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Facultad de Medicina
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DIAGNOSTICO INMUNOLOGICO DE LA AMIBIASIS INTESTINAL EN HECES Y EN SALIVA

T E S I S

Que para optar al grado de

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C O N T E N I D O:

R E S U M E N

En ensayos previos que se han efectuado con el fin de adaptar la técnica inmunoenzimática (ELISA) para detectar antigenos de E. histolytica en materia fecal, en la mayoría de los casos se han utilizado anticuerpos policlonales, los cuales mostraron cierto grado de especificidad. En otros casos se han usado anticuerpos monoclonales cuya caracterización, no ha ido debidamente establecida por lo que los resultados que se han obtenido no son confiables. Por lo anterior, se desarrolló una prueba de tipo inmunoenzimático usando anticuerpos monoclonales dirigidos contra algún epítope de la amiba. Después de ser evaluada la prueba, mostró una alta sensibilidad comparada con los métodos convencionales que normalmente se utilizan, es rápida, y de bajo costo, además de que se requiere de una sola muestra para realizar el estudio. Ahora bien tomando en cuenta la presencia de antígenos en el tejido linfoide asociado al intestino, ellos inducen una respuesta inmune de tipo secretorio, la cual puede tener cierto valor diagnóstico en una buena cantidad de enfermedades infecciosas, en este caso la amibiasis intestinal. La respuesta se caracteriza por la presencia de anticuerpos de la clase IgA y que pueden ser detectados en diferentes liquidos corporales. La técnica inmunoenzimática que se desarrolló utilizando saliva mostró una certeza diagnóstica de un 91 % y una sensibilidad de 85 % así como una especificidad del 94 % demostrándose que la detección de anticuerpos de la clase IgA en saliva es de valor diagnóstico en la amibiasis intestinal.

ABSTRACT

Polyclonal antibodies has been repeatedly applied to the inmuenzimatic assay (ELISA) with the purpose of detecting Entamoeba histolytica antigens in feces, but the technique has proved to be specific only to a certain degree. Other methologies have used monoclonal antibodies, which had not been properly caracterized, rendering the results unreliable. An immunoenzymatic test was therefore devloped using monoclonal antibodies directed to an amebic epitope.

This test is highly sensitive compered to conventional methods, it is cheap and swift, and requieres only one sample to be accomplished.

The presence of antigens in lymnphoid tissue assciated to the intestine induces a secretory immune response characterized by the presence of IgA type antibodies, which can be detected in various body fluids. These may be of diagnostic value in a number of infectious diseases such as intestinal amebiasis. An immunoenzymatica assay was concurrently developed which uses a sample of saliva to detect specific anti-Entamoeba histolytica antibodies. The assay had a diagnostic probability of 91 %, sensitivity of 85 % and specificity 94 % which makes it valuable in the diagnosis of this disease.

O B J E T I V O S

a). Desarrollar una prueba inmunoenzimática utilizando anticuerpos monoclonales para detectar antigeno de Entamoeba histolytica en materia fecal.

b). Evaluar la utilidad de la prueba de ELISA como un instrumento diagnóstico a nivel epidemiológico comparándola con el examen coproparasitoscópico.

c). Desarrollar una prueba inmunoenzimática para detectar anticuerpos de la clase IgA anti-Entamoeba histolytica en la saliva y evaluar su uso potencial en el diagnóstico de la amibiasis intestinal.

I N T R O D U C C I O N.

Las infecciones producidas por Entamoeba histolytica constituyen un problema de Salud pública importante en todo el mundo principalmente en aquellos países en vías de desarrollo. Aunque la infección ocurre por vía gastrointestinal, este parásito puede invadir otros órganos tales como hígado, pulmón, bazo,cerebro, genitales, entre otros., y por lo tanto su importancia radica no solo en la alta tasa de infectividad sino también en que es potencialmente mortal, por lo que se ha estimado que hay a nivel mundial 480 millones de personas infectadas con este protozoario presentándose cerca de 35,000 muertes anuales (1).

Los estudios epidemiológicos realizados en México en cuanto a frecuencia de amibiasis arrojan los siguientes datos: del 2 al 14 por ciento de los pacientes que ingresan a hospitales pediátricos con disentería aguda y diarrea con sangre, son causados por E. histolytica (2), y se han considerado como las formas graves de la amibiasis a la colitis fulminante, el ameboma del colon y la apendicitis amibiana, las cuales son poco frecuentes comparadas con la amibiasis hepática mejor conocida como absceso hepático amibiano. En estudios que se han efectuado, se ha observado en algunos casos un predominio en cuanto a sexo particularmente en el caso del absceso hepático, en donde individuos del sexo masculino desarrollan tres veces más esta patología que los del sexo femenino (3), siendo la mortalidad más frecuente en adultos que en niños. (4,5).

E. histolytica es un protozoario altamente dinámico y móvil que adopta diferentes formas por su capacidad para emitir pseudópodos de manera explosiva de acuerdo a la dirección en que se desplaza; posee una parte posterior que se denomina uroide y algunas prolongaciones citoplásmicas sobre su superficie llamadas filopodios; suelen encontrarse vesículas pinocíticas y vacuolas fagocíticas en número variable, lo que refleja la importante actividad endocítica de este microorganismo. El trofozoíto posee un núcleo prominente con un endosoma central y cromatina la cual está distribuída de manera uniforme y es fácilmente observable al microscopio compuesto mediante el uso de tinciones especiales. El citoplasma contiene gran cantidad de vacuolas, vesículas, gránulos de glucógeno y polirribosomas, estos últimos pueden formar agrupamientos visibles a los que se les denomina cuerpos cromatoides. Por otra parte, los trofozoítos carecen de retículo endoplasmático, complejo de Golgi, centriolos y mitocondrias (6).

Desde el punto de vista metabólico se ha considerado clásicamente como anaerobio, aunque el trofozoíto es capaz de utilizar oxígeno a pesar de la falta de mitocondrias y del ciclo de los ácidos tricarboxílicos; en la cadena respiratoria existen como componente funcional flavoproteínas y proteínas que contienen hierro y azufre (7).

Durante su migración por el tracto intestinal y su establecimiento en el intestino grueso, el trofozoíto se multiplica y se

diferencia en las formas de resistencia denominadas quistes, los cuales poseen una cubierta rígida de quitina. Estas formas son eliminadas con las heces y al ser ingeridas en los alimentos y agua contaminadas originan una nueva infección en el huésped susceptible. La enfermedad puede desarrollarse acompañada de cierta sintomatología, con invasión de la mucosa del colon lo que es fácilmente detectable mediante rectosigmoidoscopía; las heces de estos pacientes pueden contener trofozoítos con eritrocitos en su citoplasma. A todos aquellos pacientes en cuyas heces encontramos quistes en materia fecal, en ausencia de datos clínicos y/o de invasión tisular, se les conoce como portadores sanos ó portadores asintomáticos (8).

Hasta hace unos años el método más certero para el diagnóstico de amibiasis intestinal era el examen microscópico directo de la materia fecal con el que se demuestra la presencia de trofozoítos de la amiba o bien la forma quística. Al respecto Salazar Schettino y Col., en un estudio de frecuencia de parasitosis intestinales, desarrollado en escolares en una zona del sureste del Distrito Federal con condiciones deficientes de urbanizacion, de agua potable y drenaje; aplicaron diferentes exámenes coproparasitoscópicos y observaron que no existe diferencia entre los metodos cualitativos tanto con el directo como con el de Faust y para los cuantitativos demostraron que el Ferreira es el más afectivo, el Kato-Miura resultó ser el mejor para cuantificar huevos de helmintos así como para determinar

el grado de parasitosis; referente a la técnica de Stoll en todos las muestras observaron cifras muy bajas, por lo que concluyeron que para elegir un estudio coproparasitoscópico es necesario considerar los datos epidemiológicos y clínicos, la calidad y capacidad del laboratorio con que se dispone, siendo necesario conocer las ventajas y desventajas e indicaciones del método a implementar y con ello utilizar aquel que cubra la mayor probabilidad de encontrar el ó los parásitos presentes en el intestino de un individuo (9)

Aunque estos procedimientos de cierta manera resultan lentos y costoso ya que requieren de personal altamente calificado y con disponibilidad de mucho tiempo para poder hacer una cuidadosa observación. Al respecto Stamm presentó los resultados de cuatro investigadores quienes encontraron que la probabilidad de diagnosticar amibiasis con una sola muestra era de 30 a 33 % y de 72 a 76 % con 6 a 9 muestras (10). Esto viene a corroborar lo publicado previamente por Swartzwelder quién resaltó los problemas existentes en la identificación del parásito y sugirió además guías para la toma de muestras, su conservación así como su estudio (11). Marsden y Smith analizaron la probabilidad de diagnósticar la enfermedad basándose en una sola muestra observada al microscopio, y encontraron que la probabilidad es de un 50% en una persona que excreta 100,000 quistes diariamente, mientras que en aquella que excreta solo 1,000 quistes al día la probabilidad es de solo el 0.45 % (12,13). Sin embargo Mohapatra, estudió una población de 324 sujetos con amibiasis sintomática y asintomática

mediante métodos de tipo parasitológico e inmunológico,observando que el de concentración con formol éter (Carles) fué más útil que la microscopía directa de tres muestras seriadas, principalmente en aquellos pacientes con colitis, hepatitis y portadores considerados como asintomáticos. Además observó que la hemaglutinación indirecta (IHA) correlacionó debidamente con los resultados de la investigación parasitológica en aquellos pacientes con disentería y hepatitis, probando ser mucho más efectiva en los que presentaron absceso hepático amibiano (14).

Se ha considerado que además de los ya mencionados, existen varios problemas de carácter metodológico que se presentan en los estudios efectuados por coproparasitoscopía, y son aquellos en los que la eliminación de quistes de amibas en materia fecal es baja e irrregular principalmente en personas o en pacientes considerados como portadores asintomáticos,además, el examen microscópico de las heces requiere de una inversión de tiempo, de concentración y de experiencia en la identificación del parásito. La variabilidad intra e inter-observadores es un problema potencial de estos estudios, y no es rara la confusión de quistes de la amiba con otros parásitos o a veces los trofozoítos con macrófagos y otras células presentes en la muestra. La contaminación de la materia fecal con orina distorsiona los trofozoítos y en otros casos puede presentarse interferencia debido a la ingestión de sustancias como el bario, el cual es usado como material de contraste; con el tratamiento con ciertos antibióti-

cos, con el uso de antiácidos no absorbibles, con el tratamiento con antidiarréicos a base de bismuto, de caolín o bien por aplicación de enemas con agua corriente (15).

Uno de los avances más importantes en el estudio de la amibiasis así como en el de la biología de este protozoario, es el hecho de poder cultivar los trofozoítos en forma axénica, siendo Diamond quién desarrolló este tipo de medio (16). Esto ha permitido en los últimos años el desarrrollo de varias técnicas inmunológicas para el diagnóstico de la enfermedad con el propósito de encontrar la más sensible, específica, útil, con aplicación clínica, epidemiológica y que sea de bajo costo. Actualmente hay disponibles varias de ellas para la detección de anticuerpos anti-E. histolytica; sin embargo, existe el problema de distinguir entre anticuerpos generados por una infección nueva o activa de aquellos de tipo residual propios de infecciones anteriores (17).

Ha sido de particular importancia el uso de la contrainmunoelectroforesis para la detección de antígeno de E. histolytica en el suero de algunos pacientes con amibiasis invasora en la cual hay un ahorro de tiempo importante y en la que se presenta una disminución considerable de reacciones falsas positivas y con una mayor especificidad y sensibilidad comparada con la doble inmunodifusión (18).

El ensayo inmunoenzimático fué introducido hace aproximadamente 20 años y el principio fundamental mediante el cual se

basó dicha prueba fué en el de la inmunofluorescencia. Con esta técnica se detecta fácilmente tanto antígenos como anticuerpos. En el inmunoensayo enzimático, las enzimas son utilizadas como marcadores y en contraste con la inmunofluorescencia tienen una capacidad de amplificación muy elevadada, siendo de vital importancia en esta técnica el uso de varias fases sólidas como el poliestireno el cual tiene la capacidad de interaccionar tanto con antígenos como con anticuerpos a muy bajas concentraciones y bajo ciertas condiciones iónicas y detectar lo que se busca en alguno de los fluídos corporales. La simplicidad y bajo costo del ensayo inmunoenzimático son de gran importancia en el diagnóstico de muchas enfermedades infecto-contagiosas producidas por bacterias, protozoarios y helmintos. De hecho la parasitología tiene una posición muy prominente e importante en el desarrollo de este tipo de ensayo inmunoenzimático (19).

El método usual para identificar infecciones parasitarias se lleva a cabo examinando la sangre, o bien muestras de materia fecal para observar huevos, quistes o larvas mediante el uso del microscopio compuesto. Ahora bien, este método es muy poco práctico en infecciones extraintestinales o extravasculares o en estudios epidemiológicos en donde un gran número de muestras deben ser procesadas ofreciendo el inmunoensayo una gran ventaja en el diagnóstico de una gran cantidad de enfermedades infecto-contagiosas. Muchas de las infecciones consideradas como latentes y que se presentan en regiones donde existe un alto índice de

pacientes asintomáticos y en donde solo una minoría se encuentra infectada o en zonas en las que se presentan infecciones repetidas con ciertos parásitos; en la mayoría de ellas, se induce la producción de anticuerpos con títulos muy elevados, principalmente de las clases IgM, IgG e IgE con una amplia especificidad y una extensa reactividad cruzada; en estös casos y de acuerdo a los estudios que se han hecho, es cuando el ensayo inmunoenzimático puede ser considerado de gran ayuda en epidemiología. Esta reacción denominada ELISA (enzyme linked immunosorbent assay) tiene la ventaja de ser rápida, fácil de realizar, y que requiere para su implementación de cantidades muy pequeñas de cada uno de los reactivos que se utilizan (20).

Mediante la aplicacion de éste tipo de técnicas inmunoenzimáticas para la localización y cuantificación tanto de antígenos como de anticuerpos, se necesita para su desarrollo e implementación del uso de conjugados proteína-enzima; los cuales se han preparado mediante un acoplamiento covalente del marcador enzimático al anticuerpo (21).

Recientemente se ha usado la interacción no covalente de la biotina con la avidina la cual ha resultado ser excepcionalmente fuerte con una constante de disociación de 0.1 mM. Este complejo se ha utilizado en varios sistemas como son el de inactivación de bacteriófagos, mapeo celular de genes, absorción selectiva de células e inmovilización de macromoléculas (22).

Se han descrito varios métodos utilizados para la localización de antígeno en cortes de tejido o en otros sistemas de fase sólida basándose principalmente en la fuerte interacción de la avidina y la biotina. La avidina es una glucoproteína de peso molecular de 68,000 que tiene una alta afinidad por la biotina. Una de las principales ventajas que existen al utilizar la combinación biotina-avidina es que cualquier sustancia puede conjugarse a la biotina y ser detectada por la avidina, lo que posibilita su aplicación no solo en la técnica de ELISA sino también en otros muchos sistemas como inmunoquímica, electromicroscopía, Western-blot, citometría e hibridización de DNA entre otros, ya que hace que la reacción presente una sensibilidad razonablemente elevada. La biotina es una molécula pequeña y su conjugación a proteínas ó a otro tipo de sustancias involucra una reacción relativamente sencilla que no afecta la afinidad biológica de la sustancia. En el caso del acoplamiento a un anticuerpo, es importante tomar en cuenta el tamaño molecular, ya que su aplicación a nivel histológico facilita una buena penetración al tejido; así tenemos que en este sistema se pude usar la biotina acoplada al fragmento Fab del anticuerpo (23).

Las sustancias marcadas con biotina pueden ser detectadas por cualquier conjugado de avidina o por alguna otra más compleja como la avidina-fluoresceína, avidina-ferritina, avidina-anticuerpo ó avidina-enzima. El marcaje del anticuerpo unido a biotina da como resultado la formación de un conjugado muy es-

table que puede mantener la misma reactividad hasta por 3 años siempre y cuando se almacene a 4 C. La cristalización de la avidina con o sin biotina es muy estable en un amplio rango de pH y temperatura, particularmente cuando se combina con biotina (24).

Con las técnicas inmunoenzimáticas se pueden detectar diversos tipos de antígenos usando para ello un anticuerpo primario y/o secundario acoplado a la biotina, y haciéndolos reaccionar con peroxidasa de rábano acoplado a la avidina, revelándose a continuación con un reactivo que genere color e indicativo de la reacción enzimática, resultando con ello una prueba más sensible y rápida que aquella que se presenta cuando se usa el sistema peroxidasa antiperoxidasa (25).

Uno de los avances más importantes en la inmunología de la última década ha sido sin duda la obtención de anticuerpos monoclonales, resultado de la fusión de una célula de mieloma con otra célula linfoide proveniente de un animal hiperinmune. La técnica de los hibridomas le ha dado a la inmunoparasitología una nueva dimensión. Su uso provee de datos cuantitativos sobre anticuerpos parasitarios producidos como una respuesta del huésped a una gran cantidad de antígenos relevantes o irrelevantes (26).

Los anticuerpos monoclonales tienen numerosas aplicaciones, entre ellas: el analisis, localización y caracterización de antígenos parasitarios, la exploración de la heterogeneidad antigénica en poblaciones parasitarias; la detección de la

expresión de DNA clonado en varios vectores, la tipificación de parásitos, el desarrollo y estandarización de reactivos con una alta especificidad y con aplicación en inmunodiagnóstico. La tecnología de hibridomas, confiere la ventaja de contar con un aporte constante de reactivos potencialmente puros, aunque tiene la desventaja de reconocer un menor número de sitios antigénicos, contrario al comportamiento de los anticuerpos policlonales.

Recientemente se han utilizado diversos métodos con el propósito de determinar la presencia del antígeno de E. histolytica en diferentes muestras biológicas como en el suero, heces o bien en material necrótico; entre ellas el más utilizado es el de ELISA principalmente aplicado a la materia fecal y mediante el cual se ha establecido que dicho método revela un alto número de casos positivos (27,28).

En el desarrollo e implementación de las diferentes pruebas de ELISA para la determinación de antígenos de E. histolytica en heces se han usado en algunos casos anticuerpos policlonales y en otros monoclonales o bien una combinación de ambos (29). Root desarrolló una prueba inmunoenzimática para detectar en materia fecal antígeno de Entamoeba histolytica utilizando para ello anticuerpos de la clase IgG conjugados a peroxidasa y como soporte discos de poliestireno con lo cual pudo detectar diferentes concentraciones de antígeno pero requiriéndo para su realización de aproximadamente 35 horas, desconociéndose en su estudio los valores de sensibilidad y especificidad (30). Palacios y cola-

boradores en un estudio hecho con el fin de determinar la utilidad clínica de este método encontraron una sensibilidad de 97.5 % y una especificidad de 55 %, cifra muy baja para ser de utilidad en el diagnóstico, y además con el inconveniente de que requiere de incubaciones de toda la noche en cada uno de los pasos, lo que hace muy tardada la prueba que se propone (31). Randall empleó la misma técnica comparando sus resultados en diferentes poblaciones y laboratorios incluyendo en su estudio a un grupo de homosexuales de San Francisco y otro de la ciudad de México, con características clínicas no especificadas. En el grupo de San Francisco encontró una sensibilidad de 42 %, con una especificidad de 100 % con valores de predicción positivo de 100 % y negativo de 89.6% y en el otro grupo observó una especificidad de 54.5 % y con valores de predicción positivo de 38 % y negativo de 75 % (32).

Grundy desarrolló otra técnica inmunoenzimática en donde utiliza anticuerpos policlonales de conejo y de humano, en un doble sistema de anticuerpos. Este sistema tuvo menos reacciones inespecíficas y mejoró su sensibilidad, concluyendo que en todos aquellos estudios en los que se utiliza la prueba de ELISA usando anticuerpos policlonales, se requiere de mucho tiempo para realizarse ya que carecen de especificidad adecuada para ser considerados de utilidad clínica (33). Al respecto y aplicando de igual manera la prueba de ELISA Ungar, usó un anticuerpo policlonal combinado con un monoclonal pero sin pre-

sentar datos del origen, forma de preparación y sin la caracterización inmunológica respectiva de dicho anticuerpo monoclonal, reportó una sensibilidad de 82 %, una especificidad del 98%, con valor de predicción positivo de 86% y negativo de 98% con una prevalencia del 7 %. El reporte especifica que los individuos que fueron negativos a la microscopía y positivos a la prueba de ELISA representan probablemente casos no diagnosticados de amibiasis, proponiendo con ello una prueba más sensible para detectar trofozoítos de E. histolytica (34).

E. histolytica, induce tanto una respuesta inmune humoral como celular, pero hasta ahora no se ha establecido cual de ellas protege contra una reinfección. La respuesta humoral sirve más como un marcador de la enfermedad por la presencia simultánea de IgM e IgG específicos, lo que indica una infección reciente y activa, mientras que los títulos elevados de IgG específica, sugiere la presencia de una infección que se ha padecido y que no está activa. Durante la enfermedad los trofozoítos pueden permanecer viables aún en presencia de anticuerpos específicos (35).

En la caracterización de las proteínas del trofozoíto usando sueros inmunes de pacientes con amibiasis intestinal o con absceso hepático amibiano, se ha observado que, aunque la respuesta de los pacientes es muy variable, la mayoría de los anticuerpos reconocen antígenos de superficie, particularmente aquellos de peso molecular comprendidos entre 40 y 88 kD; sin embargo, no existe relación entre éstas y la duración de la enfermedad o la

sintomatología (36).

Se han detectado títulos elevados de anticuerpos antiamiba en el suero de pacientes con amibiasis invasora aguda, mientras que en los asintomáticos dichos títulos por lo general, se mantienen en niveles más bajos (37, 38).

Otros estudios reportan que trofozoítos incubados con suero de individuos que no han padecido la enfermedad, son destruídos, y que este daño, se lleva a cabo por activación de la vía alterna del complemento y no por la vía clásica que requiere de la presencia de anticuerpos específicos (39,40). Tanto los anticuerpos como el complemento pueden prevenir la iniciación de la enfermedad por cepas virulentas de E. histolytica pero no son suficientes para limitar la infección invasiva. Sin embargo, las observaciones clínicas en individuos que han sanado de absceso hepático amibiano sugieren el desarrollo de una inmunidad protectora contra la reinfección por este parásito (41).

Por otro lado se ha observado que las amibas presentes en las heces de sujetos con disentería, son refractarias a la lisis mediada por los anticuerpos de estos pacientes; no así las amibas provenientes de cultivos axénicos que son lisadas por estos mismos anticuerpos. No se descarta la posibilidad de que existan anticuerpos protectores dirigidos contra antígenos que no se expresan permanentemente (42,43).

En estudios serológicos realizados en pacientes con absceso hepático amibiano, se encontró que durante las fases iniciales de la amibiasis hepática los niveles séricos de IgM e IgG específica se elevan en el 70 % de los casos, los primeros disminuyen a las 32 semanas después de iniciado el tratamiento y los segundos pueden permanecer elevados por mas de un año (44). Las aglutininas y precipitinas pueden persistir por un período de 1 a 5 años. Estos hechos podrían ser tomados en consideración en la interpretación de resultados serológicos; así, una reacción positiva refleja un contacto previo o una infección presente; sin embargo la intensidad de la reacción serológica no ha sido correlacionada con la severidad de la infección (45).

Por lo anterior consideramos la necesidad de desarrollar un método diagnóstico que minimice la presencia de errores intra e inter-observadores, que sea rápida, que no dependa de la integridad del parásito al momento del estudio, que sea altamente sensible, especifico y útil en su detección ya sea en una forma directa e indirecta y en diferentes fluídos corporales, que sea costo-efectivo, que permita estudiar las secuelas de la infección por E. histolytica en el humano, que sirva como un marcador de la actividad patogénica desencadenada por la amiba y que pueda ser útil como herramienta para el estudio del parásito por los biólogos moleculares.

Desde hace varios años un buen número de investigadores estomatólogos han estado caracterizando los componentes salivales en

condiciones normales, así como explorando alteraciones en el rango salival y de acuerdo con ellas detectar una buena cantidad de componentes los cuales se correlacionan con cierta facilidad con un buen número de enfermedades.

El examen de las secreciones colectadas de las glándulas parótidas, submandibular, sublingual así como de la región crevicular de los espacios existentes entre el diente y la gingiva, han sido especialmente valoradas para el diagnóstico diferencial de una buen cantidad de enfermedades infecciosas así como para evaluar los efectos de diferentes agentes farmacológicos y/o regímenes terapéuticos que tienen una repercución importante en la función salival (47).

La presencia de anticuerpos de la clase IgA, puede ser demostrada en diferentes fluídos corporales como son la leche materna, el calostro, el suero, y la saliva y son los que con mayor frecuencia se encuentran y se pueden detectar a muy temprana edad siempre y cuando el estímulo antigénico que se dé, permita su presencia, la cual se ha demostrado que aparece en aproximadamente 14 días después de que el antigeno es presentado al tejido linfoide asociado al intestino (48).

Este tipo de respuesta puede tener valor diagnóstico si se detecta mediante la aplicación de técnicas altamente sensibles como la de ELISA aplicada en muchas enfermedades infecciosas. Así, la presencia de anticuerpos de la clase IgA en fluídos corporales como la saliva, pueden servir como un indicador de la ami-

biasis intestinal lo cual favorece que se amplien las perspectivas de diagnóstico de ésta y otras parasitosis cuya localización es el lumen intestinal.

RESULTADOS

Se anexan tres artículos en los que se comunica:

a). La técnica de ELISA reportada en este trabajo y en la que se utilizó un anticuerpo monoclonal anti-Entamoeba histolytica, se logró detectar 0.1 ug de antígeno membranal y un mínimo de 10 trofozoítos de la amiba dando lecturas lecturas de Densidad Optica en un rango de 0.095 a 0.530 para concentraciones de 0.1 a 10 ug del antígeno respectivamente corrrespondiendo para los trofozoítos usados en un rango de 10 a 10,000 amibas, dan lecturas de 0.057 a 0.307. Al aplicar la prueba a 272 muestras 20 resultaron positivas a la presencia del antígeno de la amiba. Las mismas muestras se estudiaron por microscopía observándose en dos de ellas la presencia de trofozóitos y en las 18 restantes, los quistes. De las 20 positivas, 6 contenían otros parásitos y 50 con dos o más parásitos, resultando 202 muestras negativas tanto al examen coproparasitoscópico como a la determinación de antígeno.

En otro estudio que se realizó posteriormente aplicando la prueba para demostrar la presencia de antígeno a 429 muestras, 26 fueron positivas a E. histolytica, éstas al ser sometidas al examen coproparasitoscopico, 13 resultaron positivas. Cuando se analizaron las historias clínicas de los 26 pacientes, se encontró que los síntomas fueron compatibles con la amibiasis intestinal.

b). En este trabajo también, se observó que la proporción de positividad para cada una de las pruebas que realizaron en forma comparativa (ELISA, coproparasitoscópico OBP, perfil rectal), se encontró que la de ELISA es más sensible que los otros métodos que se utilizaron para la detección de la amiba; de la muestra de pacientes estudiados, el 77.3% (116) de los casos la ELISA fué positiva, corrrespondiendo un 34.7% y 12.6% para el perfil rectal y OBP respectivamente. De los 116 pacientes que fueron positivos, todos recibieron tratamiento anti-amibiano y la sintomatología disminuyó en el 100% de los casos. Ahora bien en 11 de los 15 pacientes con absceso hepático, la ELISA fué positiva y solo en uno de ellos se demostró la presencia de Entamoeba histolytica mediante el examen copropárasitoscópico.

De los 116 pacientes positivos, solo en 52 de ellos se logró detectar E. histolytica aplicando las otras dos pruebas y en 47 se observaron otros parásitos.

c). En este proyecto, se estudió una población de 223 niños en edad escolar con el fin de determinar la presencia de anticuerpos de la clase IgA anti-amiba en la saliva, aplicando para ello el ensayo inmunoenzimático de ELISA, y demostrándose que su presencia puede revelar una infección intestinal por E. histolytica presentando la prueba una sensibilidadde 85% y una especificidaddel 98% con altos valores predictivos, dándose una correlación significativa con la presencia de antígeno en heces así como con el examen coproparasitoscópico. Esta prueba se realiza en menos

tiempo que la del coproparasitoscópico y es de bajo costo; por lo que puede ser aplicada en programas tanto de carácter epidemiológicos como de control de la amibiasis.

DISCUSION.

El diagnóstico de la amibiasis intestinal es comunmente establecida cuando el trofozoito ó el quiste son identificados en las heces, sin embargo , las limitaciones de las técnicas coproparasitoscópicas son bien conocidas; debido a ello, existe una alta proporción de sujetos con patología amibiana diagnosticada solo clínicamente, ya que no se cuenta con una metodología más objetiva, que sea de alta sensibilidad y especificidad para poder con ella detectar la enfermedad; llegándose a considerar en la mayoría de los casos como un criterio alternativo el diagnóstico clínico así como la respuesta positiva al tratamiento anti-amibiano con metronidazol y/o dihidroxiquinoleína. Se conoce perfectamente que los estudios parasitológicos pueden dar lugar a error en la clasificiación e identificación de los parásitos contenidos en una muestra de materia fecal y que pueden estar asociados a una serie de factores como son una inadecuada obtención de la muestra, deficiente conservación, eliminación intermitente de las formas del parásito, tratamiento que ha recibido la persona antes de la colecta de la muestra, la inexperiencia del observador y el tiempo limitado para realizar los exámenes, también es posible que la colección de la amiba en la muestra mediante la rectosigmodoscopía en aquellos individuos con sintomatología clínica de la amibiasis puedan destruirse y no ser detectada al microscopio; no así el antígeno el cual puede detectarse por esta técnica inmunoenzimática.

La prueba de ELISA en heces que se propone para el diagnóstico de la amibiasis intestinal, es una tecnica rápida de alta sensibilidad y especificidad tal y como quedó demostrado en los estudios que se realizaron para evaluarla, y en donde se comparó con los métodos de dignóstico que normalmente se utilizan. La prueba permite detectar E. histolytica en personas con o sin sintomatología de la enfermedad, por lo que puede ser de gran utilidad tanto en el laboratorio de rutina como en estudios de tipo epidemiológico o bien en programas preventivo y/o control de la amibiasis intestinal bajo diferentes condiciones de prevalencia.

En suma consideramos que la prueba para la detección de antígeno de E. histolytica en heces, comparada con otras de tipo parasitológico e inmunológico, constituye un método altamente confiable y práctico para el diagnóstico de la patología intestinal amibiana particularmente por su alta sensibilidad y especificidad, la rapidez en el procesamiento y su bajo costo.

Por lo que se refiere a los resultados obtenidos en la prueba inmunoenzimática desarrollada para la determinación de anticuerpos específicos de la clase IgA anti-Entamoeba histolytica en la saliva, demuestran que su presencia, puede revelar una infección intestinal por este protozoario presentando una sensibilidad mayor que el examen coproparasitoscópico. Su utilidad depende de la prevalencia de la enfermedad así como de la población a la cual pueda ser aplicada, resultando una sensibilidad de

85 % y una especificidad de 98 %, pudiendo excluir amibiasis intestinal con una certeza de 99 %, pero sin poder diferenciar entre un portador asintomático y un episodio de amibiasis invasiva.

CONCLUSIONES.

Se cuenta con una prueba de tipo inmunoenzimática, mediante la cual se puede determinar en heces, antígeno de Entamoeba histolytica utilizando un anticuerpo monoclonal acoplada a biotina y cuyas características más importantes es la de ser altamente específica y muy sensible para poder detectar tanto a pacientes con la enfermedad así como a portadores de la amiba, rápida de efectuar y de bajo costo.

Así mismo se propone una prueba de carácter inmunoenzimático la cual de cierta manera simplifica el diagnóstico de la amibiasis intestinal y se basa en la búsqueda de anticuerpos de la clase IgA específicos anti-E. histolytica én una sola muestra de la saliva. Resultando de igual manera ser muy sensible, específica y de bajo costo pudiéndose diagnosticar individuos asintomáticos así como enfermos con la amibiasis intestinal.

PERSPECTIVAS.

En base al uso de los anticuerpos monoclonales así como de la propia prueba, puede tener diversas aplicaciones, una de ellas es la de poder hacer una diferenciación del agente etiológico con respecto al absceso hepático amibiano ya que se confunde con mucha facilidad con otro tipo de alteraciones como pudieran ser abscesos piógenos producido por microorganismos de tipo anaerobios o bien por otra causa.

Ahora bien se conoce que el perfil rectal así como la toma de la muestra mediante el uso del rectosigmodoscopio nos da un 75% de posibilidades para poder detectar la amiba mediante el examen coproparasitoscópico por lo que de igual manera la aplicación de la prueba para determinar el antígeno de la amiba a diferentes niveles del recto podría proporcionar una información más exacta de la distancia a la cual se debe de tomar la muestra para ser analizada.

Así mismo su aplicabilidad pudiese ser de gran importancia en estudios de suero en los que la determinación del antígeno se hiciese principalmente en aquellos casos en los que la amibiasis es extraintestinal con localizaciones a nivel de hígado cerebro, corazón, genitales o en piel.

Por lo que se refiere a la técnica usada en la determinación de anticuerpos específicos anti-Entamoeba histolytica de la clase

IgA puede ser aplicada en otras parasitosis cuya localización sea a nivel del tracto intestinal; en el sida, o bien para la determinación de cierto tipo de hormonas, drogas, virus, y bacterias relacionadas con cierta patología oral, etc.

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Diagnosis of *Entamoeba histolytica* in Feces by ELISA

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A diagnostic test for intestinal amebiasis was developed by using an ELISA that incorporated a well-defined monoclonal antibody specific for a membrane antigen of *Entamoeba histolytica*. The ELISA was found to be sensitive and quite specific for *E. histolytica*, since there were no cross reactions with other parasites, especially *Entamoeba coli*, with which *Entamoeba histolytica* is often confused. In addition, this test detected not only *E. histolytica* trophozoites but also cysts. This technique will be of great value in the rapid and accurate diagnosis of *E. histolytica* in human fecal material.

Key words: Monoclonal antibody, parasitic diagnosis

INTRODUCTION

Worldwide, infections caused by *Entamoeba histolytica* are an extremely important public health problem. Although this protozoan infects the large intestine, it can invade other organs such as the liver, brain, skin, genitals, lung, etc. (1). Diagnosis of intestinal amebiasis by microscopic identification of cysts or the vegetative form of *E. histolytica* has the disadvantages of requiring not only personnel with experience in such identification but also samples taken on three different days which, in practice, often means a delay in diagnosis owing to lack of adequately supplied samples. Many attempts have been made to adapt an immunoenzymatic assay (ELISA) to the detection of *E. histolytica* antigen(s) in fecal material (2–4). In the majority of such attempts, the use of polyclonal antibodies produced relatively unreliable results because of lack of specificity (5,6). In one report, monoclonal antibodies were used; however, the monoclonals were not immunochemically characterized to determine specificity (7).

In this work, we report the development of a diagnostic test for intestinal amebiasis by means of an ELISA in which a well-defined monoclonal antibody, specific for a membrane antigen of *E. histolytica* (8) was used.

MATERIALS AND METHODS

Trophozoites of *E. histolytica*

Trophozoites of the pathogenic *E. histolytica* strain HM1:IMSS were maintained in axenic culture in Diamond TY-1 (9) and were harvested for use in the exponential phase of growth.

Isolation of Plasma Membrane From *E. histolytica*

Cytoplasmic membranes from *E. histolytica* were obtained by the method of Aley et al. (10).

Production of Polyclonal Anti-*E. histolytica*

New Zealand white rabbits were immunized by injection in the footpads with 1.0 ml of an emulsion consisting of 5×10^6 viable trophozoites in 0.5 ml 0.15 M NaCl and 0.5 ml Freund's incomplete adjuvant (Difco, Detroit, MI). These animals were again injected intramuscularly and subcutaneously with the same quantity of viable trophozoites (without adjuvant) 8 and 15 days later. When these animals demonstrated the production of serous antibodies against membrane antigen as determined by double immune diffusion (11), they were bled. The immunoglobulin fraction was purified from the separated sera by precipitation with ammonium sulfate (12).

Production of Monoclonal Anti-*E. histolytica*

BALB/c mice were immunized by intraperitoneal (i.p.) injection of 2×10^6 viable *E. histolytica* trophozoites. One

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month later, they were injected i.p. with 100 μg of *E. histolytica* membrane antigen. Four days after this last injection, the animals were killed and the spleens removed. These immune spleen cells were fused with SP2/0 myeloma cells (ratio 100:1) by using polyethylene glycol 4,000 (Sigma, St. Louis, MO). Production of antibodies by hybrid cells was determined by ELISA. Those hybrid cells producing specific anti-*E. histolytica* were cloned in soft agar (13). Production of specific immunoglobulin in the resulting colonies was determined by ELISA. Positive clones were expanded in vitro and thereafter, in vivo, in ascitis form in BALB/c mice previously inoculated i.p. with Pristan (Sigma). Eight days later, the ascitis liquid was obtained and the immunoglobulin fraction was recovered.

That this monoclonal antibody is specific to strains of *E. histolytica* has been demonstrated (8). In an ELISA, this monoclonal antibody was shown to react with soluble antigen from *E. histolytica* strains HM1:IMSS, HM3:IMSS, HM38:IMSS, and HK9:NIH but not from *E. moshkovskii*, *E. invadens*, or *E. histolytica*-related Laredo strain. This specificity was also shown in immunofluorescence studies, in which this monoclonal antibody reacted with trophozoites from *E. histolytica* but not from *E. moshkovskii*, *E. invadens*, or *E. histolytica*-related Laredo strain.

Preparation of the Anti-*E. histolytica*-Biotin Conjugate

The monoclonal antibody was dialyzed against 0.1 M $NaHCO_3$, pH 8.0. Thereafter, the protein concentration was determined (14) and, was adjusted to 100 μg/ml with 0.1 M $NaHCO_3$. For each 100 μg of antibody, 100 μl of DMSO containing 1.1 mg-hydroxysuccinimido-biotin (Sigma) was added. After the mixture had been incubated (2 h, 25°C), the reaction was stopped by the addition of 0.1 volume of 1 M NH_4Cl, pH 7.2. The resulting mixture was dialyzed against 0.01 M phosphate buffer containing 0.15 M NaCl (PBS) pH 7.2 (4°C, 24 h) (15).

Preparation of Fecal Samples

Frozen fecal samples (272) were received from the Hospital General del Centro Medico del IMSS and from the Instituto Nacional de Pediatria, Mexico City. The samples were coded in a double-blind study. After the data from ELISA were obtained, the results were compared to that from microscopic studies of samples prepared by the Faust method (16). For ELISA, each fecal sample was homogenized in PBS, pH 7.4, which contained sodium dodecyl sulfate (0.05%), and three concentrations (50 mg, 5 mg, and 0.5 mg per ml) were tested.

ELISA Determinations of *E. histolytica* in Feces

Each well of polystyrene plates (Immulon II, Dynatech Laboratories, Alexandria, VA) was coated by the addition of 10 μg of polyclonal anti-*E. histolytica* in 100 μl 0.1 M carbonate buffer, pH 9.6 and by drying the plates in a dessicator under vacuum. Previous testing of varied amounts (0.01, 0.1, 1.0, 10, and 100 μg) of this antibody showed 10 μg to be optimal. At the start of the assay, the wells were washed three times by adding PBS, pH 7.4 containing 0.05% Tween 20 (PBS-Tw) (200 μl per well, 3 min, 20°C). The wells were then blocked by the addition of 1% (w/v) bovine serum albumin (BSA) in PBS (200 μl per well; 4h; 20°C; gentle agitation).

The sensitivity of the test was determined by parallel experiments in which *E. histolytica* membrane antigen (0.1–100 μg) and *E. histolytica* trophozoites (1–1,000) were used. Therefore, to the appropriate plates, known quantities of either membrane antigen, trophozoites, or feces were added. After incubation for 1 h at 37°C and 2 h at 20°C (with agitation), the plates were washed three times with PBS-Tw containing 0.05% BSA (PBS-Tw-BSA) for 3 min before each change. The monoclonal anti-*E. histolytica*-biotin conjugate (50 μl) was added to each well and the plates were incubated 1 h at 37°C and 2 h at 20°C, with gentle agitation. The plates were then washed with PBS-Tw-BSA as before. Immediately thereafter, 50 μl of streptavidin-peroxidase (Amersham International, UK) which had been diluted 1:400 in PBS containing 1% BSA was added to each well and the plates were incubated for 1 h at 37°C. The plates were then washed five times for 3 min with PBS-Tw and 50 μl of substrate was added to each well. The substrate was prepared by adding 10 mg o-phenylenediamine and 4 μl 30% H_2O_2 to 10 ml of 0.1 M citrate buffer, pH 4.5. The plates with substrate were incubated for 4 min at 20°C in the dark. To stop the reaction, 200 μl of 1 M H_2SO_4 was added to each well. The plates were then read at 495 nm in an ELISA processor M (Behring, Marlburg, West Germany).

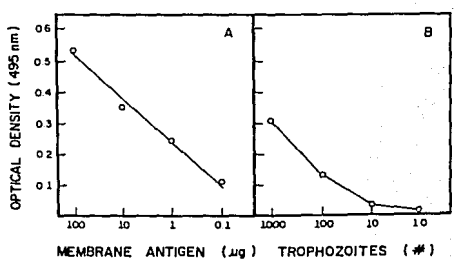

Fig. 1. Sensitivity of the ELISA. Points indicate reactivity of the monoclonal anti-*E. histolytica* against *E. histolytica* membrane antigen ***(A)*** or trophozoites ***(B)***.

TABLE 1. Determination of the Presence of *E. histolytica* in Feces by Microscopy and by ELISA

Parasite	Microscopy	ELISA
Parasites absent	202/272	252/272
Parasites present	70/272[a]	20/272
E. histolytica	20/70[b]	20/70[a]
Other parasites	50/70	0/70[c]
E. coli	14/70	0/70
G. lamblia	14/70	0/70
E. nana	11/70	0/70
I. butschlii	4/70	0/70
Ch. mesnili	4/70	0/70
A. lumbricoides	3/70	0/70
T. trichura	1/70	0/70

[a]Some samples contained more than one parasite.
[b]Same 20 samples which were positive in ELISA.
[c]Negative ELISA implies no cross reaction.

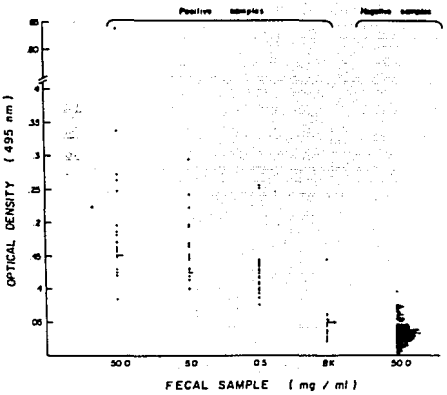

Fig. 2. Range of reactivity of the ELISA in *E. histolytica*-positive and -negative human fecal samples used at the concentrations shown on the abscissa.

RESULTS

The ELISA in which our monoclonal anti-*E. histolytica* was used was sensitivite, detecting as little as 0.1 μg of *E. histolytica* membrane antigen and ten *E. histolytica* trophozoites. ELISA reading ranged from 0.095 to 0.530 for the amounts (0.1 to 10 μg) of membrane antigen used (Fig. 1A). Although the use of one trophozoite produced a reading close to background values, 10–1,000 trophozoites gave detectable readings (0.057 to 0.307) (Fig. 1B).

This ELISA proved useful for the detection of *E. histolytica* in feces. Of the initial 272 samples studied, only 20 were positive for *E. histolytica* as determined by ELISA. These findings were confirmed by microscopy: of the 20 samples that were positive for *E. histolytica*, two contained trophozoites and 18 contained *E. histolytica* cysts. Also, of these 20, six also contained other parasites (Table 1). Fifty other samples were found to be infected with one or more parasites but these were free of *E. histolytica*. No parasites were detected in the remaining 202 samples.

The optical density readings of the three dilutions of the 20 *E. histolytica* positive fecal samples were compared among themselves and to the readings of the negative controls. Although the readings of the three dilutions gave values that were significantly different from those of the negative controls ($P < 0.001$), they did not differ among themselves ($P > 0.05$) (Table 2; Fig. 2).

Since these preliminary studies indicated that the quantity of stool sample to be analyzed was not critical for evaluating the presence of *E. histolytica*, in a later study the amount of feces was not weighed. In this study, 429 samples were examined and 26 were positive for *E. histolytica* as determined by ELISA. By microscopy, only 13 of these 26 samples and no others were positive (Table 3). When the clinical records were examined to determine the reliability of the ELISA test, it was found that the symptoms of those patients with the positive stool samples were compatible with a diagnosis of amebic infection. Here again, by microscopy, some samples positive for *E. histolytica* contained other parasites, while

TABLE 2. Sensitivity of ELISA to Different Concentrations of *Entamoeba histolytica*-Positive Feces

Group	Fecal sample (mg/ml)	No. of samples assayed	Optical Density$_{495\ nm}$ ± S.E.	P[a]
1	50.0	20	0.151 ± 0.024	< 0.001
2	5.0	20	0.111 ± 0.011	< 0.001
3	0.5	20	0.086 ± 0.011	< 0.001
4	Blank[b]	20	0.025 ± 0.005	—
5	Blank[c]	202	0.025 ± 0.002	—

[a]The P value was compared to the background values obtained in group 4 or 5.
[b]Background value of *E. histolytica*-positive feces in control plates in which either polyclonal or monoclonal antibody was not used.
[c]Background values from the 202 *E. histolytica*-free fecal samples.

TABLE 3. Determination of the Presence of *E. histolytica* in Feces by Microscopy and by ELISA[a]

Parasite	Microscopy	ELISA
None	213/429	0
E. histolytica	13/429[b]	26/429[c]
Other parasites	216/429	(0/216)
E. coli	29	0
G. lamblia	57	0
E. nana	59	0
I. butschlii	9	0
Ch. mesnili	8	0
A. lumbricoides	30	0
T. trichura	1	0

[a]The sample for the ELISA assay was taken without weighing the feces.
[b]Thirteen of the 26 found positive by ELISA and no others.
[c]Some samples contained more than one parasite.

other negative samples were found to be infected with one or more parasites but to be free of *E. histolytica*. No parasites were detected in the remaining 213 samples. Therefore, in addition to being sensitive, the ELISA assay is quite specific for *E. histolytica* since there was no cross-reaction with other parasites and there were no false positives or false negatives.

DISCUSSION

The ELISA method used in this work showed that the monoclonal anti-*E. histolytica* could detect the presence of amoebic antigen in fecal samples and was sufficiently sensitive to detect 0.1 μg of this antigen or ten or more *E. histolytica* trophozoites. As stated in Materials and Methods, this monoclonal antibody has been shown to have the necessary specificity to distinguish between *E. histolytica* and other *Entamoeba* species (8). This specificity was also demonstrated here by the lack of reactivity with the other parasites, especially *Entamoeba coli*, with which *Entamoeba histolytica* is often confused. Repetition of assays on samples which had been frozen for over 30 days showed no change in the results.

This technique, therefore, is not only sensitive and highly specific but also rapid and precise. Since it obviates the need of microscopic studies and serial samples, a greater number of samples can be processed in less time. Because this ELISA also detected *E. histolytica* cysts, the identification of asymptomatic carriers is facilitated. This technique will be of great value in the rapid and accurate diagnosis of *E. histolytica* in fecal material.

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Evaluation of the ELISA Test for Detection of *Entamoeba histolytica* in Feces

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The clinical utility of an ELISA test with monoclonal antibodies to detect antigen of *Entamoeba histolytica* in feces was evaluated in 150 patients with gastrointestinal symptoms. Each subject was examined by rectosigmoidoscopy with rectal smear and/or a triple stool search for ova-bacteria-parasite (OBP); in addition, one stool sample was collected for the ELISA test. All the tests were independent and double blind. *E. histolytica* was detected by OBP and/or rectosigmoidoscopy in 66 patients; 61 patients had other parasites; and in 23, no parasites were identified. Of all patients, 116 were positive for the ELISA test. Of these, *E. histolytica* was identified in 52. In 47, other parasites were identified and in 17, no parasites were found. The ELISA test with a monoclonal antibody against *E. histolytica* antigen showed higher sensitivity than the standard diagnostic methods: the ability to detect the presence of *E. histolytica* antigen regardless of the destruction of the parasite or of the error due to misidentification of the parasite resulting from faulty preparation of the samples.

Key words: Monoclonal antibodies, amebiasis, parasitic diagnosis

INTRODUCTION

Infection due to *Entamoeba histolytica* remains an important public health problem around the world, particularly in underdeveloped countries. The importance of amebiasis is related not only to the high infection rate but also to the potentially fatal complications associated with invasion of other organs by *E. histolytica* (1,2).

The standard diagnostic method for intestinal amebiasis is the detection of *E. histolytica* by direct microscopic examination of stools. However, asymptomatic carriers who are the major source of infection are not easily diagnosed by this method. In these cases, examination of at least three samples with the use of concentration techniques is usually needed (3,4).

Immunological tests using ELISA methods have been proposed as a diagnostic alternative (5–9), with the advantage of being highly sensitive, specific, clinically useful, and cost-effective. Del Muro et al. (10) developed an ELISA test to detect antigen of *E. histolytica* in stool using monoclonal antibodies. This test yielded a 100% sensitivity and specificity, and it had the ability to detect the presence of the parasite even when samples were frozen for up to 30 days. It should be noted that in the latter study the control group included asymptomatic patients without amebiasis.

The aim of the present study was to assess the clinical utility of the ELISA test with monoclonal antibodies for antigen of *E. histolytica* in stool in the differential diagnosis of patients with vague abdominal complaints.

MATERIALS AND METHODS

Patients

Upon their initial visit to any of the out-patient clinics of the Hospital General de Mexico, Secretaria de Salud, 150 consecutive patients were invited to participate in the project provided they met the study criteria. Criteria for patient eligibility was a minimum age of 15 years and at least one of the following groups of symptoms: a) colitic syndrome, defined by at least three out of four symptoms, namely abdominal pain, constipation and diarrhea, and adbominal distention; b) dysentery or dysentery-like symptoms regardless of the evolution time; or c) amebic abscess of the liver characterized by pain in the right upper abdominal quadrant, with fever, hepatomegaly, ultrasound image compatible with hepatic abscess, and IHA titer greater than 1:256 (11). The ameba was considered responsible for the clinical symptoms if the

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coproparasitoscopy and/or rectal profile and/or ELISA were positive.

Patients were excluded from the study if they had received antiparasitic or antimicrobial drugs during the two weeks prior to the study, or if they did not have the results of either a sigmoidoscopy with rectal smear or a triple stool search for ova-bacteria-parasite (OBP).

The average age of the patients was 37 ± 14 years (range 15–70). Sixty-seven percent (101 cases) of the sample were females. On average, the patients had been without treatment for 21 days before collecting the fecal sample (range 15–120 days).

All patients with amebic pathology received specific treatment with metronidazol (30 mg/kg a day in three doses for 10 days) and/or diiodohydroxiquinoline (650 mg three times a day for 15 days). Subjects with results negative for *E. histolytica* did not receive treatment until the etiology of their disease was determined.

Fecal Samples

Fecal samples for the ELISA test were obtained within 48 hours following the first visit of the patient. The samples were coded in a double-blind study. Samples for OBP search were obtained on consecutive days until three were collected. The parasitologic and the immunologic study were done independently.

ELISA Technique

A well-defined monoclonal antibody, specific for *E. histolytica* (12), was employed for the ELISA technique. In brief, each fecal sample was homogenized in 0.01 M phosphate buffer containing 0.15 M NaCl (PBS), pH 7.4, which contained sodium dodecyl sulfate (0.05%), and added on wells of polystyrene previously coated with 10 μg of polyclonal anti-*E. histolytica* (100 μl) in 0.1 M carbonate buffer, pH 9.6. After incubation for 1 hour at 37°C and 2 hours at 20°C (with agitation), the plates were washed three times with PBS, pH 7.4, containing 0.05% Tween 20 (PBS-Tw) and 0.05% bovine serum albumin (BSA) (PBS-Tw-BSA) (200 μl per well) for 3 minutes before each change. Afterwards, a monoclonal anti-*E. histolytica*-biotin conjugate (50 μl) was added to each well and the plates were incubated for 1 hour at 37°C and 2 hours at 20°C, with gentle agitation. The plate were then washed with PBS-Tw-BSA as before. Immediately thereafter, 50 μl of streptavidin-peroxidase (Amersham International, UK) which had been diluted 1:400 in PBS containing 1% BSA was added to each well. The substrate was prepared by adding 10 mg of o-phenylene-diamine and 4 μl 30% H_2O_2 to 10 ml of 0.1 M citrate buffer, pH 4.5. The plates with substrate were incubated for 4 minutes at 20°C in the dark. To stop the reaction, 200 μl of 1 M H_2SO_4 were added to each well. The plates were then read at 495 nm in an ELISA processor M (Behring, Marburg, FRG) (10).

This ELISA test with monoclonal antibody against *E. histolytica* detects the presence of quantities as small as 0.1 μg of *E. histolytica* membrane antigen, and 10 trophozoites of *E. histolytica* (10). In this study we found that at 0.120 O.D., the true positive cases could be optimally discriminated from the true negative cases (sensitivity, 99%; specificity, 100%) (13). With this test, the presence of both cysts and trophozoites of *E. histolytica* are detected. The monoclonal antibody used is specific against *E. histolytica* strains HM1:IMSS, HM3:IMSS, HM38:IMSS, and HK9:NIH, and it does not react against *E. moshkovskii*, *E. invadens*, *E. histolytica* of the Laredo type, nor against *Escherichia coli*.

OBP Search

Triple stool search for OBP was done by direct microscopic examination using the standard Faust's concentration method (14).

Rectal Profile

A rectosigmoidoscopy with direct parasite search in rectal mucus was performed in all patients except in cases of amebic liver abscess.

RESULTS

The proportion of positivity for each of the diagnostic tests (ELISA, OBP, rectal smear) and for each clinical category is shown in Table 1. The ELISA tests proved more sensitive than OBP and rectal profile in detecting amebic pathology, since 77.3% of the cases were ELISA positive and only 34.7% and 12.6% were positive by rectal profile and OBP, respectively. The 116 ELISA positive patients received antiamebic treatment and the symptomatology disappeared in 100% of the cases.

In 11 of the 15 patients with amebic liver abscess, the ELISA test was found positive whereas only in one of them, the presence of *E. histolytica* in feces was demonstrated by OBP. In these patients, no rectal profile was performed (Table 1).

TABLE 1. Relation Between the Presence of Several of the Clinical Entities Considered as Amebic and the Positivity of the Different Diagnostic Methods Used

Clinical diagnosis	No. of patients (n = 150)	Assay performed (positive no.)		
		ELISA	O.B.P.	Rectal profile
Liver abscess	15	11	1	N.D.[a]
Diarrhea	16	14	0	1
Colitic syndrome	117	89	17	49
Dysenteric syndrome	2	2	1	2

[a]N.D., not done.

TABLE 2. Parasites Found in Rectal Profile or Fecal Samples

Parasite	No. of patients (n = 150)
E. histolytica	66
Alone	41
With other protozoa	16
All other nematodes	9
E. coli	14
Giardia lamblia	5
Enteromonas hominis	8
Endolimax nana	1
Chilomastix mesnili	2
Trichuris trichiura	3
Strongyloides stercoralis	1
Ascaris lumbricoides	5
Hymenolepis nana	5
Enterobius vermicularis	14
Multiple nematodes	3
Without demonstrated parasites	23

Of the 116 patients with *E. histolytica* detected in feces by ELISA, the ameba was identified by other tests in only 52 cases. In 47 of the samples examined, other parasites were also found (Table 2), and, in the remaining 17 samples, no parasites were demonstrated.

Among the 34 subjects with a negative ELISA test (23%), *E. histolytica* was found in 22 subjects (65%) by coproparasitoscopy and/or rectal profile (data not shown). In two of these subjects, the symptomatology did not improve after the specific treatment, confirming afterward the diagnosis of Crohn's disease and intestinal tuberculosis. In the remaining 20 patients, no organic diagnosis was confirmed; the patients continue under study and none of them has shown a satisfactory response to antiamebic treatment with metronidazol plus diiodohydroxiquinoline or dehydroemetine. In the 12 remaining cases, no evidence of *E. histolytica* was found.

DISCUSSION

In the present study we found, that the ELISA test for the determination of the *E. histolytica* antigen with monoclonal antibodies showed higher sensitivity than the standard diagnostic methods in the population studied, in conditions where differential diagnosis in patients with nonspecific gastrointestinal symptoms is important.

The diagnosis of amebiasis is commonly established when the cyst or trophozoite is identified in feces (11). However, the limitations of these diagnostic techniques are well-known (15–18), particularly because there is a high proportion of subjects with amebic pathology from a clinical standpoint who show a positive response to specific treatment in spite of having a negative coproparasitoscopy and/or rectal profile. As a consequence, due to the absence of a standard diagnosis with adequate sensitivity for all the positive cases, we considered the presence of clinical symptoms suggestive of amebic pathology associated to a positive response to antiamebic treatment with metronidazol and/or diiodohydroxiquinoline as an alternate diagnostic criterion.

The ELISA test for antigen of *E. histolytica* was shown to be more sensitive to diagnose intestinal amebiasis than the standard diagnostic methods, since it detected 77% (116/150) of the subjects with symptomatology suggestive of amebiasis. Only in 52 cases was the diagnosis of amebiasis also confirmed by the OBP search and/or the rectal profile. Moreover, when the alternate clinical criterion of response to treatment was used as diagnostic criterion, the ELISA test was able to detect 100% of the cases. On the other hand, in subjects with a negative ELISA test, but in whom *E. histolytica* was identified by other diagnostic methods (22/34 subjects), we consider that the positivity of the parasitological studies may be due to errors in the classification of parasites such as *E. coli* or *E. hartmanni* and *E. histolytica* (3,15–18).

The possible reasons for the low accuracy obtained with the standard diagnostic methods, OBP and rectal profile are inadequate sample collection, deficient conservation of the samples, intermittent elimination of the parasite in feces, and antiparasitic or antibacterial treatment prior to sample collection. Inexperience and lack of knowledge, as well as the limited time available for performing the examinations are also sources of error. In fact, the efficiency of the microscopic detection of *E. histolytica* by skilled operators has been reported to be 10–33% (15–18). Furthermore, it is also possible that the ameba collected in the sample from rectosigmoidoscopy in individuals with clinical symptoms of amebiasis could be destroyed or escape microscopic detection but not the antigenic discovery by the ELISA method.

In summary, we believe that the ELISA test for detection of antigen of *E. histolytica* in feces, compared with other present parasitological diagnostic methods, constitutes a potentially useful tool in the diagnosis of intestinal amebic pathology because of its high sensitivity, speed in processing, and lower test costs.

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Diagnosis of Intestinal Amebiasis Using Salivary IgA Antibody Detection

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This investigation sought to determine whether detection of salivary IgA antibodies to *Entamoeba histolytica* could identify intestinal amebic infections among 223 school children. Four groups of children were identified through coproparasitoscopic examination: *E. histolytica* as the only parasite (33%); *E. histolytica* and other parasites (22%); other parasites only (20%); and parasite-free (25%). The diagnostic accuracy of salivary IgA antibodies to an *E. histolytica* membrane extract was 91.5% (sensitivity, 85%; specificity, 98%), maintaining high predictive value at different prevalences. Also, a positive correlation ($r = .753$, $P < .001$) was observed between fecal *E. histolytica* membrane antigen levels and salivary IgA antibody activity. Measurement of IgA antibodies in saliva may be useful in diagnosing intestinal infections with *E. histolytica* within a wide range of prevalences. Moreover, sampling of saliva may be a useful non invasive test for immunoepidemiologic surveys.

Entamoeba histolytica infection is distributed worldwide and is a public health problem in many developing countries, where it causes severe morbidity and mortality [1]. Moreover, amebiasis is not uncommon in industrialized nations, particularly among homosexual men [2–4] and immigrants from endemic areas [5].

At present, diagnosis of intestinal amebiasis, including in asymptomatic carriers, relies on finding the parasite in feces [1]. However, coproparasitoscopic (CPS) examination is time-consuming, requires expertise, and may have limited use in massive screening. As an alternative to direct observation of the parasite in feces, various ELISAs for the identification of *E. histolytica* antigens in stool have been introduced [6–9]. Nevertheless, collecting fecal material may still be cumbersome in epidemiologic surveys. Moreover, some ELISAs may rely on the availability of well-characterized monoclonal antibodies [8, 9].

The presence of microbial antigens in the gut-associated lymphoid tissue induces immune responses at all mucosal sites, without microbial invasion [10, 11]. Moreover, secretory immune responses have diagnostic value in infectious diseases that affect the mucous membranes, even in the absence of serum antibodies [12]. Therefore, salivary IgA antibodies to *E. histolytica* may contribute to the diagnosis of intestinal amebiasis whether invasive or asymptomatic, as both pathogenic and nonpathogenic amebae that colonize the human intestine may induce secretory IgA responses. It has been reported [13] that secretory antibodies to *E. histolytica* are present in human milk, and it was proposed that these antibodies could be of value in epidemiologic studies on amebiasis. However, milk or colostrum antibodies are relevant only to lactating women.

On the other hand, salivary antibody activity to *E. histolytica* has been observed, although a poor relation was found between intestinal amebic infection and salivary antibodies [14, 15]. Nevertheless, given the potential advantages of the secretory immune response as an indicator of infectious diseases affecting mucosal sites, we attempted to determine whether salivary IgA antibodies could identify current intestinal infection due to *E. histolytica*.

Materials and Methods

Individuals. We studied 223 school children (116 boys, 107 girls) 5–14 years old (mean, 9.6) living in an area without public services or sanitation. Children had no symptoms or signs of amebic liver abscess or dysentery. This population was chosen because of its expected high exposure to *E. histolytica* and because school authorities were willing to participate in the study. The sampled population does not differ from other children living in slums in Mexico City.

CPS examination. Parents were given suitable containers and instructed to obtain the children's early morning fecal sample. Stools were transported and processed within 6 h after collection.

A thorough CPS examination was carried out on a single, fresh unstained sample, concentrated by Faust's flotation method [16]. Fecal

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The children agreed to participate in this investigation and informed consent was obtained from their parents or guardians in accordance with the International Guidelines for Biomedical Research Involving Human Subjects as contemplated in the Mexican legislation on this subject and under the approval from the Biosafety and Ethical Committees at the Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

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smears were studied by an experienced parasitologist who, when compared with findings of five experts, had 100% agreement (κ = 1), over a set of 27 test slides.

CPS and immunologic evaluations were done in a double-blind fashion: neither the parasitologist nor the immunologist knew the other's results until completion of the study.

Four groups of children were identified on the basis of this examination: group Eh, whose feces contained *E. histolytica* as the only identifiable parasite; group Eh + OP, those with amebiasis and at least one other intestinal parasite; group OP, those harboring various intestinal parasites, not *E. histolytica*; and group NP, those apparently free of intestinal parasites.

Saliva collection. Nonstimulated saliva was obtained as previously described [17]. Briefly, each child was asked to dribble into a disposable funnel from which ~5 ml of whole saliva was collected into a test tube immersed in ice. The saliva was centrifuged at 2500 *g* and the supernatant was frozen at −20°C. At testing, the sample was thawed at 4°C and clarified by centrifugation at 14,000 *g*.

Preparation of E. histolytica *plasma membranes.* Trophozoites of the pathogenic *E. histolytica* strain HM1:IMSS were harvested at log-phase growth from axenic cultures in TYI-S-33 medium developed by Diamond [18]; membranes were isolated as previously described [19]. Briefly, 10^7 cells were washed in a buffer containing 19 m*M* phosphate and 0.27 *M* NaCl (pH 7.2). The cells were resuspended at 2×10^6 cells/ml in the same buffer plus 10 m*M* $MgCl_2$, mixed for 5 min with an equal volume of concanavalin A (1 mg/ml), and centrifuged at 50 *g* for 1 min. The cell pellet was resuspended in 12 ml of 10 m*M* Tris-HCl buffer (pH 7.5), containing 2 m*M* phenyl-methyl-sulfonide-fluoride and 1 m*M* $MgCl_2$, ground in a glass homogenizer and centrifuged through a sucrose gradient (250 *g* for 30 min). The pellet was resuspended in 1 ml Tris-HCl, 1 *M* α-methylmannoside, and stored at −20°C. Total protein concentration in the membrane preparation was measured by Bradford assay [20] using the microprotein assay (Bio-Rad, Richmond, CA) and bovine serum albumin as a standard.

Antibody analysis. The search for salivary IgA antibodies to *E. histolytica* by IgA ELISA was done according to standard procedures [21]. Each well in an Immulon II microtiter plate (Dynatech, Alexandria, VA) was coated with 100 μl of a 10 μg protein/ml solution of the *E. histolytica* membrane preparation in 0.1 *M* carbonate buffer (pH 9.6). After overnight incubation at 4°C, the wells were washed three times with PBS (10 m*M* phosphate, 0.14 *M* NaCl) containing 0.05% Tween 20 and blocked for 1 h at 37°C with 1% serum albumin. The wells were incubated for 2 h at 36°C with undiluted clarified saliva, then washed and incubated with alkaline phosphatase–conjugated goat anti-human IgA (A-3400, Sigma, St. Louis). Subsequently the wells were incubated (30 min at 37°C) with freshly prepared *p*-nitrophenylphosphate in diethanolamine buffer (pH 9.8). Substrate conversion was assessed at 410 nm in a MR-650 EIA reader (Dynatech, Chantilly, VA), and antibody activity was expressed as the mean of the absorbance readings in triplicate wells, relative to a blank sample containing substrate solution. Results were obtained using amebic membrane antigens as substrate. The reactivity of a crude soluble amebic extract was compared with that of the membrane preparation, using five saliva samples, and no differences in reactivity were observed.

Detection of E. histolytica *in stools by ELISA. E. histolytica* antigen in feces was detected by ELISA as previously described [9].

Table 1. Intestinal parasite infections and salivary IgA antibody activity to *Entamoeba histolytica* in 223 school children.

Group	No. of patients (%)	Absorbance at 410 nm (mean ± SD)
Eh	75 (34)	.389 ± .16
Eh + OP*	48 (21)	.433 ± .19
Other protozoa	30	.405 ± .20
Nematodes	13	.461 ± .16
Nematodes and protozoa	5	.478 ± .15
OP†	45 (20)	.151 ± .06
Entamoeba coli alone	18	.159 ± .05
Endolimax nana alone	3	.124 ± .02
Giardia lamblia alone	14	.156 ± .06
Nematodes	4	.170 ± .07
Multiple parasites	6	.204 ± .24
NP†‡	55 (25)	.163 ± .05

NOTE. Eh, *Entamoeba histolytica*; OP, other parasites; NP, no parasites.
* Antibody activity not significant against Eh.
† Antibody activity significant at $P < .001$, Student's *t* test, against Eh and Eh + OP.
‡ Antibody activity not significant against OP.

In brief, the feces were homogenized in PBS containing 0.05% SDS and centrifuged at 250 *g*. The supernatant was incubated in ELISA wells previously coated with the immunoglobulin fraction of rabbit hyperimmune serum to *E. histolytica*. Antigen captured in the wells was tagged by incubation with a biotin-labeled monoclonal antibody that recognizes a membrane protein specific for *E. histolytica* [22]. The wells then were incubated with avidin-peroxidase conjugate, followed by development with orthophenilendiamine in the presence of hydrogen peroxide. Substrate conversion was measured at 490 nm.

Statistical analysis. The sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of the IgA-ELISA were calculated as described previously [23], where: SE = proportion of children with a positive IgA-ELISA for children with a positive CPS; SP = number of children with a negative IgA ELISA among the total children with a negative CPS; PPV = the number of positive CPS cases among all positive IgA ELISA cases; NPV = the number of negative CPS children among all negative IgA ELISA cases. False-positive rate = 1 − specificity. False-negative rate = 1 − sensitivity. Diagnostic accuracy is the number of correct diagnoses (true-positive and true-negative cases) over the total number of cases. The optimum A_{410} cut off value for the IgA-ELISA was selected by means of a receiver operating characteristic (ROC) curve. Student's *t* test was applied to the comparison of means. The correlation coefficient was obtained through computerized means (Program 6D; BMDP Statistical Software, Los Angeles). *P* values correspond to two-sided tests, and a critical value of $P < .05$ was considered significant.

Results

Of the 223 children studied, 168 (75%) had intestinal parasites, as determined by CPS examination (table 1). The prevalence of infection with *E. histolytica* was 55% (123 cases). In 75 (34%) *E. histolytica* was the only parasite identified and in 48 (21%) was associated with other parasites. In 45

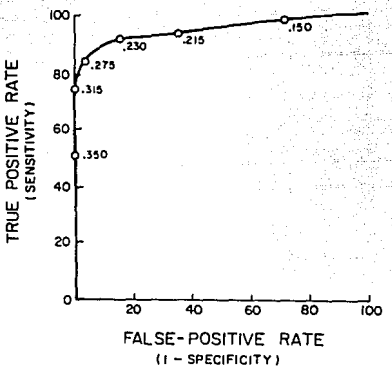

Figure 1. Receiver operating characteristic (ROC) curve showing that 0.275 is the optimal A_{410} cut off value for detection of anti-*E. histolytica* IgA antibodies in saliva.

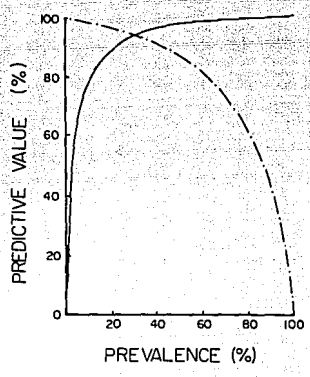

Figure 2. Effect of prevalence on positive (– – –) and negative (– • –) predictive values of ELISA for anti-*E. histolytica* IgA antibodies in saliva.

children (20%) parasites other than *E. histolytica* were found, while 55 children (25%) were apparently free from intestinal parasites.

Mean absorbance values for IgA salivary antibody activity obtained from groups Eh (.389 ± .16) and Eh + OP (.433 ± .19) were significantly higher ($P < .001$) than those from groups OP (.151 ± .06) and NP (.163 ± .05). No significant difference in IgA antibody activity was observed between those children infected only with *E. histolytica* and those who had intestinal amebiasis associated with other parasites, such as *E. coli* (.159 ± .05) or *Giardia lamblia* (.156 ± .06) (table 1).

IgA antibodies to *E. histolytica* were present in saliva samples from 85% of children infected with this parasite. The diagnostic accuracy for the presence of salivary IgA antibodies to *E. histolytica* was 91.5%. Sensitivity and specificity values for this IgA ELISA are shown in figure 1. The IgA ELISA confirmed infection with *E. histolytica* in 105 of 123 children with amebiasis (SE = 85%); 98 of 100 children with a negative CPS result were confirmed by ELISA (SP = 98%). IgA antibody activity to the membrane extract was found in saliva samples from 2 children whose stools were negative for *E. histolytica*, yielding a false-positive rate of only 2%. On the other hand, 18 children infected with *E. histolytica* showed no IgA antibody activity in saliva, a false-negative rate of 15%.

The predictive values of the IgA ELISA at different prevalence levels of amebiasis are shown in figure 2. For a prevalence of 55%, as observed in this study, the probability of having intestinal amebiasis for an individual with a positive ELISA was 98%, whereas the corresponding NPV was 85%.

A significant correlation ($r = .753$, $P < .001$) was observed between the parasitic load, as evaluated from the levels of *E.*

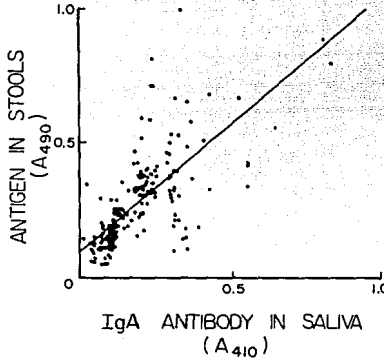

Figure 3. Correlation between ELISA results to detect *E. histolytica* membrane-specific antigen at A_{490} and ELISA values for salivary IgA antibody activity at A_{410} in 223 school children studied, as determined by Pearson's correlation coefficient; $r = .753$, $P < .001$.

histolytica antigen in feces and salivary IgA antibody activity (figure 3). A good association was found also between the diagnosis of amebiasis through CPS examination and the presence of amebic antigen in feces; 119 of 123 CPS-positive children tested positive for amebic antigen, and only 6 CPS-negative children had *E. histolytica* antigen in their stools.

Discussion

These results demonstrate that IgA antibodies to *E. histolytica* in saliva can reveal a current intestinal infection with this parasite. Further, this secretory immune response may also reflect the parasitic load, as a positive correlation was observed between salivary IgA antibody activity and the levels of *E. histolytica* membrane antigen in stools. However, this secretory immune response to *E. histolytica* is not expected to differentiate between an asymptomatic carrier and an individual with invasive amebiasis, because secretory immune responses to microbial antigens are elicited in gut-associated lymphoid tissue without the need for invasiveness [10, 11]. Whether the secretory immune response to *E. histolytica* plays a protective role in intestinal amebiasis remains to be clarified.

Secretory antibodies to *E. histolytica* have been found in the colostrum [13] and saliva [14, 15] from individuals infected with this parasite. However, the reported findings seem inadequate for diagnostic purposes. Speelman and Ljungstrom [14] reported a poor correlation between salivary antibodies and intestinal amebiasis, but only 8 of 251 individuals studied were infected with *E. histolytica*. Moreover, the sensitivity and specificity of the ELISA used were not reported and could not be calculated from their data. In another study [15] of 33 lactating women among whom asymptomatic amebiasis was confirmed in 61%, the sensitivity and specificity of ELISA for secretory antibodies in saliva was 32% and 55%, respectively, as calculated from the reported results.

In contrast, in the present study, based on a single CPS examination as the reference standard, the IgA ELISA correctly classified, either as true positive or true negative, 91.5% of 223 children with a high sensitivity (85%) and specificity (98%).

Nevertheless, saliva samples from 18 individuals whose feces contained *E. histolytica* showed no IgA antibody activity. These false-negative results could be attributed to recently acquired infections in which the antibody response had not yet appeared. In humans, IgA antibodies appear in external secretions ~14 days after presentation of antigen to the gut-associated lymphoid tissue [11].

The consequences of a false-negative IgA ELISA in a symptomless carrier would be associated with the risk of *E. histolytica* transmission and the possibility of developing the disease. However, in endemic areas, it might not be cost-effective to treat all infected individuals, because of the high risk of reinfection [1]. In these areas, pathogenicity markers may be useful in selecting asymptomatic carriers for treatment. Zymodeme analysis has been proposed as a tool to differentiate pathogenic from nonpathogenic isolates. However, it has been shown that isolates carrying nonpathogenic zymodemes may change and express pathogenic markers [24, 25]. Nevertheless, the clinical relevance of these findings remains to be determined.

IgA antibody activity to the membrane extract was found in saliva samples from two children whose stools were negative for *E. histolytica*. This false-positive rate of 2% could be associated with infections that were cured shortly before the examination; in these cases the test may have detected residual IgA antibodies. Nevertheless, the reported short duration of the human IgA antibody response [10, 11, 25] may pose further advantages in the assessment of amebiasis, because serology cannot distinguish between antibodies generated by a current active infection and long-lasting residual antibodies from an earlier episode [26]. Although these children were not treated and followed to evaluate this possibility, preliminary findings of a cohort study of 42 adults with intestinal amebiasis show a decrease of salivary anti-*E. histolytica* IgA antibodies over 45 days after treatment (unpublished data).

The diagnostic performance of the IgA ELISA will depend on this test's capability for detecting *E. histolytica* infections and on how good the CPS examination is as the reference standard. The sensitivity of three consecutive CPS examinations with Faust's method is 60%–80% [27]. Even if the new test is better than the reference standard, the inaccuracy of the standard will make the IgA ELISA appear inferior, when in fact it might reflect more closely the true prevalence of the disease [23]. Moreover, the sensitivity of the IgA ELISA was at least as good as that of a single CPS examination; therefore, it may be suitable for epidemiologic surveys.

The sensitivity and specificity of a diagnostic test remain constant at different prevalences [23]; however, the clinical utility of the IgA ELISA described depends on the prevalence of amebiasis for the population in which the test will be applied (as shown in figure 2). For instance, in communities with an amebiasis prevalence <5%, as in the USA, a negative IgA ELISA will exclude intestinal amebiasis with a certainty >99%, whereas only 74% certainty is obtained at a prevalence of 70%. However, at a prevalence <5%, a positive IgA ELISA will confirm the diagnosis of amebiasis with a certainty <60%, whereas 99% certainty is obtained at a prevalence of 70%.

The results obtained in children living in a highly endemic area may be applicable to other high-risk groups, even in industrialized nations, where the prevalence of amebiasis is as high as 36% among male homosexuals, inmates at mental institutions, or immigrants [28].

The IgA ELISA offers various advantages over other diagnostic procedures. CPS examination is time-consuming, labor-intensive, and requires a skilled parasitologist [1], whereas the use of enzyme-linked assays to detect amebic antigens in stool reduces the time required to examine fecal material and inter- and intraobserver variability. However, handling of feces remains cumbersome. Therefore, epidemiologic surveys based on the screening for salivary antibodies can be carried out with greater ease and lower cost than can stool or serum examinations.

These results demonstrate a high diagnostic accuracy for the IgA ELISA and indicate that this test can be useful in immunoepidemiologic and preventive programs for the control of intestinal amebiasis under different prevalence conditions.

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