Part II - Parasite Remains Preserved in Various Materials and Techniques in Microscopy and Molecular Diagnosis

17. Paleoserology

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Paleoserology

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The application of serological techniques to paleobiological material from ancestral populations is highly relevant for the identification of antigenic molecules from parasites, allowing the diagnosis of extant infections in these populations. The main molecular targets are the proteins present in ancient material, of both animal origin and human origin.

Working with samples in a good state of preservation, containing proteins or peptides with preserved epitopes, it is possible to identify these molecules with serological techniques using polyclonal or monoclonal antisera against specific target antigens.

Before the emergence of modern serological techniques, pioneering studies were developed by Boyd & Boyd (1933, 1934a, 1934b), aimed at determining the ABO blood group in preserved muscle tissue. The same authors (Boyd & Boyd, 1937), applying the precipitin test to tissues from 94 Egyptian mummies, found only four positive samples and one indeterminate sample in the ABO system. Using the same approach in 159 samples from ancient Americans, they only found two positive samples and seven indeterminate ones.

Candela (1936) observed inconsistent reactions in A and B group serotyping, working with ancestral human skeletons and using high-titer antisera that did not react with A or B antigens present in low concentrations. The conclusion in these cases was false-positive group O.

These results showed the need to improve the serological methods used at that time. Thus, serological techniques were developed that allowed more precise determination of the ABO system, the identification of which can now be done with greater precision in mummified tissues. Given that this identification depends on specific antigens present in the red blood cells and other tissues, and since optimized production became possible for antisera with specific antibodies using the Freund adjuvant in their elaboration, anti-ABO antisera with higher sensitivity were developed.

The use of these antisera in precipitation, agglutination inhibition, and cell mixture agglutination tests serves as a precious tool when applied to ancestral tissues, aimed at finding this system's antigen markers.

Rather than components of the ABO system, in some circumstances anti-ABO antibodies are found in the materials tested, and this procedure is thus called a reverse or indirect test. The agglutination test in a cell mixture, developed by Coombs, Bedford & Rouillard (1956), aimed at identifying the blood group in blood-deficient material, allowed Otten & Florey (1963) to determine the blood group in tissues from Chilean mummies.

Lippold (1971) later used the agglutination technique applied to cell mixture and demonstrated the perpetuation of the ABO system among current indigenous populations and prehistoric populations from the Aleutian Islands, Southwest United States, Peru, and Chile.

Stastny (1974), using agglutination inhibition for characterization of the HL-A system, found similarities between prehistoric Amerindian populations and modern indigenous populations. The author also observed that the profile of the HL-A system in mummies found on the Peruvian coast perfectly matched the general profile of Amerindian populations.

The principal modern immunodetection techniques feature immunoenzymatic, immunofluorescence, and immunochromatography (dipstick) assays, which have been used in paleoserology with high sensitivity and specificity in the diagnosis of parasitic and non- parasitic diseases.

Faulkner, Patton & Johnson (1989) used indirect immunofluorescence to examine eight desiccated human fecal samples from a cave in Van Buren County, Tennessee, USA, and identified *Giardia* cysts, among other enteroparasites. These findings were in material from prehistoric populations dated to approximately 2,177 BP. According to the authors, this was the first report of this finding in the New World.

Cattaneo et al. (1992) used ELISA (enzyme-linked immunosorbent assay) with monoclonal anti-albumin IgG and with sensitivity to detect 10 µg of protein, applied to bone extracts from periods corresponding to the English Civil War (1644 AD), Middle Age (1100-1400 AD), early Saxon period (450-600 AD), Roman period (100-200 AD), Iron Age (approximately 400 BC), and Bronze Age (2200-1700 BC). The method proved sensitive and reproducible and can be used for the identification of other molecules such as HLA and ABO and as an ancillary tool in the identification of racial origin and predisposition to diseases in ancestral populations.

Immunoprecipitation tests by Lewin (1995) suggest, according to the author, that the lesions found on the face of the mummy of Pharaoh Ramses V (1156 to 1151 BC) were due to smallpox.

Allison, Bergman & Gersten (1999), using direct immunofluorescence, identified *Cryptosporidium* sp. and *Giardia* sp. in feces from the intestine of mummies ranging from 500 to 3,000 BP. These findings were confirmed by immunoenzymatic tests in the same material. In a fecal sample of the same origin, the authors concurrently demonstrated the presence of *Helicobacter pylori* antigens in paleofeces dated circa 3,000 BP.

Cerutti et al. (1999), examining skin, muscle, and bone samples from Egyptian mummies dated to the pre-Dynastic period (3200 BC) from the Gebelen region, found a *Plasmodium falciparum* antigen, histidine rich protein-2 (Pf HRP-2). The analysis was done with immunoenzymatic assay, and the results suggest malaria prevalence on the order of 40% in mummies from the Gebelen group. The data are consistent with populations living in ecological conditions similar to malaria-endemic areas.

Gonçalves et al. (2002) examined feces from latrines situated in Medieval archaeological sites in France and diagnosed the presence of *Giardia lamblia* coproantigens using ELISA. Ortega & Bonavia (2003) used immunofluorescence to examine 22 human coprolites from excavations along the northern coast of Peru and found three positive samples for *Giardia* sp. cysts in material dated between 2375 and 1525 BC. Another coprolite, dated between 770 and 830 AD, contained *Cryptosporidium* sp. oocysts, and a third coprolite, dated between 500 and 900 BP, also contained *Giardia* sp. cysts.

Also using ELISA, Gonçalves et al. (2004), working with the same material used in the diagnosis of *Giardia* sp (Gonçalves et al., 2002), identified *Entamoeba histolytica* coproantigens. The target antigen was Gal-lectin from *Entamoeba histolytica*, an adhesin present in large amounts in the parasite membrane. In this case, a commercial *Entamoeba histolytica* anti-lectin monoclonal antibody kit was used (an antibody that specifically recognizes a single epitope of immunogenic proteins). This test is normally used in the parasite's specific routine laboratory diagnosis to distinguish between *Entamoeba histolytica* and *Entamoeba dispar*, morphologically identical species that constitute the so-called "*histolytica* complex".

Biannucci et al. (2007) used immunochromatography as a rapid diagnostic test for plague in ancient material, for the detection of *Yersinia pestis* F1 antigen.

Józsa (2008) made an interesting finding of glomerular lesions consistent with Berg's sub-acute focal glomerulonephritis in preserved tissues from Prince Joseph Palatinus of the Habsburg Dynasty, who died in 1847 at 71 years of age. Using immunohistochemistry, the author observed an IgA precipitate in both the glomerular mesangium and Bowman's capsule in the kidneys preserved in rosemary oil and stored with other organs in metal canisters. The precipitate was located by marking with avidin-biotin-peroxidase, conjugate, with diaminobenzidine as the chromophore.

BASIS FOR THE TECHNIQUES USED IN PALEOSEROLOGY

The most widely cited techniques for identifying antigens in paleobiological material have been ELISA and immunofluorescence. Their main advantage is the existence of diagnostic kits that facilitate laboratory procedures, with either polyclonal antibodies or monoclonal antibodies targeting a single antigen epitope, which in this case guarantees high sensitivity and specificity.

Each variant of ELISA has modifications described in the manufacturer's instructions for performing the respective technique.

As described above, the serological techniques used to identify parasite antigens in ancestral material prove sensitive and specific. The results thus allow a reliable diagnostic approach in materials of ancestral origin in order to make important paleoepidemiological inferences on currently observed parasitic diseases.

REFERENCES

- ALLISON, M. J.; BERGMAN, T. & GERSTEN, AND. Further studies on fecal parasites in antiquity. *American Journal of Clinical Pathology*, 112: 595-596, 1999.
- BIANUCCI, R. et al. A rapid diagnostic test for plague detects *Yersinia pestis* antigen in ancient remains. *Comptes Rendues de Biologie*, 330: 747-754, 2007.
- BOYD, W. C. & BOYD, L. G. Blood grouping by means of preserved muscle. *Science*, 78: 578-579, 1933.
- BOYD, W. C. & BOYD, L. G. Group specificity of dried muscle and saliva. *Journal of Immunology*, 26: 489-494, 1934a.
- BOYD, W. C. & BOYD, L. G. An attempt to determine the blood groups of mummies. *Proceedings of the Society of Experimental Biology and Medicine*, 31: 671-672, 1934b.
- BOYD, W. C. & BOYD, L. G. Blood grouping tests on 300 mummies. *Journal of Immunology*, 32: 307-319, 1937.

- CANDELA, P. B. Blood group reactions in ancient human skeletons. *American Journal of Physical Anthropology*, 21: 429-432, 1936.
- CATTANEO, C. et al. Reliable identification of human albumin in ancient bone using ELISA and monoclonal antibodies. *American Journal of Physical Anthropology*, 87: 365-372, 1992.
- CERUTTI, N. et al. Immunological investigation of malaria and new perspectives in paleopathological studies. *Bollettino della Società Italiana di Biologia Sperimentale*, 75: 17-20, 1999.
- COOMBS, R. R. A.; BEDFORD, D. & ROUILLARD, L. M. A and B blood group antigens on human epidermal cells demonstrated by mixed agglutination. *The Lancet*, 270: 416-463, 1956.
- FAULKNER, C. T.; PATTON, S. & JOHNSON, S. S. Prehistoric parasitism in Tennessee: evidence from the analysis of desiccated fecal material collected from Big Bone Cave, Van Buren County, Tennessee. *The Journal of Parasitology*, 75: 461-463, 1989.
- GONÇALVES, M. L. C. et al. Detection of *Giardia duodenalis* antigen in coprolites using a commercial available enzyme-linked immunosorbent assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 96: 640-643, 2002.
- GONÇALVES, M. L. C. et al. Amoebiasis distribution in the past: first steps using an immunoassay technique. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98: 88-91, 2004.
- JÓZSA, L. G. Histologic diagnoses of tissues from two nineteenth century Habsburgs. *Paleopathology Newsletter*, 141: 12-18, 2008.
- LEWIN, P. K. Ventures in paleopathology: 'mummy' riddles unraveled. *Germs Ideas*, 1: 10-14, 1995.
- LIPPOLD, L. K. The mixed cell agglutination method for typing mummified human tissue. *American Journal of Physical Anthropology*, 34: 377-383, 1971.
- ORTEGA, Y. R. & BONAVIA, D. *Cryptosporidium*, *Giardia*, and *Cyclospora* in ancient Peruvians. *The Journal of Parasitology*, 89: 635-636, 2003.
- OTTEN, C. M. & FLOREY, L. L. Blood typing of Chilean mummy tissue: a new approach. *American Journal of Physical Anthropology*, 21: 383-385, 1963.
- STASTNY, P. HL-A antigens in mummified pre-Columbian tissues. *Science*, 183: 864-846, 1974.