subcommittee on that charge. So this subcommittee is led by our members Dr. Kathleen Glass and Dr. Elisabetta Lambertini. Dr. Glass will provide the updates for this subcommittee.

DR. GLASS: All right. Thank you very much. I'm not seeing being able to go forward. Yep.

MS. LOCKEY: You should be able to click on the screen there and then be able to control the slides. I gave you presenting rights.

DR. SOUTHERN: Kathy, if you look at the top

of this slide, do you see a number 7 with an arrow --

DR. GLASS: Okay. There.

MS. LOCKEY: Yeah. And you (indiscernible) that --

DR. GLASS: We have it. Okay.

MS. LOCKEY: -- too. Yeah.

DR. SOUTHERN: We can skip that slide.

DR. GLASS: All right. Thank you very much. I would like to thank the -- specifically the subcommittee on behalf of Elisabetta and myself for working on this in a very short time period. We received this charge in March and were able to have an in-person meeting in May and all of the rest of it has been done online.

What we are going to be giving today is an interim report that is only going to include charge question number one which is a charge from FDA about cronobacter species in powdered infant formula.

So as the background that we got from FDA is that cronobacter contaminated powdered infant formula has been associated with infections in infants, specifically it's cronobacter sakazakii. That's the one that's most often associated with illness.

We know that cronobacter species can be isolated from powdered and rehydrated formula, from

utensils, environment, animals, and other types of foods. We also know that cronobacter can survive for long periods of time in low-moisture foods such as powdered infant formula.

Given that background, we look at it in perspective of illnesses, specifically that which occurred in 2021 and '22, and eventually resulted in a large recall of powdered infant formula. And as the manufacturer was looking through methods to be able to mitigate this ended up having a powdered infant formula shortage.

So with that, FDA came up with a strategy to prevent cronobacter species illnesses associated with powdered infant formula and released that in November of 2022. To go forward, FDA is seeking advice from NACMCF to address knowledge gaps in key issues related to cronobacter species in four specific areas which are our charge questions.

Our subcommittee will only take a look at phase one, which is charge question one, which is current prevalence and levels of cronobacter contamination in powdered infant formula in the U.S. market, what's known about cronobacter in other foods and other home environment, and the frequency with which these foods and environmental sources might

contribute to human infections.

Because this committee will be rotating off, the rest of the charge will be on -- with the next term. So phase two, which will be completed by next year, is going to be what kind of factors specifically virulence factors, host factors, dose of exposure that place an infant at greater risk of cronobacter infection and severe health outcomes.

Next thing is what kind of food safety management practices can be applied at the manufacturing level of powdered infant formula to help reduce the risk of cronobacter species contamination in the formula or in the production environment.

And lastly, given that powdered infant formula is not sterile, how could food safety messaging be improved for infant care providers with an emphasis on sterile ready-to-use formula for infants at greater risk, and also safe infant formula preparation and storage for infant formulas in general.

So as we said, this committee is charged with question one, which, as we break it down, comes into three sub-questions. What is the current prevalence and level of cronobacter species contamination in powdered infant formula specifically

in the U.S. market. Secondly, what is known about cronobacter species and other foods and the home environment, and the frequency with which these foods or the environmental sources can contribute to human infections.

As the committee looked at this, we realized that questions one, two, three were actually related. So there are going to be some partial answers to questions two and three that are going to be incorporated into our interim report.

Next, due to very little data on prevalence and level of cronobacter species in powdered infant formula, or other foods and environment in the U.S. market, we have expanded the review to include information from outside the United States.

Next, not all cronobacter species are going to be pathogenic, so the interim report has expanded the review to evaluate evidence on epidemiology and risk factors contributing to illness, and which is going to be a partial answer to question two.

Because as we take a look at the prevalence and level information on powdered infant formula and other foods, we need to understand that there's going to be differences in identification and enumeration and detection protocols. And those methods are going

to be at different stages of refinement that might actually effect what the prevalence data is and what the identification's going to be.

So the report has expanded the review to evaluate current methodologies. We were also asked by FDA to review a correlation between its presence of cronobacter and other indicator organisms in processing facilities because some of the information that we reviewed for cronobacter specifically did include information about indictor organisms.

We also took a look at what were going to factors that were associated with the occurrence of cronobacter in the review. We did not address cronobacter infections in elderly populations even though they are still going to be cronobacter species that are going to be included in all of the epidemiology numbers.

In that response, we organized it in these different levels. One being the epidemiology. Next, it's going to be the occurrence in powdered infant formula, ingredients that are used in powdered infant formula, and the production environments that are used both in the dry diary situation as well as powdered infant formula.

Next, what is going to be the occurrence in

other foods and in the home, and institutional environments. And then as we were able to provide data that are going to be in tables as well as in appendices.

As we mentioned with epidemiology and the nature of the pathogen, even though that specifically wasn't in question one, it provided a lot of background for us to be able to evaluate the further questions. Specifically, when we're looking at the occurrence of powdered infant formula -- in powdered infant formula, we were looking at prevalence data, but also expanded it to understanding survival and growth characteristics, indicator organisms, and factors that are associated with occurrence in these facilities.

For a little bit of background, the epidemiology and risk factors, organisms were previously classified as Enterobacter sakazakii, and they were reassigned to a new genus cronobacter in 2007. Along with sakazakii are seven species. Cronobacter sakazakii is the one that's most linked to illness, but less frequently with malonaticus.

So, once again, as we're looking at prevalence data, and if it's only including cronobacter species, is not necessarily going to be

identifying the ones that are most associated with illness.

One of the difficulties in identifying what are going to be some risks is that we have very few cases that are going to be reported in the United States every year with estimated incidence in the United States of about 18 cases of invasive illness in infants. The mortality rate is going to be high, however, and that depending upon the disease manifestation, where the outbreak was, where the location and what kind of treatments were then, the mortality rate may range between 20 to 80 percent.

Currently, it is only a reportable disease in two states in the United States. However, this will change effective January of 2024 where all states will be required to report it. And this may be beneficial to be able to trigger more detailed investigations and get more information. Once again, the committee did not include information about infections related to elderly.

With other risk factors, it was determined that the highest risk infants are going to be those that are going to be premature or low -- and/or low birth weight. Typically, the illnesses are going to occur within 28 days of birth, but it can occur later.

Greatest risk factors have been associated so far with the use of reconstituted powdered infant formula. However, there have been reports of illnesses associated with breast milk that was contaminated with breast pump milk parts.

Now there's little on other information about how other risk factors and correlations between other foods and environmental and illness can occur probably mainly because there -- we have a very low rate of reported illnesses and it's difficult to make that type of correlation.

Thus far, it's unclear what the dose -- infectious dose is going to be. Earlier reports suggested that it was going to be 1,000 CFU. However, it did not include what might be more and repeated dosages within a short period of time. Enumeration from outbreaks suggested that the number of colony forming units were one to 10 colony forming units per hundred grams of powdered infant formula, and that there was a suggestion that there was potential growth after reconstitution that may be contributing to infection.

Effective in 2002, the recommendation that neonate ICUs would use sterilized formula, or at least reduce the hang time, which is basically the full time

that reconstituted formula would be used while it is going to be fed to the infants to be no more than four hours, which is in line with the food code.

However, CDC's advice to parents to reduce the risk is to prepare the formula with hot water, 70 degrees Celsius and then cool, and then use within two hours of preparation, or refrigerate immediately and use within 24 hours.

Now as far as the detection methods, we do now that the methods can affect the reporting of the prevalence data. The detection methods that were used for the various surveys were going to vary. Mostly, they were going to be cultured based for presumptive but also were whole genome sequencing for confirmation. However, there are other phenotypic methods that may be unreliable. So it was clear that there was going to be research that's going to be needed for rapid isolation, identification, and quantification protocols for cronobacter at the species level.

As far as occurrence in powdered infant formula and dried dairy ingredients and the production even related to these, all the published studies that we could find specifically for powdered infant formula are from outside the United States. Also, typically

they're going to be reported as cronobacter species rather than specific pathogenic species.

Regardless, when we take a look at the prevalence rates in the powdered infant formula, it's going to be a wide range. In countries such as the Netherlands, Switzerland, and South Korea, the reported positive rates in powdered infant formula range between 2 and 7 percent. However, ranges of -- in some countries range all the way up to 96 percent of powdered infant included cronobacter.

We have very few enumeration data available. Most of the information is from outbreaks. When they did have surveys, they were less than one colony forming unit per gram, and from the outbreaks, as I mentioned before, was going to be one to ten colony-forming units per 100 grams.

When we take a look at other dry powder, milk powder facilities in the United States, there was a good survey with that, but, once again, it was not specifically for powdered infant formula. There was a wide range of positive samples. Up to 69 percent of the samples were found to have cronobacter species, and about 4.4 percent of the environmental samples were found to be positive. About 1 percent of the one, which is food contact surfaces were going to have

cronobacter. Overall, the prevalence of cronobacter was greater than what was found for salmonella.

What the survey also found out that there was on specific correlation between specific type of dried dairy ingredient and cronobacter. But rather it was -- might be associated with the type of manufacturing environment.

As far as understanding the cronobacter survival and the harborage, we know that cronobacter does not survive through milk pasteurization, but if it is recontaminated, it will survive through the spray drying. They have substantially longer D-values with the dry heat than with wet heat.

More than likely there is going to be the cross-contamination in the manufacturing environment specifically spray drying towers, harborage sites in air filters, transfer by air, in personnel. And if there are any water events in the building or its moisture accumulation, there is the potential of exacerbating the situation. Once it is in the product though, it is very tolerant to desiccation with long survival times reported in diary powders and in powdered infant formula for two years or longer.

Looking at correlation between indicator organisms in cronobacter, we find out that

Enterobacteriaceae is a weak indicator. But currently in the industry, there is found to be no better indicator, and because they're both going to be gram negative organisms, it is what is being used currently as indicators.

As far as identifying cronobacter in other foods and in the home environment, once again, most of the evidence is from outside the United States. We know that cronobacter's home is really in plant-based foods, but it is going to be found in animal-sourced foods and animals.

It has been found in teas, flowers, herbs and spices, and cereals which could serve as cross-contamination for powdered infant formula. It has also been isolated from vacuum cleaners, specifically dust, water, and from outbreaks, open bottled nursery water. Also tap and bottled water in other countries.

Because cronobacter is so ubiquitous, it suggests that other foods and the environment may serve as a source of cross-contamination with powdered infant formula even if the infant formula itself came up as being negative. However, due to the low numbers of illnesses and the difficulty to determine the frequency with which these foods and environmental sources contribute to human infections, we cannot

necessarily make a conclusion at this point.

So this is going to be the conclusion for this particular term. However, there will be further refinement to question one as new evidence become available, and also listen to any comments that might be coming through. And then we still have the response to questions 2, 3, and 4 within the next year. And with that, I'd like to open it up for any kind of questions or comments from the committee.

DR. SOUTHERN: Thank you, Dr. Glass. And thank you for opening it up. So are there any -- are there any questions from the executive committee or members of the committee on the cronobacter charge and the presentation today?

DR. PRATER: Yes. It's Don Prater here from FDA. I want to thank the committee for working on this question. This is such an important issue for us, and really appreciate your work on this. The responses I think will be extremely valuable to us, and so look forward to the questions ahead. But thank you so much for the terrific work on this and very much appreciate it.

DR. ESTEBAN: And, Kristal, this is --

DR. SOUTHERN: Thank you, Dr. Prater.

DR. ESTEBAN: This is Emilio. Kathy,

excellent job as usual. This is a very significant issue as Don just stated and the advice of this committee will give -- will really carry a lot of weight. So I look forward to your recommendations for the other questions, and hopefully won't have to face another crisis like we did here in the month past. So thank you very much for your work.

DR. GLASS: Thank you. Kristal, back to you.

DR. SOUTHERN: Thank you. Thank you, Drs. Prater and Esteban, as well as you, Dr. Glass, for the presentation. And thank you, Dr. Lambertini and Glass, for serving as the subcommittee co-leads on this.

So we did not receive requests to comment on the -- any pre-registration requests to comment on the cronobacter charge. So we'll open it up to the audience. We are a little past our 1:00 o'clock period, but I do want to make sure that those who want to comment have an opportunity to do so.

So just a reminder, each person, if you want to comment, you can raise your hand to get in the queue. You'll have three minutes to make your comment, and then we'll move on to the next person. I'll now hand it over to the Event Producer to receive

public comments on the cronobacter charge.

MS. LOCKEY: And if you'd like to make a comment, you can please use the raise hand icon located at the bottom of your screen to enter the queue. If you're on the phone only, you can press pound two. You will have three minutes, and then please provide your name and affiliation before speaking.

I do not see any hands raised at this time. There is a comment in the chat from Carol Coulane (ph.). Is there a link to the cronobacter report of the NACMFC?

DR. SOUTHERN: So thank you for that comment. Because this is just an interim update on the first question, there is not a full report yet. Once the committee, which will be in the next committee term, will complete the remaining questions, then a full report will be available similar to what we did with the Cyclospora report.

However, this is being recorded, and once the video is prepared and ready to -- we'll be posting it online and you can go back and review it. And we'll also have a transcript of this meeting available on our website as well. Thank you. Are there -- sorry, are there any other comments?

MS. LOCKEY: I do not see any in the queue at this time.

DR. SOUTHERN: Okay. So thank you. I think we'll move on. Just want to say thank you to everyone that participated in today's meeting, especially our committee members and our commenters. It was a great discussion. This brings us to the end of our agenda, but before we go, we have a special presentation for the committee. For that, I'll now turn it over to Dr. Esteban.

DR. ESTEBAN: Thank you, Kristal. And clearly, I've said this a couple times, and I want to say it one more time, which is I want to show my appreciation for NACMCF and the -- not only for the NACMCF members because you guys are good scientists and that's why you were appointed to this job. But also for the NACMCF staff team.

It's not easy to hold these meetings, to moderate the meetings. And you cannot even imagine the paperwork that goes behind all the work to this committee to get this committee really -- to work out. So I want to actually thank in particular some of the NACMCF members that have worked on the charges for -- those three charges for the last three years.

Both USDA and FDA rely on you to provide a

lot of evidence-based pathogen control and prevention recommendations. Really you are actually our guideline for our agency and department. We thank you for your outstanding contributions to the national advisory committee on micro bacteria and foods.

But if you look at the list here, many people are giving tremendous amount of time and expertise to help us making our food supply safer. Your work on the committee and the advisory board is instrumental for us to control pathogens, and so we appreciate it immensely. Thank you. Thank you very much. I see a lot of very good friends on this list, I hope to continue to work with you, and thank you again. Thank you very much. I'll leave it at that.

DR. SOUTHERN: Thank you, Dr. Esteban. I also want to thank all of the NACMCF members for your commitment to the work of the committee, and a special thank you to our outgoing members for this 2021/2023. It's truly been a pleasure working with you over the last several months. And I want to say thank you to the executive committee for supporting NACMCF.

So as you all know, we put out a call for nominations earlier this year. We're still going through that process. And the next time that we meet, we will -- after the new committee members are

appointed by the secretary, the next plenary, which should be later this year, that will be our opportunity to present to you those new members.

We're still going through that process, so if you did apply, just know that that process is still ongoing and decisions, appointments have not yet been made and we'll be updating you all shortly.

So, again, I just want to say thank you to everyone. And then also thank you to our subject matter experts for consulting with the committee and helping them with information that they need to answer the charge questions, and also to the public for continuing to support NACMCF.

A special thanks to our NACMCF secretariat, and especially our advisory committee specialist, Ms. Shantel Williams. I greatly appreciate all of you and your efforts and energy invested to supporting us.

So we have completed the purpose of today's NACMCF Plenary meeting. If there is no objection, we will adjourn. I don't think anyone will object. So I don't hear any, raised hands or anything. Being there is no objection, we now stand adjourned. Thank you and have a wonderful rest of the day.

COURT REPORTER: Off the record at 1:09.

(Whereupon, at 1:09 p.m., the meeting was

concluded.)

C E R T I F I C A T E

This is to certify that the attached proceedings in the matter of:

NATIONAL ADVISORY COMMITTEE ON

MICROBIOLOGICAL CRITERIA FOR FOODS

PLENARY SESSION

August 30, 2023

were held as herein appears, and that this is the original transcription thereof for the files of the United States Department of Agriculture, Food Safety and Inspection Service.

TOM BOWMAN, Reporter

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