A STUDY ON THE SEROCONVERSION RATES IN AN AREA ENDEMIC FOR HISTOPLASMA CAPSULATUM

MARK JOHN WOLCOTT

A Study on the Seroconversion Rates in an Area Endemic for *Histoplasma capsulatum*

An Abstract

Presented to the

Graduate and Research Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Biology

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by

Mark John Wolcott

January 1989

ABSTRACT

In this study, some of the serological responses to Histoplasma by soldiers that were recently assigned to Fort Campbell, Kentucky were examined. The purpose of this study was to determine the percentage of soldiers that arrived at Fort Campbell with no prior serological evidence of exposure to Histoplasma who later acquired a detectable serological reaction. This was done because no studies have been conducted on seroconversion rates for persons who move from a non-endemic locale into an endemic area. The period of sample collection was three months. A modified enzyme-linked immunoassay for detecting three classes of anti-histoplasmin antibody levels was used to measure seroconversion.

Eighty two participants initially enrolled in the study, and thirty four completed all sampling requirements. Thirty-one of the thirty-four participants (91%) gave no indication of exposure to Histoplasma as a result of three months of service at Fort Campbell. Twenty-three of the thirty-four participants (68%) had results suggesting anti-histoplasmin antibody formation as a consequence of exposure to Histoplasma before arriving at Fort Campbell. Sera from four participants (12%) had an increase in anti-histoplasmin antibody levels. The results from these four participants suggested that they either

might have been re-exposed to Histoplasma or the increase might be normal variation in antibody titers. None of the eight participants that arrived at Fort Campbell with no detectable anti-histoplasmin antibodies seroconverted to a detectable anti-histoplasmin antibody level by the end of this study. The possibility of histoplasmosis seroconversion from a seronegative status as a sole result of being stationed at Fort Campbell for three months is negligible for this small population sampled. The thirty four participants that completed the study represent approximately one-tenth of one percent of the military personnel stationed at Fort Campbell at the time of this study.

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APPENDIX

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To the Graduate and Research Council:

I am submitting herewith a Thesis written by Mark J. Wolcott entitled "A Study on the Seroconversion Rates in an Area Endemic for Histoplasma capsulatum." I have examined the final copy of this paper for form and content, and I recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science, with a major in Biology.

R. H. McCoy
Major Professor

We have read this thesis
and recommend its
acceptance:

Daniel W. Bath
Second Committee Member

B P Stone
Third Committee Member

Accepted for the Graduate and
Research Council:

William H. Ellis
Dean of the Graduate School

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INTRODUCTION

Histoplasma capsulatum is a dimorphic, saprophytic soil fungus. At room temperature (approximately 23° Celsius) the mycelial form predominates, but at body temperature (approximately 37° Celsius) the yeast form develops and becomes predominant (Penn and George 1983; Macher 1980; Goodwin et al. 1981; Kaufman and Riess 1986). Consistent with the optimum temperature for Histoplasma infections, the yeast-phase is the principal form that occurs in tissue macrophages and leukocytes (Kaufman and Riess 1986; Goodwin et al. 1981; Goodwin and Des Prez 1978). The infectious disease process causes progressive pulmonary cavitation that can spread to the reticulo-endothelial system. The acute form is normally benign. Fatalities are rare, except in substantial infections or in immunosuppressed patients (Goodwin et al. 1981; Berkow and Talbott 1977). Riley (1983) stated that histoplasmosis is the most common systemic fungus infection in humans and causes more deaths than any other systemic mycosis.

Histoplasmosis is endemic in 31 of the 48 continental states. This endemic area includes the Mississippi and Ohio river valley areas (Edwards et al. 1969; Goodwin et al. 1981). In these areas, over 80% of the population reacts to the skin test (Penn and George

1983). Based on a study in 1943 (Riley 1983), inductees into the military services from the Mississippi and Ohio river valley areas (including Tennessee) had a higher incidence of positive skin tests for Histoplasma than the inductees from other areas of the United States. The histoplasmosis organism is especially plentiful in soil containing fowl and bat manure (Penn and George 1983; Macher 1980). Chicken houses and other areas of avian use have often been documented as point sources of the fungus (Goodwin et al. 1981; Chick et al. 1980a; Campbell 1980; Chick et al. 1981; Anderson 1980; MacVandiviere et al. 1981; Schlech et al. 1983; Sathapatayavongs et al. 1983).

Immune reactions to Histoplasma are typical of both cellular and humoral mechanisms. Histoplasmosis is distinguished by a B-cell hyperplasia (Goodwin et al. 1981; Cox and Arnold 1980). Chandler et al. (1969) reported that Histoplasma initiated a sequential response characterized by the rise in immunoglobulin M (IgM) antibodies followed by an increase in immunoglobulin A (IgA) and slightly later by an increase in immunoglobulin G (IgG). Anti-histoplasmin IgM levels peak at 14-27 days post-infection with a return to normal by 3.5-6 months. Anti-histoplasmin IgA levels increase during the 14-27 day period when IgM levels are the highest, but do not peak until 1-3 months after the onset of illness. Levels of

anti-histoplasmin IgA do not return to normal until 6-13 months after the onset of illness. Anti-histoplasmin IgG levels increase over a 1-3 month period, with a slow return to normal within one year (Chandler et al. 1969; Kaufman 1971; Goodman 1982). McMurray et al. (1982) demonstrated that this sequence of immunoglobulin response is neither unique to histoplasmosis nor to humans; it has also been observed in bats infected with Histoplasma.

Lymphadenopathy and granulomatous lesions in lungs and other tissues mark the cellular response to histoplasmosis infection (Macher 1980; Goodwin et al. 1981; Goodwin and Des Prez 1978). These cellular responses to an infectious agent are typical of the immune response to a respiratory or gastrointestinal route of entry (Hood et al. 1978). According to Goodwin et al. (1981) and Cox (1979), the cellular immune response is the method by which immunity is conferred in the host.

Benign and clinically insignificant infections with Histoplasma are very common (Goodwin et al. 1981; Jacobson and Straus 1981; Berkow and Talbott 1977). Problems in the detection of histoplasmosis occur because the symptoms are often subtle and there is difficulty in detecting presence of the disease. Many studies of the prevalence of histoplasmosis have been undertaken by using a standardized skin test to detect cellular immune responses. In one study (Edwards et al. 1969), nine of

ten people tested by using a skin test were sensitive to Histoplasma. A skin test sensitivity study of residents of Hopkinsville, Kentucky and Fort Campbell, Kentucky showed that positive rates were as high as 59% (Caudill et al. 1975). Although high rates were obtained, skin tests do not measure humoral responses and may, therefore, fail to detect the full range of immunological responses involved in histoplasmosis infection (Goodwin et al. 1981; Cox 1979). The production of a positive skin test result is not permanent; hypersensitivity could decrease with time and then be re-established on new exposure to the organism (Wheat et al. 1982; Goodwin et al. 1981; Davies and Sarosi 1987a; Zeidberg et al. 1951). This complicates the use of skin testing for detecting or monitoring exposure to Histoplasma (Davies and Sarosi 1987a).

According to Drutz (1986), "In the management of a suspected fungal infection, no diagnostic test is superior to the isolation of the causative agent from a relevant clinical specimen, or to its unequivocal physical identification in a histopathological setting of tissue invasion." Culturing Histoplasma is at present the best method of diagnosing an active infection (Berkow and Talbott 1977; Drutz and Graybill 1987; Goodman 1982). The culturing procedure, however, is time consuming and prone to failure in detecting the organisms when they are present (Goodwin and Des Prez 1978). According to Wheat

et al. (1982), only ten percent of diagnostically positive histoplasmosis cases yield positive culture results. In part, by virtue of the failure of the cultural techniques to detect active infections, serologic tests are often used to diagnose acute histoplasmosis (Davies and Sarosi 1987a). Serological tests have been relied on, with varying degrees of success, in diagnosing histoplasmosis. The most commonly employed tests are the standardized complement fixation (CF), micro-ID procedure (ID) and latex agglutination (LA) method (Macher 1980; Jacobson and Straus 1981; Cox 1985; Sarosi et al. 1984). The most important serological test has been complement fixation because it has been standardized and is available in most state health department laboratories (Goodwin et al. 1981; George and Lambert 1984; Goodman 1982). Newer methods are being employed to detect antibody responses to histoplasmosis; these include radioimmunoassay (RIA) (George and Lambert 1984; Wheat et al. 1983; Sprouse et al. 1981; George et al. 1981; Wheat et al. 1986a; Schlech et al. 1983; Davies 1986; Sarosi et al. 1984; Penn and George 1983) and enzyme-linked immunosorbent assay (ELISA) (Boyer and Scalarone 1983; Sharma et al. 1982; Gabal and Mohammed 1985; Davies and Sarosi 1987a; Davies and Sarosi 1987b; Lambert and George 1987; Richardson and Warnock 1983; Sarosi et al. 1984). Enzyme-linked immunosorbent assays are about twenty times more sensitive

than complement fixation tests (Sharma et al. 1982) for detecting histoplasmosis, but have not been standardized for routine clinical laboratory use. Wheat et al. (1986b) developed a radioimmunoassay test to detect histoplasmin antigens that may prove to be the best method of detecting an acute infection.

Although histoplasmosis is normally a benign infection, its incidence in endemic areas is cause for concern. With increased numbers of immunocompromised patients because of human immunodeficiency virus (HIV) and increased number of elderly, more frequent encounters and more serious infections may occur (Kauffman et al. 1978; Mandell et al. 1986). The most common form of histoplasmosis, the primary acute form, is often hard to distinguish from respiratory influenza-like illnesses (Berkow and Talbott 1977). The incidence of this form of infection is, therefore, difficult to determine accurately.

Fort Campbell exists in an endemic area. This is of potential concern. Partially due to the threat of dissemination of Histoplasma by fowl, Fort Campbell undertook a blackbird eradication project in 1975 (Department of the Army 1975). This action was deemed necessary to protect the health of both the military population and their families at Fort Campbell. Any service member not capable of deploying with their

military unit becomes a serious combat loss. After the deployment of the 101st Air Assault Division, the facilities at Fort Campbell will become a training center for replacement personnel. The training will be short in duration because of the immediate need for personnel if an armed conflict should arise. The loss of trainees, attributable to any illness, again poses a critical loss of manpower. If histoplasmosis contributes to the overall rate of illnesses because it is endemic in the training area, the planners of military actions need to know the extent of the incapacitation in order to outline alternatives.

The purpose of this study was to determine the percentage of soldiers that arrived at Fort Campbell with no prior serological evidence of exposure to Histoplasma who later acquired a detectable serological reaction within a three month period. The percentage of soldiers at Fort Campbell that develop a detectable serological reaction to Histoplasma should be an indication of the degree of exposure. An enzyme-linked immunosorbent assay system was used to determine serum levels of anti-histoplasmin antibodies. By using different anti-human conjugates to detect the immunoglobulins M and G, the immunoglobulins produced by the serological responses to Histoplasma could be differentiated. Assays were also conducted to differentiate antibodies to the two forms of

Histoplasma, the yeast-phase and the mycelial-phase.

Chapter II

MATERIALS AND METHODS

Samples

Approximately seven-milliliter volumes of venous blood were obtained from voluntary participants under the rules and regulations of the Army Medical Department as an approved clinical investigation study. Voluntary participation was elicited from military personnel during entrance into Fort Campbell, Kentucky on 10 and 11 September 1985. The participants were volunteers and should be representative of the personnel being assigned to Fort Campbell. Participants were briefed on the nature of their participation. The informed consent of all participants was obtained in accordance with the regulations of the United States Army that cover clinical investigation and research programs involving human volunteers and was approved by local and regional command representatives (Appendix 1). The informed consent form included information on the nature of the study, risks, inconveniences and discomforts, benefit, number of participants, duration of study, duration of participation, and any supplemental information requested. Participants were also requested to provide supplemental information on the informed consent form. This information included two questions about the possibility of prior exposure to Histoplasma. Collection of samples

followed standard venipuncture procedures (Stockblower et al. 1984). Vacuum collection tubes without anticoagulant or preservative were used. The samples were consecutively numbered with four digit numbers to insure confidentiality in that all later information pertained to the participant's number only. Cells and debris were removed within two hours of collection by centrifugation at approximately 850g for five minutes. The serum was decanted into polystyrene tubes and frozen at a temperature of approximately -35° Celsius. The frozen samples were transferred to a temperature of approximately -70° Celsius within a week and stored until used. Collection of a second sample from the participants occurred 28-35 days after the first sample, and a third sample was collected 88-98 days after the first sample was collected. The second and third samples were processed in the same manner as the first sample, retaining the same four digit identification number as initially assigned. Participants varied in their compliance with specimen collection requests after the initial sample collection and only thirty four participants gave all three samples for analysis.

Antigens

Antigens were obtained commercially from Meridian Diagnostics, (Cincinnati, Ohio) and consisted of Histoplasma yeast-phase whole-cell concentrated antigen, (product number 102001, lot number HY02A22) and histoplasmin mycelial-phase "H" and "M" antigen (product number 101901, lot number HC02A18).

Conjugated Antibodies

The conjugated antibodies were commercial preparations of goat anti-human polyvalent immunoglobulins peroxidase conjugate (product number A-8400, lot number 66F-8846), goat anti-human IgG peroxidase conjugate (product number A-6029, lot number 17F-8852), and goat anti-human IgM peroxidase conjugate (product number A-6907, lot number 97F8885). All three conjugates were purchased from Sigma Chemical Company, St. Louis, Missouri.

Substrate

The substrate indicator was 5-aminosalicylic acid (Boorsma and Streefkerk 1978; Tijssen 1985; Van Weeman and Schuurs 1971). Sufficient 5-aminosalicylic acid (5AS) (product number A-3537, Sigma Chemical Company, St. Louis, Missouri) was prepared by dissolving one milligram per milliliter in warm (56° Celsius), 0.02-molar monosodium

phosphate buffer at pH 6.8. The solution was decolorized with approximately 1-5 milligrams of activated charcoal for each one hundred milliliters of solution. The solution was then filtered through Whatman #1 filter paper. Shortly before use, one-tenth milliliter of one percent hydrogen peroxide (H_2O_2) (hydrogen peroxide AR, catalog number 5240, lot number KPLP, Mallinckrodt, Paris, Kentucky) was added as the substrate to each ten milliliters of solution. Excess substrate solution was discarded at the end of the day.

Enzyme-linked immunosorbent assay

A modified version of the heterogeneous indirect enzyme-linked immunosorbent assay technique (Voller and Bidwell 1986) was used. Rigid polystyrene Microtiter plates (Microtiter Plate, catalog number 1-221-24, lot number G152, Dynatech Laboratories, Inc. Alexandria, Virginia) were coated with fifty microliters per well of 1:1000 antigen in phosphate-buffered saline (PBS) (PBS Buffer, product number 0008, lot number AK652, Zeus Scientific, Raritan, New Jersey). The plates were covered and incubated overnight at 4° Celsius. The antigen was removed and the plates were washed three times with room temperature PBS/Tween20 (Voller and Bidwell 1986). All washings were done by using a Dynatech Dynadrop SR1 dispenser, and the plates were blot-dried between washings

to minimize carry over. The wells were blocked with five percent bovine serum albumin (BSA) (Albumin, Bovine, Fraction V, 4503, Sigma Chemical Company, St. Louis, Missouri) in PBS, covered and incubated overnight at 4° Celsius. The bovine serum albumin was removed and the plates were washed three times with room temperature wash solution (Wash Solution, 50-63-00, Kirkegaard and Perry Laboratories Incorporated, Gaithersburg, Maryland). Fifty microliters of a 1:500 dilution of serum or control specimen (diluted in one percent BSA/PBS) were added to each well. The plates were covered and incubated for approximately twenty-four hours at room temperature. The sera and control specimens were removed and the plates were washed seven times with room temperature wash solution. Fifty microliters of appropriate conjugate were added. The plates were covered and incubated approximately twenty-four hours at room temperature. The conjugate was removed and the plates were washed seven times with room temperature wash solution. Fifty microliters of substrate were added. The plates were incubated, uncovered, for a minimum of twenty-five minutes at room temperature and then read manually within forty-five minutes of substrate addition. Results were recorded as visual estimates of the intensity of the purple-brown color developed, on a scale of 0 (negative), weak (w) (a reaction with some background tint as compared with a

negative that was visibly clear), borderline (?) (a reaction with discernible color but significantly less than full color development; usually discrete spots of color) 1 (full diffuse color development in entire well at a low visual intensity), 2 and 3 (varying by degrees of color intensity) to 4 (the visual intensity of a strong-positive control specimen; see Quality Control below).

Standardization of the Assay

The assay was optimized by using a checkerboard titration pattern (Voller and Bidwell 1986) with the antigen diluted at 1:500, 1:1000 and 1:2000. To provide the complete range of positive and negative reactions, for the determination of optimal working concentrations of reagents to be used, three specimens were used. A specimen that was positive for anti-histoplasmin antibodies, from a confirmed disease positive patient (provided by Dwight David Eisenhower Army Medical Center, Department of Pathology, Virology Section, Fort Gordon, Georgia), a specimen that was negative or had a very low antibody level for anti-histoplasmin antibodies (a newborn cord blood sample), a specimen that was a high level of anti-histoplasmin antibodies (from a volunteer exhibiting a positive skin test for histoplasmin) and a blank (10% BSA) were diluted 1:200, 1:500, 1:1000 and 1:2000. Conjugate (anti-human polyvalent immunoglobulin peroxidase

conjugate) was diluted according to the instructions of the manufacturer (1:1000). The optimum dilutions of the reagents were determined to be the highest antigen dilution in combination with the serum dilution that gave a +1 (subjective quantity based on the strong-positive maximum reaction) with minimal background color in either the negative or blank specimen reactions. The titration tests were run three times on three separate days to verify the dilutions selected. A combination of a 1:1000 dilution of antigen and a 1:500 dilution of serum/control were determined to be the optimal working dilutions.

Quality Control

After the checkerboard titration series, each run was compared to the control specimens included in each plate to ensure accurate performance. A single strong histoplasmin antibody positive specimen (to verify performance and establish the semi-quantitative interpretation), a duplicate specimen negative for histoplasmin antibodies (to establish the background color of any non-specific binding or inadequate washing), and a duplicate specimen weakly positive for histoplasmin antibodies were included in each run. All tests exhibited appropriate reactions in the strong-positive and negative control specimens before being accepted as valid.

Data Handling

The semi-quantitative reactions from all three test procedures were averaged and index value determined, based on their numerical score (0 to 4). To ensure inclusion of all reactions, a weak (w) reaction was assigned a value of 0.25 and a borderline (?) reaction was assigned a value of 0.5. The weak and borderline reactions were included because of the varying color intensities from one assay to the next and because of the subjective semi-quantitative readings. A cut-off index value of 0.7 was used as determining a positive anti-histoplasmin antibody response. This cut-off value represents the average of detectable antibody levels showing a number 1 reaction in at least two of three runs. "Significant" antibody levels were determined to be an index level of greater than 1.3. A change in the antibody levels from one participant's specimen to the following specimen collected, was considered if the index value changed by 0.3 or more.

Chapter III

RESULTS

Each assay was run three individual times by using each of the conjugates and both of the antigens in combinations: a total of eighteen assays. Table 1 shows the results of testing participants that did not provide all three specimens. Presented in Table 2 are the results from the participants that provided all three specimens. Table 5 summarizes the general class of antibody reactions detected during this study from the thirty four participants that submitted all three specimens. Information about the participants' prior exposure to Histoplasma is presented in Tables 4, and 5. Table 6 summarizes the thirty four participants that submitted all three specimens and their possible prior exposure to Histoplasma.

Tables 1 and 2 show that thirty-four of the initial eighty-two participants that volunteered for the study submitted to collection of all three specimens. Of the eighty-two participants, sixty-two (76%) had an initial specimen that had detectable anti-histoplasmin antibody levels. Detectable anti-histoplasmin antibody levels (index greater than 0.7), in one or more of the anti-human immunoglobulin tests, were present in twenty-six of the thirty-four participants (76%) who provided all three samples (Table 2). All twenty-six specimens were positive

for anti-histoplasmin antibodies to either the histoplasmin yeast-phase and/or mycelial-phase tests with anti-human polyvalent immunoglobulins conjugate.

The results presented in Table 3 show that twenty-six of the thirty-four participants that submitted all three specimens, had no change in their anti-histoplasmin antibody levels during this study period. Four of the thirty-four participants that submitted all three specimens, had a rise in their anti-histoplasmin antibody levels. The remaining four, of the thirty-four participants that submitted all three specimens, had a fall in their antibody levels to Histoplasma.

According to Tables 4 and 5, only eighteen to twenty percent of all participants did not indicate either a farm acquired nor endemic area acquired exposure to Histoplasma. According to Table 6, those that did indicate a possible exposure, either through living in an endemic area or living on a farm that raised fowl, did not fall into any specific category of anti-histoplasmin antibody response classes.

Table 1

Index Values of Anti-human Conjugates For Participants

Without All Samples Collected

Participant Number	*Histoplasma* Mycelia Phase Antigen									*Histoplasma* Yeast Phase Antigen								
	Anti-human Conjugates									Anti-human Conjugates								
	Polyvalent			IgM			IgG			Polyvalent			IgM			IgG		
	Sample			Sample			Sample			Sample			Sample			Sample		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1352	1.1	1.4	ND[a]	0.8	1.4	ND	0.1	0.1	ND	0.8	1.1	ND	0.1	0.1	ND	0.3	0.8	ND
1355	0.4	ND	0.4	0.8	ND	0.5	0.0	ND	0.1	0.7	ND	1.0	0.2	ND	0.1	0.0	ND	0.0
1356	1.4	ND	ND	0.3	ND	ND	0.3	ND	ND	1.3	ND	ND	0.1	ND	ND	0.0	ND	ND
1357	1.0	ND	ND	0.3	ND	ND	0.3	ND	ND	0.5	ND	ND	0.0	ND	ND	0.0	ND	ND
1359	1.3	1.3	ND	0.1	0.4	ND	0.3	0.1	ND	0.4	0.7	ND	0.1	0.1	ND	0.0	0.0	ND
1360	1.2	ND	ND	1.2	ND	ND	0.0	ND	ND	0.5	ND	ND	0.3	ND	ND	0.0	ND	ND
1364	0.3	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND

Table 1

Continued

1367	0.1	ND	ND	0.1	ND	ND	0.0	ND	ND	0.3	ND	ND	0.0	ND	ND	0.0	ND	ND
1370	0.5	ND	ND	0.2	ND	ND	0.2	ND	ND	0.5	ND	ND	0.1	ND	ND	0.2	ND	ND
1371	1.0	1.0	ND	0.3	0.7	ND	0.1	0.1	ND	0.4	0.4	ND	0.1	0.1	ND	0.0	0.0	ND
1372	0.6	1.0	ND	1.0	0.8	ND	0.3	0.2	ND	0.5	0.5	ND	0.7	0.8	ND	0.0	0.0	ND
1373	0.3	0.1	ND	0.2	0.3	ND	0.0	0.0	ND	0.1	0.0	ND	0.0	0.0	ND	0.0	0.0	ND
1374	0.3	ND	ND	0.5	ND	ND	0.2	ND	ND	0.1	ND	ND	0.2	ND	ND	0.0	ND	ND
1377	1.3	ND	ND	1.1	ND	ND	0.5	ND	ND	1.1	ND	ND	0.3	ND	ND	0.1	ND	ND
1378	0.7	ND	ND	1.0	ND	ND	0.2	ND	ND	1.0	ND	ND	0.5	ND	ND	0.1	ND	ND
1379	0.7	ND	0.7	0.0	ND	0.0	0.0	ND	0.0	0.1	ND	0.4	0.0	ND	0.0	0.0	ND	0.0
1380	1.3	ND	1.1	0.5	ND	0.4	0.0	ND	0.0	0.5	ND	0.7	0.2	ND	0.3	0.0	ND	0.0
1381	1.3	ND	1.3	1.3	ND	1.3	0.0	ND	0.0	0.4	ND	1.0	0.4	ND	0.5	0.0	ND	0.0
1382	1.7	ND	ND	2.3	ND	ND	0.3	ND	ND	0.7	ND	ND	0.8	ND	ND	0.0	ND	ND
1383	1.1	ND	1.4	1.3	ND	0.8	0.4	ND	0.2	1.3	ND	1.0	0.4	ND	0.4	0.8	ND	0.8
1384	1.2	0.7	ND	0.1	0.1	ND	0.5	0.3	ND	0.6	0.6	ND	0.0	0.0	ND	0.0	0.0	ND
1386	0.9	0.7	ND	0.1	0.2	ND	0.3	0.3	ND	0.7	0.7	ND	0.1	0.1	ND	0.0	0.0	ND

Table 1

Continued

1387	0.7	ND	0.8	0.0	ND	0.0	0.0	ND	0.0	0.4	ND	0.4	0.0	ND	0.0	0.0	ND	0.0
1388	1.3	ND	0.8	0.9	ND	0.9	0.1	ND	0.1	1.1	ND	1.0	0.2	ND	0.2	0.0	ND	0.0
1389	1.7	ND	2.0	2.0	ND	2.3	0.6	ND	1.0	1.5	ND	1.7	0.5	ND	1.1	0.0	ND	0.0
1390	1.0	ND	ND	1.0	ND	ND	0.0	ND	ND	0.4	ND	ND	0.2	ND	ND	0.0	ND	ND
1451	1.1	ND	ND	0.2	ND	ND	0.2	ND	ND	0.5	ND	ND	0.1	ND	ND	0.0	ND	ND
1453	0.2	ND	0.5	0.1	ND	0.2	0.0	ND	0.0	0.3	ND	0.3	0.0	ND	0.3	0.0	0.0	ND
1456	0.9	ND	ND	0.1	ND	ND	0.5	ND	ND	0.4	ND	ND	0.0	ND	ND	0.0	ND	ND
1459	1.7	0.8	ND	0.0	0.0	ND	0.8	0.5	ND	1.0	0.8	ND	0.0	0.0	ND	0.0	0.0	ND
1460	1.8	ND	2.1	0.8	ND	0.8	2.0	ND	2.0	1.4	ND	1.7	0.4	ND	0.3	0.0	0.0	ND
1462	1.1	ND	1.0	0.4	ND	0.5	0.2	ND	0.3	0.8	ND	0.7	0.1	ND	0.1	0.0	0.0	ND
1464	0.5	ND	0.5	0.4	ND	0.5	0.4	ND	0.4	0.7	ND	0.7	0.1	ND	0.1	0.0	0.0	ND
1465	0.5	0.7	ND	0.0	0.2	ND	0.2	0.2	ND	1.0	1.3	ND	0.0	0.0	ND	0.0	0.0	ND
1470	0.6	ND	ND	0.1	ND	ND	0.2	ND	ND	0.5	ND	ND	0.0	ND	ND	0.0	ND	ND
1471	0.5	ND	0.4	0.1	ND	0.3	0.0	ND	0.0	1.0	ND	1.1	0.1	ND	0.2	0.0	0.0	ND
1473	1.7	ND	1.7	2.0	ND	2.3	0.5	ND	0.3	1.7	ND	1.7	1.7	ND	1.7	0.0	0.0	ND

Table 1

Continued

1474	1.1	ND	ND	2.0	ND	ND	0.8	ND	ND	1.1	ND	ND	0.9	ND	ND	0.0	ND	ND
1475	0.7	ND	ND	0.3	ND	ND	0.0	ND	ND	0.3	ND	ND	0.0	ND	ND	0.0	ND	ND
1476	1.2	ND	1.2	0.4	ND	0.8	0.2	ND	0.2	0.8	ND	0.8	0.0	ND	0.0	0.0	0.0	ND
1477	0.3	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0	ND	ND
1480	1.3	ND	1.3	0.2	ND	0.0	0.1	ND	0.1	0.4	ND	0.5	0.0	ND	0.0	0.0	0.0	ND
1481	0.7	ND	0.5	0.1	ND	0.4	0.1	ND	0.1	0.3	ND	0.3	0.1	ND	0.2	0.0	0.0	ND
1483	1.7	ND	ND	0.1	ND	ND	1.4	ND	ND	1.0	ND	ND	0.0	ND	ND	1.1	ND	ND
1484	0.5	ND	0.3	0.0	ND	0.0	0.3	ND	0.1	0.4	ND	0.4	0.0	ND	0.0	0.0	0.0	ND
1487	2.4	2.0	ND	3.3	3.3	ND	0.1	0.1	ND	2.3	2.3	ND	2.0	2.0	ND	0.0	0.0	ND
1490	1.1	ND	ND	0.8	ND	ND	1.7	ND	ND	1.0	ND	ND	0.2	ND	ND	0.3	ND	ND
1491	0.2	0.1	ND	0.0	0.1	ND	0.0	0.0	ND	0.0	0.4	ND	0.0	0.0	ND	0.0	0.0	ND

[a]ND indicates no sample received for analysis.

Table 2

Index Values of Anti-human Conjugates For Participants

With All Samples Collected

Participant Number	*Histoplasma* Mycelial Phase Antigen									*Histoplasma* Yeast Phase Antigen								
	Anti-human Conjugates									Anti-human Conjugates								
	Polyvalent			IgM			IgG			Polyvalent			IgM			IgG		
	Sample			Sample			Sample			Sample			Sample			Sample		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1351	2.0	2.0	1.5	1.1	1.2	0.8	1.7	1.7	0.8	1.3	1.3	1.0	0.8	0.5	0.3	0.0	0.0	0.0
1353	0.9	0.4	0.8	0.0	0.0	0.0	0.5	0.5	0.8	0.5	0.3	0.5	0.0	0.0	0.1	0.1	0.0	0.1
1354	1.1	1.3	1.3	0.8	1.7	2.3	0.5	0.5	0.5	1.0	1.7	1.3	0.5	1.2	1.2	0.0	0.0	0.0
1358	0.4	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0
1361	2.0	1.4	1.1	1.4	1.2	1.8	1.3	1.0	1.2	1.1	1.1	1.1	0.8	0.3	0.8	0.0	0.1	0.0
1362	1.1	0.9	1.0	2.0	2.0	2.0	0.1	0.1	0.1	0.8	1.0	0.8	1.7	1.7	1.7	0.0	0.0	0.0
1363	0.5	0.3	0.3	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0

Table 2

Continued

1365	2.0	2.0	2.0	0.8	0.5	1.3	1.1	0.8	1.3	0.8	1.3	1.3	0.6	0.6	0.8	0.2	0.1	0.3
1366	2.3	2.0	1.7	2.0	2.0	2.0	0.8	1.0	0.8	2.0	1.7	2.0	1.7	1.7	1.7	0.1	0.0	0.0
1368	0.6	0.6	0.8	0.1	0.1	0.0	0.1	0.2	0.1	0.3	0.3	0.4	0.0	0.0	0.0	0.0	0.1	0.0
1369	0.4	0.4	0.5	0.1	0.1	0.0	0.0	0.1	0.0	0.4	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0
1375	1.7	0.0	0.3	0.8	0.3	0.3	2.0	0.0	0.0	1.1	0.1	0.1	0.2	0.0	0.1	0.2	0.0	0.0
1376	1.3	1.7	1.3	0.6	0.5	0.3	0.0	0.0	0.0	0.3	0.5	0.4	0.1	0.2	0.0	0.0	0.0	0.0
1385	1.2	0.8	0.8	0.8	0.8	0.5	0.1	0.2	0.1	1.4	1.4	1.1	1.1	0.8	0.4	0.0	0.0	0.0
1452	1.3	1.3	1.3	1.7	1.7	1.5	0.0	0.0	0.0	1.1	1.3	1.1	1.1	1.3	1.0	0.0	0.0	0.0
1454	2.3	2.3	2.3	0.0	0.0	0.1	2.7	3.0	3.3	1.0	1.3	1.7	0.0	0.0	0.0	0.8	1.1	1.4
1455	0.4	0.7	0.3	0.3	0.5	0.5	0.0	0.2	0.2	1.0	1.0	1.0	0.1	0.1	0.1	0.0	0.0	0.0
1457	0.8	0.7	1.1	0.4	0.5	0.3	0.0	0.2	0.2	0.8	1.0	0.8	0.1	0.2	0.0	0.0	0.1	0.0
1458	0.8	1.1	1.1	0.7	1.4	1.2	0.0	0.1	0.0	0.8	1.0	1.0	0.4	1.1	1.1	0.0	0.0	0.0
1461	1.0	1.0	0.4	0.6	0.5	0.2	0.3	0.3	0.3	1.0	0.8	0.8	0.8	1.0	0.1	0.0	0.0	0.0
1463	0.4	0.7	0.7	0.8	1.4	0.8	0.0	0.1	0.0	0.8	1.0	0.8	0.4	0.7	0.2	0.0	0.0	0.0
1466	0.8	1.0	1.0	0.8	0.8	0.3	1.1	0.8	0.5	0.5	0.5	0.5	0.2	0.2	0.1	0.2	0.2	0.0

Table 2

Continued

1467	0.4	1.0	0.3	0.8	0.8	0.8	0.2	0.2	0.2	0.5	0.5	0.5	0.1	0.1	0.1	0.0	0.0	0.0
1468	1.1	0.8	0.8	0.2	0.0	0.0	0.5	0.4	0.0	0.8	0.3	0.6	0.0	0.0	0.0	0.1	0.0	0.0
1469	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
1472	0.8	0.5	1.0	0.3	0.3	0.3	0.6	0.8	0.8	1.7	1.4	1.7	0.1	0.1	0.1	0.1	0.1	0.1
1478	0.4	0.8	0.5	0.3	0.3	0.3	0.2	0.2	0.1	0.4	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0
1479	0.3	0.5	0.5	0.2	0.2	0.1	0.0	0.0	0.1	0.2	0.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0
1482	0.5	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0
1485	0.5	0.5	0.5	0.1	0.3	0.1	0.0	0.0	0.0	0.4	0.3	0.4	0.3	0.0	0.0	0.0	0.0	0.0
1486	0.4	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0
1488	0.4	0.3	0.3	0.1	0.1	0.0	0.5	0.5	0.3	0.8	0.7	0.7	0.0	0.0	0.0	0.1	0.1	0.1
1489	1.7	2.0	1.7	0.3	0.5	0.4	0.6	0.8	1.0	0.8	0.5	0.7	0.0	0.0	0.0	0.0	0.0	0.0
1492	2.0	1.7	2.0	1.7	1.5	2.0	0.8	0.3	0.5	1.7	2.0	1.7	1.0	1.0	1.0	0.2	0.1	0.2

TABLE 3

Summary of Anti-Histoplasmin Antibody Test Results For Participants With All Three Samples Collected

Immunoglobulins Detected	No Change* in index	Rise in index	Fall in index
None detected	8		
Polyvalent Antibodies detected	7		
Polyvalent and IgM Antibodies detected	4	2	2
Polyvalent and IgG Antibodies detected	3	1	
Polyvalent, IgM, and IgG Antibodies detected	4	1	2
TOTAL	26	4	4

* This characteristic was based upon any one of the six immunoglobulin conjugate tests showing the indicated trend. No participant had mixed trends (ie. a rise in one immunoglobulin class with a fall in another).

TABLE 4

Determination of Possible Exposure Prior to Study

All Participants

Possible Farm Exposure	Living in an Endemic Area Exposure		Both Possible Farm and Endemic Area Exposure	Neither Possible Exposure
8(10%)	1 month	3(4%)	6(7%)	17(21%)
	6 months	5(6%)	0	
	12 months	7(9%)	2(2%)	
	60 months	18(22%)	16(20%)	
	TOTAL	37(46%)	28(35%)	

TABLE 5

Determination of Possible Exposure Prior to Study Participants With All Three Samples Provided

Possible Farm Exposure	Living in an Endemic Area Exposure		Both Possible Farm and Endemic Area Exposure	Neither Possible Exposure
3(9%)	1 month	1(3%)	3(9%)	6(18%)
	6 months	1(3%)	0	
	12 months	5(15%)	1(3%)	
	60 months	7(21%)	7(21%)	
	TOTAL	14(41%)	11(32%)	

TABLE 6

Summary of Anti-Histoplasmin Antibody Test Results With The Number That Had Possible Exposure Prior to Study For Participants With All Three Samples Provided

Immunoglobulins Detected	No Change* in index	Rise in index	Fall in index
None detected	8/7		
Polyvalent Antibodies detected	7/7		
Polyvalent and IgM Antibodies detected	4/3	2/2	2/1
Polyvalent and IgG Antibodies detected	3/2	1/1	
Polyvalent, IgM, and IgG Antibodies detected	4/1	1/1	2/2

Chapter IV

DISCUSSION

General

As seen in Table 2, the overall specificity of the assay was such that the anti-human polyvalent immunoglobulins conjugate could detect all the tests in which immunoglobulins class G or M were also positive when the individual immunoglobulins were tested for. Nineteen of the thirty-four participants (56%) had the combination of a detectable anti-histoplasmin antibody level with the anti-human polyvalent and the anti-human IgG and/or IgM conjugates. This is in contrast to only seven of the thirty-four participants (21%) having detectable anti-histoplasmin antibodies with the anti-human polyvalent immunoglobulins conjugate alone. This detection by polyvalent immunoglobulins conjugate suggests that antibodies to other immunoglobulin classes, A (IgA), D (IgD) or E (IgE), were detected. The detection of any class of anti-Histoplasma immunoglobulins, M, G or those detected with the polyvalent immunoglobulins conjugate, are considered as positive indicators for exposure to the Histoplasma organism. A negative anti-Histoplasma antibody response does not necessarily indicate that the participant was not exposed to the Histoplasma organism, but rather that the exposure was not sufficient to elicit an antibody response (Kaufman and Riess 1985). Antibody

classes and the levels of the antibodies will also vary with the type of disease (acute, disseminated, etc.) (Kaufman and Riess 1985). The detection of different immunoglobulin classes and the amount of antibody detected could also be the result of normal antibody titer variations within the participant or this testing system (Wells 1980).

Polyvalent Immunoglobulins Detection

Seven of the thirty-four participants (21%) had detectable anti-histoplasmin antibody levels with only the anti-human polyvalent immunoglobulin conjugate.

Of these seven, one participant (number 1488) had detectable anti-histoplasmin antibody levels to the histoplasmin yeast-phase antigen alone. Three participants (numbers 1368, 1376 and 1478) had detectable anti-histoplasmin antibody levels to the histoplasmin mycelial antigen alone. Three participants (numbers 1488, 1455, and 1457) had detectable anti-histoplasmin antibody levels to both histoplasmin mycelial-phase and histoplasmin yeast-phase antigens. Only one of the seven (number 1376) had a significant (index of greater than 1.3 for one or more samples) anti-histoplasmin antibody level. The remaining six had low, detectable anti-histoplasmin antibody levels (index of less than 1.1 for one or more samples).

The anti-histoplasmin antibody levels detected with anti-human polyvalent immunoglobulin conjugate without anti-histoplasmin antibody levels in the IgG and IgM tests represent anti-histoplasmin antibodies of the IgA, IgD or IgE class, or some combination of these later classes (Goodman 1987). The results from anti-human polyvalent conjugate represent only the IgA class for two reasons. The first reason is the vastly greater amount of antibody that IgA represents; approximately 15-21% of the total serum immunoglobulins (Goodman 1987). This is compared to IgD representing approximately 0.2% and IgE representing approximately 0.002-0.004% of the total serum immunoglobulins (Goodman 1987) making detection of these two classes of antibodies extremely difficult. The second reason for declaring the result being the sole detection of the IgA class, in the absence of the IgM and IgG classes, is the manufacture's declaration of specificity for the conjugate used. According to the manufacturer, in the absence of immunoglobulin classes IgG and IgM, the conjugate should be reactive only with IgA and the Bence Jones antibody fragments (Sigma Chemical Co. 1986). The Bence Jones fragments will not typically show up in otherwise healthy histoplasmosis positive participants (Wells 1980). The anti-human polyvalent immunoglobulins conjugate having detected mainly IgA is consistent with the premise that histoplasmosis induces significant IgA

responses even when IgG and IgM levels are relatively normal (Chandler et al. 1969; Cox and Arnold 1980). In general, the clearing time for IgA in an adult is approximately 6 days (Hood et al. 1978). In histoplasmosis infections, IgA and IgM titers rise 14-27 days post infection and start clearing approximately three months later (Chandler et al. 1969; Kaufman 1971; Wheat et al. 1983). This suggests that histoplasmosis produces a long IgA secretion period.

As established in Table 2, the majority of the participants with only anti-histoplasmin immunoglobulin detected by the anti-human polyvalent conjugate, did not have any indicative change in detectable antibody level during this study. The detectable levels of anti-histoplasmin IgA without concurrently detectable anti-histoplasmin immunoglobulin M or G levels is not typical of any previous report of immunoglobulin responses to histoplasmosis (Chandler et al. 1969; Cox and Arnold 1980; Sprouse et al. 1981). As Sprouse et al. (1981) noted, the IgA role in serological tests is varible and difficult to compare between different methods of detection. Additional research is needed to determine the significance of these elevated IgA antibodies with concurrently non-detectable levels of IgG or IgM antibodies.

Polyvalent Immunoglobulins and Immunoglobulin M Detection

Sera from eight of the thirty-four participants (24%) produced detectable anti-histoplasmin antibody levels with both the anti-human polyvalent immunoglobulin conjugate and anti-human IgM conjugates. Of these eight, anti-histoplasmin antibodies to both the mycelial-phase and yeast-phase of histoplasmosis were detected in seven participants. Six of the seven participants exhibited a significant (index of greater than 1.3) anti-histoplasmin antibody level for one or more samples (Table 2).

One participant (number 1354) had a notable increase in anti-histoplasmin IgM levels in both the histoplasmin yeast-phase and mycelial-phase testing during this study period. The increase was from an index of 0.8 in the first sample to an index of 1.7 in the second sample to an index of 2.3 in the third sample for the anti-histoplasmin mycelial phase IgM immunoglobulins detection. The same degree of antibody titer increase was also seen in the anti-histoplasmin yeast phase IgM immunoglobulins detection. The index values for this test changed from 0.5 (negative) in the first sample to 1.2 for both the second and third samples. The anti-histoplasmin immunoglobulin detected with the anti-human polyvalent immunoglobulins conjugate also showed some increase in the histoplasmin yeast-phase tests' second sample, but not as

great as in the anti-histoplasmin IgM detection. The anti-histoplasmin IgM index more than doubled from the first to the third sample indicating a large change in antibody level. This large change in antibody level is the type of reaction that would be typical for a person that had became benignly infected and produced a normal humoral response to histoplasmosis (Goodman 1982; Chandler et al. 1969; Kaufman 1971; Wheat et al. 1983). The change in values could also be the result of normal antibody titer variations within the participant and test system, but this is considered unlikely due to the large degree of change seen and the reactions of other participants' samples in these tests.

One other participant had an overall rise in antibody levels. Participant number 1458 demonstrated an anti-Histoplasma antibody increase in both the histoplasmin yeast-phase and mycelial phase testing for the IgM class and the polyvalent immunoglobulins conjugate. The antibody level increase was not as great as seen with the previous participant (1354) but was consistent over all four tests.

Four participants had no essential change in the antibody levels for their three samples. One participant (number 1362) had very high anti-histoplasmin yeast-phase and mycelial-phase antibody levels (index greater than 1.7) in the IgM class of immunoglobulins without

commensurate levels in the other classes of immunoglobulins (Table 2). Wheat et al. (1983) previously observed similar elevations of IgM that were not explainable based on current knowledge of the disease. One participant had detectable anti-histoplasmin antibody levels to only one of the antigens and not both antigens together. This participant, number 1467, had detectable anti-histoplasmin antibody levels with both anti-human polyvalent and anti-human IgM conjugates in the histoplasmin mycelial-phase antigen test only. A higher anti-histoplasmin antibody level was detected in the second sample with the anti-human polyvalent immunoglobulin conjugate than the anti-histoplasmin antibody level in either the first or third sample. It can not be determined if the higher second sample index value for the anti-human polyvalent immunoglobulin conjugate is a true IgA mediated response, an artifact in the testing system or the normal variation of antibody levels in a participant. The anti-histoplasmin IgM had index values that were detectable but not significantly elevated when compared to the samples of the other participants. The anti-histoplasmin IgM levels also remained relatively constant throughout the study period (Table 2). Participants 1452 and 1463 have detectable antibody levels in the histoplasmin mycelial-phase and yeast-phase testing with both anti-human polyvalent

conjugate and with the anti-human IgM conjugate (Table 2). During the duration of this study, the antibody levels for both of these participants did not change (less than 0.3 index value difference). Only the antibody levels of participant 1452 were consistently high enough to be considered significant (greater than 1.3) and represent exposure to Histoplasma prior to arrival at Fort Campbell.

Two participants had anti-histoplasmin antibody levels that decreased from the index level of the first two samples to the third sample. Participant number 1385 had detectable anti-histoplasmin immunoglobulin levels in the initial first and second samples, then declining levels to the third sample. This was detected in both histoplasmin mycelial-phase and yeast-phase testing with both anti-human polyvalent conjugate and with the anti-human IgM conjugate. Participant number 1461 had detectable anti-histoplasmin antibody levels in the first two samples that decreased to undetectable levels in the third sample. These immunoglobulins were detected with both anti-human polyvalent conjugate, in the histoplasmin mycelial-phase and yeast-phase tests, and with the anti-human IgM conjugate, in the histoplasmin yeast-phase test. These patterns suggest exposure that triggered the IgM (and IgA in participant 1461 for anti-histoplasmin mycelial-phase) to rise before the first sample was collected (Chandler et al. 1969; Kaufman 1971). As seen

in Table 2 for these two participants, the majority of the immunoglobulin classes are returning to undetectable levels (less than 0.7) without the anti-histoplasmin IgG level being detected (greater than 0.7). The lack of anti-histoplasmin IgG level is most likely due to the lag time between loss of anti-histoplasmin immunoglobulins A and M and formation of the detectable anti-histoplasmin IgG level (Chandler et al. 1969; Kaufman 1971).

Polyvalent Immunoglobulins and Immunoglobulin G Detection

Four of the thirty-four participants (12%) had detectable anti-histoplasmin antibody levels with both the anti-human polyvalent immunoglobulin and anti-human IgG conjugates.

Of these four, only one participant had detectable anti-histoplasmin antibody levels with both immunoglobulins in the histoplasmin mycelial-phase antigen test alone. Participant number 1353 had only anti-histoplasmin antibody levels in the histoplasmin mycelial-phase antigen and the anti-histoplasmin antibody levels detected were very low (indexes of 0.8). This is a IgG response in which the levels are detectable but constant for a period of time (Chandler et al. 1969; Kaufman 1971).

Two participants had anti-histoplasmin IgG levels in the histoplasmin mycelial-phase test increase from the first sample to the third sample. At the same time, their anti-histoplasmin immunoglobulins detected with the anti-human polyvalent immunoglobulins conjugate remained constant. Participant number 1489 had very high anti-histoplasmin immunoglobulin levels (index greater than 1.7) detected by the anti-human polyvalent immunoglobulin conjugate compared with the anti-histoplasmin immunoglobulin levels detected by anti-human IgG conjugate having index levels of 1.0 or less. The anti-histoplasmin yeast-phase antibody level detected by the anti-human polyvalent immunoglobulin conjugate was very low (index less than 1.0). This participant's response was typical of the IgG response (Chandler et al. 1969; Kaufman 1971) except that the anti-histoplasmin mycelial-phase immunoglobulin level for the third sample showed a slight increase from the first sample. This is possibly due to a mild, stimulating exposure to Histoplasma (Barrett 1974). Another participant (number 1472) had higher anti-histoplasmin yeast-phase antibodies, as detected by the anti-human polyvalent immunoglobulin conjugate, than those determined in the histoplasmin mycelial-phase antigen tests. This reaction would be typical of a subclinical pulmonary infection (Buechner et al. 1973). This participant, number 1472, did not have any exposure due to

having lived in an endemic area or near a farm with fowl (see discussion on Previous Exposure, page 50). The participant's military occupational specialty, field artillery, does not place him at a higher risk for exposure than any other military occupational specialty.

The remaining participant in this group (1454) had detectable anti-histoplasmin antibody levels with both anti-human polyvalent and M immunoglobulin conjugates in both the histoplasmin mycelial-phase and yeast-phase testing. The anti-histoplasmin mycelial-phase antibody level was extremely high (index greater than 2.0) in this participant. The anti-histoplasmin yeast-phase antigen antibodies were also higher than seen in many other participants (index 0.8-1.7), but not as high as in the histoplasmin mycelial-phase test. The IgG levels detected against both yeast and mycelial antigens showed a rise from the first to the third sample suggesting a previous exposure from one to three months earlier (Chandler et al. 1969; Kaufman 1971). This participant had obvious exposure to Histoplasma that continued to increase the anti-histoplasmin antibody levels either through normal progression or through continued exposure on arrival at Fort Campbell. The change in values could also be the result of normal antibody titer variations within the participant and test system, but this is considered unlikely due to the degree of change seen and the

reactions of other participants samples in these tests. Due to the anti-histoplasmin IgM being non-detectable (index 0), continued exposure is ruled out. This participant indicated previous exposure of greater than five years in endemic areas and exposure due to having lived on or near a farm that raised fowl (see Previous Exposure discussion section). The participant's military occupational specialty does not place him at a higher risk for exposure than any other military occupational specialty. This participant was the only one of the thirty-four participants and one of three from all eighty-two participants enrolled to have any IgG anti-histoplasmin yeast-phase antibody detected. This anti-histoplasmin yeast-phase IgG antibody most likely represents the long term response of the IgG class of immunoglobulins to a primary pulmonary infection that produces a yeast-phase response (Kaufman and Riess 1985).

Combined Immunoglobulins Detection

Seven of the thirty-four participants (21%) had detectable anti-histoplasmin antibody levels detected with all three anti-human immunoglobulin conjugates: anti-human polyvalent immunoglobulin, anti-human IgM and anti-human IgG conjugates. All participants had detectable anti-histoplasmin antibody levels with all three anti-human immunoglobulin conjugates in the histoplasmin mycelial-

phase tests. None of the participants had detectable anti-histoplasmin antibody levels with all anti-human immunoglobulin conjugates in histoplasmin yeast-phase tests. In five of the seven participants (numbers 1361, 1375, 1466, 1351 and 1366) the anti-histoplasmin mycelial-phase IgG decreased from the first sample to the third.

In participants number 1361 and 1366, the anti-human polyvalent immunoglobulins conjugate detected higher levels of anti-histoplasmin immunoglobulin antibodies than the levels detected by either anti-human IgG or anti-human IgM conjugates and possibly represents IgA in these participants. The anti-histoplasmin IgA level was decreasing in both participants and the anti-histoplasmin immunoglobulins G and M remained constant. Both participants (numbers 1361 and 1366) had high anti-histoplasmin antibody levels in most of the classes. It would be difficult to determine what the changes in immunoglobulin levels represent in these participants without clinical correlation. Possibly the results show the natural clearing of the anti-histoplasmin antibodies that were detected with the anti-human immunoglobulins conjugate. The anti-histoplasmin immunoglobulins G and M remaining constant does not match the previously stated clearing patterns for anti-histoplasmin antibodies (Chandler et al. 1969; Kaufman 1971).

In participant number 1375, the anti-histoplasmin antibody levels decreased from a significant antibody level (index greater than 1.3) to essentially zero in anti-histoplasmin IgG and M in the histoplasmin mycelial-phase test and in the immunoglobulins detected with anti-human polyvalent immunoglobulins conjugate. The latter anti-histoplasmin immunoglobulins most likely represent anti-histoplasmin IgA. This antibody response most likely represents the final clearing of the anti-histoplasmin antibodies from a prior exposure many months or perhaps a year ago (Chandler et al. 1969; Kaufman 1971). This participant showed no serologic evidence of re-exposure at Fort Campbell.

Participant 1466 was the only participant to have both detectable anti-histoplasmin IgM and IgG levels to the mycelial-phase antigen tested, but no detectable anti-histoplasmin yeast-phase immunoglobulin. The anti-histoplasmin immunoglobulins G and M antibodies showed a decrease from a detectable level in the first two samples to an undetectable level (index less than 0.7) anti-histoplasmin IgG or IgM in the third sample. The very constant anti-histoplasmin immunoglobulin levels again confuse what would otherwise be seen as normal clearing of the anti-histoplasmin antibodies (Chandler et al. 1969; Kaufman 1971).

The anti-histoplasmin mycelial-phase IgG level decreased in one participant (number 1492). The remaining detectable anti-histoplasmin immunoglobulins were of a constant index level (Table 2). This decrease of anti-histoplasmin mycelial-phase IgG level could represent the normal clearing of this immunoglobulin from an exposure approximately one year ago and a re-exposure that has stimulated the other immunoglobulin classes more recently but before arrival at Fort Campbell. This participant did not suggest any previous exposure attributable to either living in an endemic area or living on or near a farm that raised fowl (see Previous Exposure discussion section, page 50). The participant's military occupational specialty does not place him at a higher risk for exposure than any other military occupational specialty.

The last participant in this group (number 1365) had an increase in at least two of the five anti-histoplasmin antibodies detected. The mycelial-phase immunoglobulins G and M and the yeast-phase anti-histoplasmin immunoglobulins detected with the anti-human polyvalent immunoglobulin conjugate and anti-histoplasmin IgM were all elevated in the third sample compared with the levels obtained in the first or second samples. Due to the antibodies detected and their formation times, this participant's response is most likely the reaction to re-exposure to Histoplasma slightly preceding arrival at Fort

Campbell. The change in values could also be the result of normal antibody titer variations within the participant and test system due to the relatively low changes in antibody levels.

In three of the seven participants, the IgM level detected was higher than the corresponding IgG level detected. All three showed no meaningful change in anti-histoplasmin antibody levels detected between the first and third samples. Five of the seven participants had detectable anti-histoplasmin antibody levels with both anti-histoplasmin mycelial-phase immunoglobulins A, G and M, and anti-histoplasmin yeast immunoglobulin M and A. The IgA was determined by the anti-human polyvalent immunoglobulin conjugate detecting higher amounts of total antibody than the corresponding antibody detected with anti-human IgM conjugate. All five participants had extremely high (index greater than 2.0) anti-histoplasmin antibody levels, as detected by the anti-human polyvalent immunoglobulin conjugate (Table 2).

Antigen Responses

Contrary to previous reports (George and Lambert 1984; Davies 1986; Buechner et al. 1973), more participants had anti-histoplasmin mycelial-phase antibody levels without corresponding levels of anti-histoplasmin yeast antibody levels. Six of the participants shown in

Table 2 had anti-Histoplasma mycelial-phase antibodies as compared to only one participant (number 1488) that had anti-histoplasmin yeast-phase antibodies with no anti-histoplasmin mycelial-phase antibodies. In only two of the participants (10%) that had anti-histoplasmin yeast-phase antibody levels (1488 and 1385), were the anti-histoplasmin yeast-phase antibody levels higher than the corresponding anti-histoplasmin mycelial-phase antibody levels. Even considering those participants with significant anti-Histoplasma antibody levels (index greater than 1.3), there were more anti-histoplasmin mycelial-phase than yeast-phase positive participants. There were five anti-histoplasmin mycelial-phase participants (1361, 1375, 1376, 1458, 1463, and 1489) with significant antibody levels and only two with significant yeast-phase antibodies (1385 and 1472) that did not have the other corresponding yeast-phase or mycelial-phase (respectively) antibody at a significant level.

Previously published reports suggest that anti-histoplasmin antibodies occur more frequently to yeast antigen than to mycelial antigen (Davies 1986; George and Lambert 1984). The more frequent occurrence of histoplasmosis yeast-phase antibodies than anti-histoplasmin mycelial-phase antibodies is difficult to explain given the typical clinical course of the infection. The infectious process involves a dimorphous

fungus. In the soil, the fungus exists in its mycelial form. When the soil becomes disturbed, the fungus spores become airborne and may be inhaled. The spores enter the lungs and become stuck in the distal air spaces where they convert into the invasive yeast form of the fungus (Penn and George 1983; Macher 1980; Goodwin and Des Prez 1978). This route and method of infection should have the host developing immunoglobulin levels to the mycelial antigens before the yeast-phase infection occurs.

It is possible that in the report by Davies (1986), which was based on complement fixation testing, the response to the histoplasmosis mycelial-phase antigens could not be detected as well as with an enzyme-linked immunosorbent assay test or other system that tests for all classes of immunoglobulins. This is supported in that IgG is the major activator of complement fixation (Hood et al. 1978; Goodman 1987; Stansfield 1981) and the other classes of immunoglobulins may be missed during complement fixation testing (Sprouse et al. 1981; Chandler et al. 1969). Enzyme-linked immunosorbent assay tests, when used quantitatively, have been reported to be more than twenty times as sensitive as complement fixation testing (Sharma et al. 1982). Enzyme-linked immunosorbent assay tests thus may be able to detect the other classes of immunoglobulins and/or smaller amounts of antibody, that

demonstrate histoplasmosis mycelial-phase infection, better than complement fixation tests.

George and Lambert (1984), reported the results of testing healthy blood donors by both complement fixation and RIA. The complement fixation testing showed more positive yeast-phase antibodies than were detected in the mycelial-phase testing. Using RIA, the opposite was seen; there were more mycelial-phase antibodies detected than yeast-phase antibodies. Both complement fixation and RIA had more yeast-phase antibodies detected in the lower levels of detection. This report was based on healthy blood donors whose previous exposure to Histoplasma was not known. Since the subjects appeared healthy, it would be reasonable to suspect that the acute infection with mycelial forms was not present at the time of sample collection.

Sharma et al. (1982) demonstrated that yeast-phase antibodies had a higher early titer than mycelial-phase antibodies; the antibody difference changed approximately nine weeks after infection when mycelial-phase antibodies reached high titers. The higher mycelial levels in this study group could therefore be explained by the difference in duration since infection or exposure.

IgG production is stimulated by protein antigens and IgM production is stimulated by antigens composed of polysaccharide materials (Stansfield 1981). Histoplasma

organisms, both mycelial and yeast, contain a predominance of polysaccharide containing materials in comparison with the protein content (Bradley et al. 1974; Drutz 1986; Brock et al. 1984). The variation in the stimulation of anti-histoplasmin antibodies, based on the difference in antigen composition, supports the more frequent detection of anti-histoplasmin IgM and hence the lack of some complement fixation sensitivity. More (eight verses four) participants had detectable anti-histoplasmin IgM levels without concurrently detectable anti-histoplasmin IgG levels than the number of participants with detectable anti-histoplasmin IgG levels without concurrently detectable anti-histoplasmin IgM levels. Again, this may signify a higher detection rate of histoplasmosis for enzyme-linked immunosorbent assay testing than previous complement fixation testing may have achieved. Campbell (1960) pointed out that different antibodies could also be due to the antibody responses of different stages of the infection producing different antigenic components.

Previous exposure

Two questions were asked of each of the participants to determine the possibility of a prior exposure to Histoplasma (Appendix 1). The first question included a map of the United States and had the area considered endemic for histoplasmosis shaded (Edwards

et al. 1969). The first question asked if the participant had ever lived in the shaded area for one, six, twelve and/or sixty months. The second question asked about the participant living on or near a farm that raised fowl.

Table 4 shows that of the eighty-two participants, only sixteen participants (18.8%) gave no history of any of these type exposure routes. The exposure results given in Table 5 were very similar to the results noted in Table 4. Fifteen percent of the thirty-four participants that completed the study indicated no known exposure to Histoplasma. This was determined by the supplemental information about prior exposure that each participant provided at the start of the study. Those participants identified as having detectable anti-histoplasmin antibody levels did not come from any one particular exposure group based on the supplemental information. Fifty percent of the participants that did not signify any known exposure and had significant (index value greater than 1.3) anti-histoplasmin antibody levels. This included both anti-histoplasmin yeast-phase and anti-histoplasmin mycelial-phase immunoglobulins G and M antibodies. This high rate of significant antibody levels in "unexposed" participants is probably due to the increased sensitivity of the enzyme-immunoassay system used.

Inapparent infection with histoplasmosis is very common in the central portion of the United States and

over 99% of those infected experience benign disease courses (Jacobson and Straus 1981). According to Jacobson and Straus (1981), reactivity to a single antigen, even at high levels of anti-histoplasmin antibody, has been shown unreliable for the diagnosis of histoplasmosis. The presence of serum antibodies is therefore only suggestive or supportive of the diagnosis because they can result from previous infection or the infection of a related fungus (Lambert and George 1987). Serologic findings in histoplasmosis, as contrasted to coccidioidomycosis, consist of studies from separate geographical areas (Campbell 1960). Almost one-third of the participants that had detectable anti-histoplasmin antibodies indicated no exposure to what is considered the most common routes of infection, specifically living in a geographic area and/or fowl. This high incidence of anti-histoplasmin antibodies occurring among those that should be considered as having a low likelihood of exposure suggests that histoplasmosis might be a widespread and benign infection. People who think they have low probability for exposure to Histoplasma may have a higher than expected exposure to the organism. Histoplasmosis should not be ruled out of a diagnosis based on a negative history alone.

Previous skin tests

Not included was the possibility of detectable anti-histoplasmin antibodies resulting from prior skin testing. This should be considered as a source of possible interference to the results obtained. Several reports (Heusinkveld et al. 1967; McDearman and Young 1960; Sigrest et al. 1963; Davies and Sarosi 1987a) have stated that histoplasmin skin testing will produce an anti-histoplasmin antibody response that can be detected by complement fixation. It is therefore possible that the enzyme-linked immunosorbent assay used also could have detected antibodies formed as a result of skin testing. According to Scalarone et al. (1985), the newer skin test reagent, Histolyn-CYL, has been reported not to produce a significant serological response in complement fixation or micro-ID. Zeidberg et al. (1951) and Penn and George (1983) have noted that the skin test response decreases over time. The possibility of the participants having detectable anti-histoplasmin antibody levels due to a previous skin test therefore cannot be determined. Possible effects of prior skin testing on enzyme-linked immunosorbent assays needs to be studied.

Cross-reactions

Cross-reactions with sera positive for other fungal infections, including blastomycosis and coccidiomycosis, have limited the specificity of the serological tests for

histoplasmosis (Kaufman and Reiss 1986; Drutz and Graybill 1987; Wheat et al. 1986a). Previous reports indicate that from 2-50% of samples from other study's participants with other diseases, including lung and sexually transmitted diseases, had detectable histoplasmosis assay results (Davies and Sarosi 1987a; Lambert and George 1987; Davies 1986; Wheat et al. 1983; George et al. 1981). Both the histoplasmosis radioimmunoassay screening test (George et al. 1981) and enzyme-linked immunosorbent assay showed similar lack of specificity comparable to the lack of specificity with the complement fixation tests (Lambert and George 1987; George et al. 1981). Davies and Sarosi (1987b) described the cross-reactivity of their enzyme-linked immunosorbent assays for histoplasmosis and blastomycosis as having almost perfect cross-reactions with the opposite infection. Calculations of specificity varied widely depending on the control group (Penn et al. 1983; Terry et al. 1978; Wheat et al. 1982; Lambert and George 1987; George and Lambert 1984; Bauman and Smith 1975; Davies 1986). In addition to the problem of cross-reactions with heterogenous antigens from other organisms, additional explanations for incorrect test results include testing not done properly, undefined acute phase reactants including rheumatoid factor, or clinically undetected histoplasmosis (Terry et al. 1978). The heterogeneous nature of the antigens from histoplasmosis, blastomycosis

and coccidiomycosis potentially limit the current usefulness of serological testing for these mycoses.

The cross-reactions in histoplasmosis testing with blastomycosis has been reported not to restrict the usefulness of the assays due to the low incidence of blastomycosis (Davies and Sarosi 1987b; Wheat et al. 1983) in spite of the shared endemic areas. This approach may be useful in treating patients, but causes difficulty in interpreting the results of testing.

Coccidioidomycosis cross-reactions would normally not be of concern during histoplasmosis testing, owing to the difference in geographical distribution of the two organisms and to the difference that can be clinically determined (Wheat et al. 1983). As Campbell and Binkley (1953) pointed out however, military personnel move in and out of states endemic for different mycoses. This caveat is especially accurate since the United States Army developed the desert warfare training center at Fort Irwin, California and large numbers of military personnel have trained there annually.

Boyer and Scalarone (1983) used Histolyn CYL in their enzyme-linked immunosorbent assay and had only slight cross-reactivity with Coccidioides positive serum. Histolyn CYL, a yeast-phase skin testing reagent, may have fewer heterogeneous antigens than previously used enzyme-linked immunoassay Histoplasma antigens. In this study,

column purified Histoplasma extracts were used to reduce the occurrence of cross reactions with other fungal organisms (personal communication with Meridian Diagnostics, Inc. 1988). In addition, very limited cross-reactivity was seen in a separate study when histoplasmosis, blastomycosis and coccidiomycosis cross absorbed sera was used as specimens in the test system (Wolcott, unpublished data). This is similar to information Wheat et al. (1986a) published concerning inhibition studies. Since the actual diagnostic status of the participants is not known, the true specificity of the assay developed for this study is not known.

Chapter V

SUMMARY

Eight of the thirty-four participants that submitted three specimens (24%) (Table 2) had no seroconversion to having detectable anti-Histoplasma antibodies by the end of three months. The thirty four participants represent approximately one-half of one days newly arriving personnel and approximately one-tenth of one percent of the military personnel stationed at Fort Campbell at the time of this study.

Thirty of the thirty-four participants that submitted three specimens (88%) gave no indication of exposure to Histoplasma as a result of being assigned to Fort Campbell. The four other participants (12%) had results indicating either re-exposure to Histoplasma or normal antibody titer variations within the participant and test system. Each of these four participants had index value differences between the classes of IgM and IgG immunoglobulins indicating that none of the four responded in the same manner as the others in this group. None of the participants that arrived at Fort Campbell without serological evidence of previous infection to Histoplasma had a detectable seroconversion to Histoplasma during this study.

The serologically detectable anti-Histoplasma antibodies, detected on the first day as shown in Table 2,

suggests that those participants may have been previously exposed to Histoplasma and had the anti-Histoplasma antibodies on arrival at Fort Campbell. These twenty-two participants (65%) represent a higher than expected incidence for persons not necessarily from an endemic area and previously not considered to have a high probability for exposure to Histoplasma.

Of the twenty-six participants that had detectable anti-Histoplasma antibody levels in Table 2, more had anti-histoplasmin mycelial-phase antibody levels without the corresponding anti-histoplasmin yeast antibody level. This is in contrast to only one participant with anti-histoplasmin yeast-phase antibody levels without the corresponding anti-histoplasmin mycelial-phase antibody levels. This is contrary to previously published information and needs further research to clarify the significance and cause.

Eight of the twenty-six participants (31%) with detectable anti-Histoplasma antibody levels, shown in Table 2, had anti-histoplasmin IgM levels without concurrent anti-histoplasmin IgG levels. Four participants had anti-histoplasmin IgG levels without concurrent anti-histoplasmin IgM levels. The possibility of histoplasmosis seroconversion from a seronegative status, as a sole result of being stationed at Fort Campbell for three months, is negligible for this small population sampled.

LITERATURE CITED

Anderson, H.R. 1980. Bird roosts and histoplasmosis. Journal of the Tennessee Medical Association. 732-733.

Barrett, James T. 1974. Biological aspects of immunoglobulin formation. Pages 48-96 in Textbook of immunology. An introduction to immunochemistry and immunobiology. The C.V. Mosby Co., Saint Louis.

Bauman, David S. and Coy D. Smith. 1975. Comparison of immunodiffusion and complement fixation tests in the diagnosis of histoplasmosis. J. Clin. Microbiol. 2:77-80.

Berkow, Robert and John H. Talbott, eds. 1977. The merck manual of diagnosis and therapy. 13th ed. Merck Sharp and Dohme Research Laboratories, Rahway New Jersey.

Boorsma, D.M. and J.G. Streefkerk. 1978. Improved method for separation of peroxidase conjugates. Pages 225-235 in W. Knapp, K. Holubar and G. Wick, eds. Immunofluorescence and related staining techniques. Proceedings of the VIth international conference on immunofluorescence and related staining techniques held in Vienna, Austria on April 6-8, 1978. Elsevier/North-Holland Biomedical Press, Amsterdam.

Boyer, M.J. and G.M. Scalarone. 1983. The use of Histolyn CYL in an enzyme immunoassay to detect Histoplasma capsulatum antibodies. Sabouraudia. 21:303-315.

Bradley, Georgia, Leo Pine, Michael W. Reeves, and C. Wayne Moss. 1974. Purification, composition, and serological characterization of histoplasmin-H and M antigens. Infect. Immun. 9:870-880.

Brock, Edson G., Errol Reiss, Leo Pine, and Leo Kaufamn. 1984. Effect of peroxidation on the detection of antibodies against the M-antigen of histoplasmin by enzyme immunoassay (EIA) inhibition. Curr. Microbiol. 10:177-180.

Buechner, Howard A., John H. Seabury, Charlotte C. Campbell, Lucille K. Georg, Leo Kaufman, and William Kaplan. 1973. The current status of serologic, immunologic and skin tests in the diagnosis of pulmonary mycoses. Chest. 63:259-270.

Campbell, Charlotte C. 1980. Histoplasmosis outbreaks: recommendation for mandatory treatment of known microfoci of H. capsulatum in soils. Editorial in Chest. 77:6-7.

Campbell, Charlotte C. 1960. The accuracy of serological methods in diagnosis. Ann. N. Acad. Sci. 89:163-177.

Campbell, Charlotte C. 1967. Serology in the respiratory mycoses. Sabouraudia. 5:240-259.

Campbell, Charlotte C. and Grace E. Binkley. 1953. Serologic diagnosis with respect to histoplasmosis, coccidiomycosis, and blastomycosis and the problem of cross reactions. J. Lab. Clin. Med. 42:896-906.

Caudill, Georgia, Stephen Deak, William Medina, and Stephen Ross. 1975. Histoplasmin sensitivity rates among children in Hopkinsville and Fort Campbell, Kentucky. Student Report for the University Of Kentucky.

Chandler, John W., Terrill K. Smith, W. Marcus Newberry, Jr., Tom D.Y. Chin, and Charles H. Kirkpatrick. 1969. Immunology of the mycoses. II. Characterization of the immunoglobulin and antibody responses in histoplasmosis J. Infect. Dis. 119:247-254.

Chick, Ernest W., Suzanne B. Compton, Ted Pass III, Bill Mackey, Calixto Hernandez, and E. Austin Jr., Frank R. Pitzer, Clyde Flanigan. 1981. Hitchcock's birds, or the increased rate of exposure to Histoplasma from blackbird roost sites. Chest. 80:434-438.

Chick, E.W., C. Flanigan, S.B. Compton, T. Pass III, C. Gayle, C. Hernandez, F.R. Pitzer, and E. Austin Jr. 1980. Blackbird roosts and histoplasmosis An increasing medical problem. Editorial in Chest. 77:584-585.

Chick, Ernest W., Suzanne B. Compton, Ted Pass III, Frank R. Pitzer, Clyde Flanigan, Bill Mackey, Calixto Hernandez, and E. Austin Jr. 1980. A new appraisal of histoplasmin sensitivity in Kentucky. Kentucky Medical Association. 405-407.

Chick, E.W., G.L. Baum, M.L. Furcolow, M. Huppert, L. Kaufman, and D. Pappagianis. 1973. The use of skin tests and serologic tests in histoplasmosis, coccidioidomycosis, and blastomycosis. Am. Rev. Respir. Dis. 108:156-159.

Cox, Rebecca A. 1985. Serodiagnosis of coccidioidomycosis, histoplasmosis, and blastomycosis. Lab. Mgmt. 23:46-52.

Cox, Rebecca A. and Douglas R. Arnold. 1980. Immunoglobulin E in histoplasmosis. Infect. Immun. 29:290-293.

Cox, Rebecca. 1979. Immunologic studies of patients with histoplasmosis. Am. Rev. Respir. Dis. 120:143-149.

Davies, Scott F. and Georgia A. Sarosi. 1987a. Role of serodiagnostic tests and skin tests in the diagnosis of fungal disease. Clinics In Chest Med. 8:135-146.

Davies, Scott F. and George A. Sarosi. 1987b. Serodiagnosis of histoplasmosis and blastomycosis. Am. Rev. Respir. Dis. 136:254-255.

Davies, Scott F. 1986. Serodiagnosis of histoplasmosis. Sem. Respir. Infect. 1:9-15.

Department of the Army. 1975. Final environmental impact Statement: Blackbird control at two Army installations, Fort Campbell, Kentucky - Milan, Tennessee AAP. U.S. Government Printing Office, Washington, D.C.

Drutz, David J. 1986. Antigen detection in fungal infections. N. Engl. J. Med. 314:115-117.

Drutz, David J. and John Richard Graybill. 1987. Infectious disease. Pages 534-581 in D.P. Stites, J.D. Stobo, and J.V. Wells, eds. Basic and clinical immunology, 6th ed. Appleton and Lange, Norwalk Conn.

Edwards, Lydia B., Francis A. Acquaviva, Verna T. Livesay, Forrest W. Cross, and Carroll E. Palmer. 1969. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. Am. Rev. Respir. Dis. 99:1-132.

Furcolow, Michael L. 1963. Tests of immunity in histoplasmosis. N. Engl. J. Med. 268:357-361.

Gabal, M.A. and K.A. Mohammed. 1985. Use of enzyme-linked immunosorbent assay for the diagnosis of equine Histoplasmosis farciminosi (epizootic lymphangitis). Mycopatha. 91:35-37.

George, Ronald B., Ronald S. Lambert, Marie J. Bruce, Jerry W. Pickering, and R. Michael Wolcott. 1981. Radioimmunoassay: A sensitive screening test for histoplasmosis and blastomycosis. Am. Rev. Respir. Dis. 124:407-410.

George, Ronald B. and Ronald S. Lambert. 1984. Significance of serum antibodies to Histoplasma capsulatum in endemic areas. S. Med. J. 77:161-163.

Goodman, Joel W. 1987. Immunoglobulins I: Structure and function. Pages 27-36 in Daniel P. Stites, John D. Stobo, and J. Vivian Wells, eds. Basic and clinical immunology, 6th ed. Appleton and Lange, Norwalk Conn.

Goodman, Norman L. 1982. Current concepts in the diagnosis of systemic mycoses. Kansenshogaku Zasshi. 56:908-911.

Goodwin, Robert A., James E. Loyd, and Roger M. Des Prez. 1981. Histoplasmosis in normal hosts. Medicine. 60:231-266.

Goodwin, Robert A. and Roger M. Des Prez. 1978. State of the Art; Histoplasmosis. Am. Rev. Respir. Dis. 117:929-956.

Heusinkveld, Robert S., Fred E. Tosh, W. Marcus Newberry Jr., Pasquale J. Ciccone, and Tom D.Y Chin. 1967. Antibody response to the histoplasmin skin test. Am. Rev. Respir. Dis. 96:1069-1072.

Hood, Leroy E., Irving L. Weissman and William B. Wood. 1978. Immunology. The Bejamin/Cummings Publishing Company, Inc., Menlo Park, California. 467 pp.

Jacobson, Eric S. and Stephen E. Straus. 1981. Reevaluation of diagnostic Histoplasma serologies. Am. J. Med. Sci. 281:143-151.

Jacobson, Eric S. and Stephen E. Straus. 1983. Serologic tests for histoplasmosis. Ann. Intern. Med. 98:560-561.

Kauffman, Carol A., Karen S. Israel, James W. Smith, Arthur C. White, Jan Schwarz, and George F. Brooks. 1978. Histoplasmosis in immunosuppressed patients. Am. J. Med. 64:923-932.

Kaufman, Leo and Errol Riess. 1986. Serodiagnosis of fungal disease. Pages 446-466 in Noel R. Rose, Herman Friedman, and John L. Fahey. eds. Manual of clinical laboratory immunology, 3d ed. American Society for Microbiology, Washington D.C.

Kaufman, Leo and Errol Riess. 1985. Serodiagnosis of fungal disease. Pages 924-948 in Edwin H. Lennette, Albert Balows, William J. Hausler, Jr., and H. Jean Shadomy, eds. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington D.C.

Kurstak, Edouard. 1986. Enzyme immunodiagnosis. Academic Press, Inc., New York.

Lambert, Ronald S. and Ronald B. George. 1987. Evaluation of enzyme immunoassay as a rapid screening test for histoplasmosis and blastomycosis. Am. Rev. Respir. Dis. 136:316-319.

Macher, Abe. 1980. Histoplasmosis and blastomycosis. Med. Cl. N. Am. 64:447-491.

MacVandiviere, H., Norman L. Goodman, Irene G. Melvin, Raj Narain, and H. David Wilson. 1981. Histoplasmosis in Kentucky can it be prevented? Kentucky Medical Association. 719-726.

Mandell, William, David M. Goldberg, and Harold C. Neu. 1986. Histoplasmosis in patients with the acquired immune deficiency syndrome. Am. J. Med. 81:974-978.

McDearman, Sara C. and Joseph M. Young. 1960. The development of positive serologic tests with Histoplasma capsulatum antigens following single histoplasmin skin tests. Am. J. Clin. Pathol. 34:434-438.

McMurray, David N., Jane Stroud, Jana J. Murphy, Mirta A. Carlomagno, and Donald L. Greer. 1982. Role of immunoglobulin classes in experimental histoplasmosis in bats. Developmental and Comparative Immunology. 6:557-567.

Owens, R.D. and G. M. Scalarone. 1984. Immunodiffusion studies on the antigens of Histoplasma capsulatum: comparison of mycelial histoplasmin with the yeast phase reagent Histolyn-CYL. Sabouraudia. 22:487-491.

Pearson, Gary R. 1985. Introduction to immunology. Pages 230-257 in Bob A. Freeman ed. Burrows textbook of microbiology, 22nd ed. W.B. Saunders Co., Philadelphia.

Penn, Robert L., Ronald S. Lambert, and Ronald B. George. 1983. Invasive fungal infections; the use of serologic tests in diagnosis and management. Arch. Intern. Med. 143:1215-1220.

Penn, Robert L. and Ronald B. George. 1983. Respiratory Infections. Pages 403-466 in Ronald B. George, Richard W. Light, Richard A. Matthay, eds. Chest Medicine. Churchill Livingstone, New York.

Richardson, M.D. and D.W. Warnock. 1983. Enzyme-linked immunosorbent assay and its application to the serological diagnosis of fungal infection. Sabouraudia. 21:1-14.

Riley, Harris D. 1983. The history of histoplasmosis. Oklahoma State Medical Association Journal. 76:31-40.

Rippon, John W. 1985. Medical mycology: The pathogenic fungi and the pathogenic actinomycetes. Pages 874-937 in Bob A. Freeman ed. Burrows textbook of microbiology, 22nd ed. W.B. Saunders Co., Philadelphia.

Sarosi, George A., Donald Armstrong, Scott F. Davies, Ronald B. George, John R. Graybill, James E. Pennington, Glenn D. Roberts, and David A. Stevens. 1984. Laboratory diagnosis of mycotic and specific fungal infections. Am. Thor. Soc. :1373-1379.

Sathapatayavongs, Boonmee, Byron E. Batteiger, Joseph Wheat, Thomas G. Slama, and Justin L. Wass. 1983. Clinical and laboratory features of disseminated histoplasmosis during two large urban outbreaks. Medicine. 62:263-270.

Scalarone, G.M., A. Restrepo, L.R. Schacht, G.H. Rupp, and M.D. LeRoy. 1985. Lack of antibody induction by Histolyn-CYL, a new skin-testing reagent for histoplasmosis. Sabouraudia. 23:335-338.

Schlech, Walter F., L. Joseph Wheat, John L. Ho, Morris L.V. French, Robert J. Weeks, Richard B. Kohler, C.E. Deane, H.E. Eitzen and J.D. Band. 1983. Recurrent urban histoplasmosis, Indianapolis, Indiana 1980-1981. Am. J. Epidemiol. 118:301-312.

Schwartz, Jared N. 1986. Specific fungal diagnosis. Arch. Pathol. Lab. Med. 110:369-370.

Sharma, Anjana, Bhavdish N. Johri, and Shriniwas. 1982. An enzyme immunoassay for yeast and mycelial phase-specific antibodies in histoplasmosis. J. Immunol. Methods. 50:115-121.

Sigma Chemical Company. 1986. Anti-human polyvalent immunoglobulins peroxidase conjugate (IgG fraction of antisera). St. Louis, Mo. (Product number A-8400 insert sheet).

Sigrest, Marion L., Floyd L. Lummus, Guy D. Campbell, John F. Busey, and Fred Allison Jr. 1963. Effect of diagnostic skin testing on antibody levels for histoplasmosis. N. Engl. J. Med. 269:390-394.

Sprouse, Ronald F., Charles W. Caldwell, and E. Dale Everett. 1981. Radioimmunoassays that use ^{125}I-labeled protein A for determination of Histoplasma capsulatum-specific immunoglobulin G, A, and M class antibodies in histoplasmosis. J. Clin. Microbiol. 13:66-72.

Stansfield, William D. 1981. Antigens and antibodies. Pages 30-66 in Serology and immunology: A clinical approach. Macmillan Publishing Co., Inc., New York.

Stockblower, Jean M., Kathleen M. Belgeri, Erika Bruck, Roger Calam, Harald Jacoby, Jean K. Shanahan, Onno W. van Assendelft and Bette Weber. 1984. Approved standard procedures for the collection of diagnostic blood specimens by venipuncture. 2nd ed. National Committee for Clinical Laboratory Standards. Villanova, PA.

Terry, Peter B., Edward C. Rosenow III, and Glenn D. Roberts. 1978. False-positive complement-fixation serology in histoplasmosis. J. Am. Med. Assoc. 239:2453-2456.

Tijssen, P. 1985. Practice and theory of enzyme immunoassays. In R.H. Burdon and P.H. van Knippenberg ed. Laboratory techniques in biochemistry and molecular biology, volume 15. Elsevier, Amsterdam.

Van Weeman, B.K. and A.H.W.M. Schuurs. 1971. Immunoassay using antigen-enzyme conjugates. FEBs Letter. 15:232-236.

Voller, A., D.E. Bidwell, and A. Bartlett. 1979. The enzyme linked immunosorbent assay (ELISA): a guide with abstracts of microplate applications. Flowline Press, Guernsey, Channel Islands.

Voller, Alister and Dennis Bidwell. 1986. Enzyme-linked immunosorbent assay. Pages 99-109 in Noel R. Rose, Herman Friedman, and John L. Fahey, eds. Manual of clinical laboratory immunology, 3d ed. American Society for Microbiology, Washington D.C.

Walter, Jinks E. 1969. The significance of antibodies in chronic histoplasmosis by immunoelectrophoretic and complement fixation tests. Am. Rev. Respir. Dis. 99:50-58.

Wells, J. Vivian. 1980. Immunoglobulins: Biosynthesis and metabolism. Pages 64-78 in H. Hugh Fudenberg, Daniel P. Stites, Joseph L. Caldwell, and J. Vivian Wells, eds. Basic and clinical immunology, 3d ed. Lange Medical Publications, Los Altos, Ca.

Wheat, Joseph, Morris L.V. French, Susan Kamel, and Ram P. Tewari. 1986. Evaluation of cross-reactions in Histoplasma capsulatum serological tests. J. Clin. Microbiol. 23:493-499.

Wheat, Lawrence J., Richard B. Kohler, and Ram Tewari. 1986. Diagnosis of disseminated histoplasmosis by detection of Histoplasma capsulatum antigen in serum and urine specimens. N. Engl. J. Med. 314:83-88.

Wheat, Joseph, Morris L.V. French, Richard B. Kohler, Sarah E. Zimmerman, Warren R. Smith, James A. Norton, Harold E. Eitzen, Coy D. Smith and Thomas G. Slama. 1982. The diagnostic laboratory tests for histoplasmosis; Analysis of experience in a large urban outbreak. Ann. Int. Med. 97:680-685.

Wheat, L.J., R.B. Kohler, M.L.V. French, M. Garten, M. Kleiman, S.E. Zimmerman, W. Schlech, J. Ho, A. White, and Z. Brahmi. 1983. Immunoglobulin M and G histoplasmal antibody response in histoplasmosis. Am. Rev. Respir. Dis. 128:65-70.

Zeidberg, L.D., Ann Dillion, and R.S. Gass. 1951. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tennessee. Am. J. Public. Health. 41:80-89.

APPENDIX 1

VOLUNTEER AGREEMENT AFFIDAVIT

For use of this form, see AR 40-38; the proponent agency is the Office of the Surgeon General

THIS FORM IS AFFECTED BY THE PRIVACY ACT OF 1974

1. AUTHORITY: 10 USC 3012, 44 USC 3101 and 10 USC 1071-1087.

2. PRINCIPAL PURPOSE: To document voluntary participation in the Clinical Investigation and Research Program. SSN and home address will be used for identification and locating purpose.

3. ROUTINE USES: The SSN and home address will be used for identification and locating purposes. Information derived from the study will be used to document the study; implementation of medical programs; teaching; adjudication of claims; and for the mandatory reporting of medical condition as required by law. Information may be furnished to Federal, State and local agencies.

4. MANDATORY OR VOLUNTARY DISCLOSURE: The furnishing of SSN and home address is mandatory and necessary to provide identification and to contact you if future information indicates that your health may be adversely affected. Failure to provide the information may preclude your voluntary participation in this investigational study.

PART A - VOLUNTEER AFFIDAVIT

VOLUNTEER SUBJECTS IN APPROVED DEPARTMENT OF THE ARMY RESEARCH STUDIES

Volunteers under the provisions of AR 70-25 are authorized all necessary medical care for injury or disease which is the proximate result of their participation in such studies.

I, _____ SSN _____ having
(last, first, middle)

full capacity to consent and having attained my _____ birthday, do hereby volunteer to participate in
Histoplasmosis Seroconversion Study
(research study)

under direction of CPT Mark J. Wolcott conducted at Blanchfield Army Community Hospital
(name of institution)

The implications of my voluntary participation; the nature, duration and purpose of the research study; the methods and means which it is to be conducted; and the inconveniences and hazards that may reasonably be expected have been explained to me by

I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights on study-related injury I may contact
Center Staff Judge Advocate Office

at Ft Campbell, KY
(name and address of hospital & phone number (include area code))

I understand that I may at any time during the course of this study revoke my consent and withdraw from the study without further penalty or loss of benefits however, I may be ☐ required (military volunteer) or ☐ requested (civilian volunteer) to undergo certain examination if, in the opinion of the attending physician, such examinations are necessary for my health and well-being. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled.

PART B - TO BE COMPLETED BY INVESTIGATOR

INSTRUCTIONS FOR ELEMENTS OF INFORMED CONSENT: (Provide a detailed explanation in accordance with Appendix E, AR 40-38 or AR 70-25.)

NATURE OF STUDY: Ft Campbell is an area with a high occurrence of exposure to histoplasmosis (a fungal disease that resembles many flu-like illnesses). Many people will develop antibodies to the fungus without having many symptoms. The reliability of a test for these antibodies in patients with unusual pneumonias depends on how many people develop them shortly after moving to the Ft Campbell area and how soon. This study will follow 100 newly arrived volunteers initially, at one month, and at three months for any change from negative to positive in blood antibodies to this fungus.

RISKS, INCONVENIENCES AND DISCOMFORTS: The only risk involved in the study is from having approximately one-half ounce of blood drawn from a vein on three different occasions. A temporary and local discoloration or bruise is the only common risk of this procedure in basically healthy individuals having no history of bleeding disorders. The major inconvenience is coming in for the test on three separate occasions.

(CONTINUE ON REVERSE)

PART B - TO BE COMPLETED BY INVESTIGATOR

BENEFIT: You may not benefit directly from this study, but the study may provide information useful in treating newly arrived personnel with flu-like illnesses.

NUMBER OF SUBJECTS INVOLVED IN STUDY: 100.

DURATION OF STUDY: 4 months.

DURATION OF SUBJECTS' PARTICIPATION: 3 months.

IF THERE IS ANY PORTION OF THIS EXPLANATION THAT YOU DO NOT UNDERSTAND, ASK THE INVESTIGATOR BEFORE SIGNING.

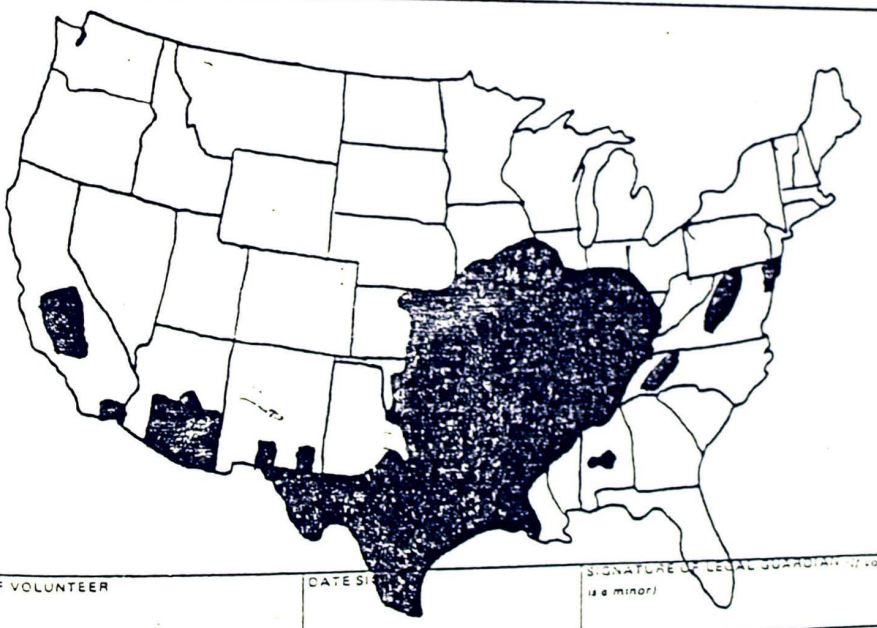
SUPPLEMENTAL INFORMATION

1. What is your age_____ sex _____ primary MOS _____

2. If you have your next unit assignment here at Ft Campbell please list it:

3. Have you ever lived on or near a farm that raised chickens, turkeys or other types of birds? _____

4. Referring to the map of the US below, have you ever stayed in an area shaded in black for more than 1 months?_____
   6 months?_____
   1 year? _____
   5 years? _____



SIGNATURE OF VOLUNTEER	DATE SIGNED	SIGNATURE OF LEGAL GUARDIAN (if volunteer is a minor)
PERMANENT ADDRESS OF VOLUNTEER	TYPED OR PRINTED NAME AND SIGNATURE OF WITNESS	DATE SIGNED

DA FORM 5303-R, Apr 84