

Modelling Human Health by Levels of Biological Organization

Wilfred R. Cuff^a, Clifford Clark^a, Paulette Richard^b, Venkata R.S.K Duvvuri^c

^aNational Microbiology Laboratory,
Canadian Science Centre for Human and Animal Health,
Public Health Agency of Canada,
Winnipeg, Manitoba

^bPublic Health Agency of Canada
Ottawa, Ontario

^cMITACS Centre for Disease Modelling,
York Institute for Health Research,
Toronto, Ontario,

Wilfred.Cuff@phac-aspc.gc.ca, Clifford.Clark@phac-aspc.gc.ca,
Paulette.Richard@phac-aspc.gc.ca, vduvvuri@yorku.ca

ABSTRACT

The hierarchy of levels in the organization of matter, or the “hierarchy of life”, is a fundamental concept in biology, and biologists are used to thinking about their work as being at a particular level. Yet medicine, in both research and application, operates somewhat independently of this concept, working almost exclusively at levels at or below the individual. Through the relatively-recent expansion of epidemiology, medicine does reach up to the level of the population - but medicine at higher levels of biological organization remains under-developed. In this paper we review the biological idea of hierarchy of life and explore what medical research and practice might look like at the higher levels. The range of therapies might be broad, including such as controlling release of toxic chemicals and various types of planning. Practitioners may also be broad at the higher levels. In terms of research, we believe that ecology will be notably important. Hence the environment, and our concern here with environmental modelling, is important to medicine operating at all levels of the hierarchy of life. Key concepts from conference papers - socio-ecological and socio-environmental systems; modelling water supply; modelling forest fires and the northern environment; energy, material and water flows; air quality; and climate change - are obviously linked to human health. Where environmental models have significant health implications, the constituency of interest in the findings can be huge. The time seems right to make such contributions.

Keywords: hierarchy of life; linkage; human health; environmental models; opportunity

1. INTRODUCTION

“Medicine is the art and science of healing. It encompasses a range of health care practices evolved to maintain and restore health by the prevention and treatment of illness. Contemporary medicine applies health science, biomedical research, and medical technology to diagnose and treat injury and disease, typically through medication, surgery, or some other form of therapy” (<http://en.wikipedia.org/wiki/Medicine>). Contemporary medicine is usually thought to have evolved in Southern Europe from the work of famous "ancients" like Hippocrates (Greece) and Galen (Roman Empire), as early as the 5th and 4th centuries B.C. (<http://lib-sh.lsuhs.edu/fammed/grounds/history.html>). In fact, much earlier developments have been traced throughout the Middle East, presented in admirable detail in an Iranian book by Dr. Tadjbakhsh [2003].

Biology students are trained at an early stage to think in terms of levels: cells, tissues, *etc* at bottom end, and biomes, biosphere, *etc* at the top end. In fact, biologists often define themselves in terms of the level of biological organization in which they have expertise, directly (*e.g.*, cell biologist, population ecologist) or indirectly (*e.g.*, cardiologist, being a specialist in the cardiovascular Organ System). This hierarchy is commonly called the “hierarchy of life” (http://en.wikipedia.org/wiki/Biological_organisation).

Oddly, this basic concept of biology is curtailed in medical research or practice. Medicine is strongly focussed on the organism (*i.e.*, individual) level, with therapies usually applied to lower levels (*i.e.*, cells, tissues, organs, and organ systems). Levels above the organism are contributed to medical research and practice only by the discipline of epidemiology.

Early epidemiology, as described by Saracci [2001], consisted of “three streams ... medical, demographic and theoretical, coalesced in an effective way only towards the end of the eighteenth and beginning of the nineteenth centuries, giving rise to epidemiology as we recognize it today, an investigation of diseases and their aetiology at the population level”. The emergence of a “new epidemiology” occurred around the Second World War and it is notable that “no text specifically devoted to epidemiological methods was available before 1960”. It is clear that medicine at the population level has seriously entered medical research and practice only very recently.

Medical research and practice at higher levels of biological organization virtually does not exist - or, more precisely, ongoing work of relevance to medicine is not recognized as such. In this paper we review the biological idea of hierarchy of life and explore what medical research and practice might look like at the various levels. Our focus is on the application of modelling human health by levels of biological organization.

2. THE HIERARCHY OF LIFE

As poignantly stated in the Web Page quoted below: “Life is organized on many structural levels with each level building on previous levels. Each level of biologic organization has emergent properties.”

The levels are:

“Atoms - building blocks of all matter.

Molecules - two or more atoms chemically bonded ...

Macromolecules - many molecules chemically bonded into large complex molecules ...

Sub-cellular organelles - mitochondria, Golgi apparatus, chloroplasts, nucleus *etc.*

Cells - make up the structure and function of living organisms.

Tissues - groups of similar cells performing a particular function.

Organs - specific arrangement of tissues forming functional structures.

Organ systems - groups of organs performing a particular function.

Organism - any living thing.

Population - Localized group of organisms belonging to the same species.

Community - Populations of species living in the same area.

Ecosystems - An energy-processing system of community interactions that include abiotic environmental factors such as soil and water.

Biomes - Large scale communities classified by predominant vegetation type and distinctive combinations of plants and animals [e.g., Taiga, Prairies]

Biosphere - The sum of all the planet's ecosystems."

<http://blue.utb.edu/rlnash/Summer2004/NotesSummer2004/Structural Hierarchy.htm>

This is the hierarchy of life. Fig. 1 shows how Contemporary Medicine relates to this hierarchy, with emphasis roughly proportional to size of text and colour (red text is part of medicine, black receives little or no interest).

3. MEDICINE AT ALL LEVELS OF THE HIERARCHY

One might ask what medicine would look like if it were considered equally at all levels of the biological hierarchy, and to stimulate interest, the following two tables present some aspects for consideration.

3.1 Issues related to human health

Table 1 considers various types of "issues" that can be related to human health, and the kinds of therapy that might be appropriate. Issues of infectious and chronic diseases and suggested therapies are well known. The effects of toxic chemicals are understandable in terms of toxin exposure - although listed "therapies" involving the control of exposure and/or release would normally be considered environmental rather than medical concerns. Eutrophication would not conventionally raise health concerns even though it can interfere with the quality of drinking water. Habitat protection would also not raise health concerns although planning is usually intended to promote quality of life and hence human health.

3.2 Medical practice and research by level

At the level of the organism the Medical Doctor is the practitioner. At the population level the practitioner is also a Medical Doctor, but one with post-graduate specialization in Community/Population Health. This is the same model as used for specialized training at sub-organism levels, e.g., cardiologists, immunologists, dermatologists, etc. (We recognize that these statements are generalizations, there being many other specialities involved

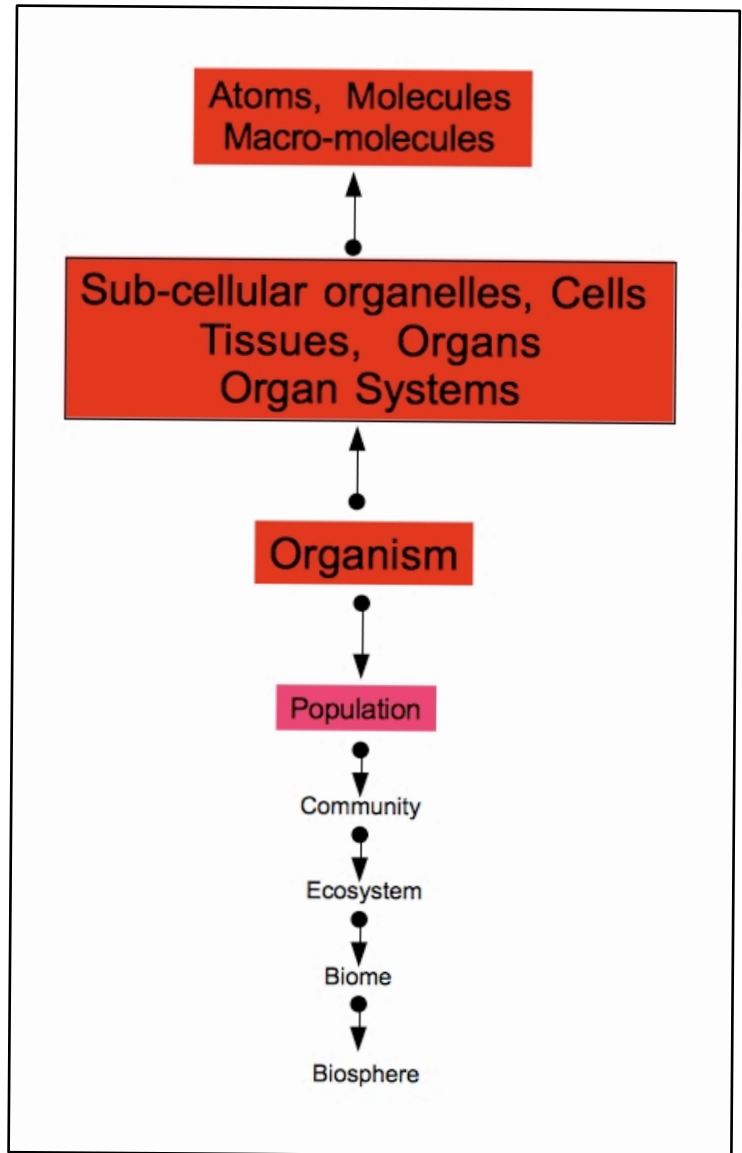


Figure 1. The hierarchy of life, with the size of text and colour intensity representing the degree of interest by Contemporary Medicine.

in medicine at the level of the organism and below, e.g., laboratory technicians, database administrators, hospital staff, and so on.)

This is what currently exists. Medical practice at the level of community, biome, ecosystem, or the biosphere are not yet defined. The role of medical doctors at the higher levels of the hierarchy is unclear. Much work done by various committees and planners working at these levels do have impacts on human health. It is interesting to speculate on whether

Table 1. Issues related to human health and plausible therapies at various levels of the biological hierarchy.

ISSUES	Levels			
	Below Organism	Organism	Population	Above Population
Infectious Diseases	Drugs	Behaviour Modification	Public Health Programs	Vector Control
Chronic Diseases	Drugs	Lifestyle	Healthy Lifestyle Promotion	Enhance Environmental Quality
Toxic Chemicals	Organ Transplant	Deaden Pain	Control Exposure	Control Release
Eutrophication		Bottled Water	Water Treatment	Land-use Planning
Habitat Protection	Treat Depression	Psychological Counseling	City Planning	Regional Planning

Medical Doctors will make their strong contributions at these levels through providing information rather than providing therapies.

Table 2 hypothesizes what might be the appropriate medical researchers at each level of the hierarchy. What is relevant to the audience addressed here is the wide variety of research disciplines that can contribute to the maintenance of human health. One might think that communities are most represented - what with virologists, bacteriologists, etc. - but to an animal or plant Community Ecologist, even these efforts are comparatively under-developed in the medical area. Work is substantially focussed on subjects related to zoonotic diseases where a “vector” (often an arthropod) transmits a pathogen (bacteria or virus) from a “reservoir” (e.g., infected animal) to a “host” (uninfected human).

3.3 Examples of medicine at the higher levels

As suggested in Table 2, ecology may be an important component of medicine at higher levels of the biological hierarchy. Human diseases caused by enteric bacteria provide a useful case study of how an “environmental approach” can be informative. Pathogens are transmitted among animal and human hosts. Disease incidence is impacted by well-defined human activities, from farm practices through food production and consumption. Bacterial genes encoding fitness are selected within a short time frame.

Escherichia coli belonging to the Shiga-toxigenic pathotype, or Shiga-toxigenic *E. coli* (STEC), can cause serious disease in humans, including ulceration and bleeding of the large intestine, damage to kidney tubules and glomeruli, and brain damage subsequent to clotting in the smaller arteries. STEC infects humans; domestic cattle, pigs, and sheep (Beutin et al. [1993]; Heuvelink et al. [1998]; Kudva et al. [1997]); and wildlife including deer (Asakura et al. [1998]), seagulls (Makino et al. [2000]), and soil-dwelling amoebae and nematodes. As a consequence, it can contaminate both soil and water - and hence any food materials that come into contact with them. Fruits (e.g., apples or apple juice; Anonymous [1997]; Steele et al. [1982]) and vegetables (e.g., lettuce; Abdul-Raouf et al. [1993]) are known sources of human infection.

There is a large and growing body of literature highlighting the relationship between STEC and the environment. For example, locations with high cattle density are associated with frequent or continuous contamination of surface water with a highly-virulent STEC serotype (Johnson et al. [2003]). Children on farms show evidence of frequent exposure to STEC from contact with cattle and sheep and their manure, and resulting in both high disease rates and increased immunity of the exposed children (Belongia et al. [2003]).

Interestingly, the interaction of STEC with human populations has not been pursued with equivalent vigor, with studies mainly following from outbreaks of disease. There is evidence, however, that human populations are a reservoir of STEC. For example, *E. coli* O157:H7 can be shed for more than 32 - 39 days in the absence of symptoms of disease (Karch et al. [1995]; Orr et al. [1994]). It has been detected in polluted river water in Japan (Kurokawa et al. [1999]), and there is evidence (Higgins et al. [2005]) that STEC may be a permanent resident of urban, polluted streams in the Boston metropolitan area. Most of the isolates recovered from wastewater treatment plants in France contained indicators of pathogenic potential for humans (Vernozy-Rozand et al. [2004]). STEC isolated from sewage in Spain, Germany, and The Netherlands suggested that a variety of strains are present in the human population (Blanch et al. [2003]; Garcia-Aljaro et al. [2005]; Holler et al. [1999]; Heijnen and Medema [2006]). STEC have been isolated from untreated human sewage, further supporting the hypothesis that they are endemic in the human population. Lastly, experiments have shown free bacteriophages are present in water, sewage, and other environmental niches, indicating the presence of STEC (Muniesa and Jofre [1998]), frequently in higher numbers than viable bacteria. The rates of carriage of STEC in human populations have not been quantified, nor have the specific STEC types responsible been identified.

More knowledge of the interactions of STEC and other components of ecosystems within which it is found are needed. Highly pathogenic STEC strains such as *E. coli* O157:H7 are known to be transmitted to humans through water, and hence could also be transmitted to cattle, pigs, and wildlife. There is evidence that the Stx-bacteriophages evolved in response to predation of *E. coli* by protozoa (Steinberg and Levin [2007]). Protozoa capable of ingesting *E. coli* O157:H7 have frequently been isolated from spinach and lettuce from retail grocery stores. Slugs (Sproston et al. [2006]) and free-living soil nematodes such as

Table 2. Medical research by level in the hierarchy of life.

Level	Researcher
Organism	Pharmacology, etc.
	Psychology, etc.
Population	Population Ecology
	Sociology
Community	Sociology
	Resource Management
Ecosystem	Geography / Geomatics
	Ecology
Biome	Geomatics
	Ecology
Biosphere	Political Science
	Ecology

Diploscapter sp. (Gibbs et al. [2005]) are capable of ingesting, growing on, and shedding *E. coli* O157:H7 bacteria. These bacteria remain viable for extended periods within *Caenorhabditis elegans*, which in turn appears to protect the bacteria from adverse environmental conditions (Kenney et al. [2005]).

There has been little or no research to quantify the relative role of each source in the cycling of these organisms through the ecosystems and biomes, nor to determine the relative importance of each source on human disease and health. Research, including modelling, is needed to clarify how ecological changes affect the presence and density of these pathogens in the environment. Furthermore, it is not clear whether the most important infectious agent is the bacterium or the bacteriophage which can, theoretically at least, create new pathogens by infecting existing *E. coli* strains with the appropriate genetic background. It is also unclear whether there are human-specific seropathotypes of STEC that are never found in non-human sources, nor is it clear how each animal host, ecological niche, or mode of transmission selects for or against human pathogenic strains. The field may be at the point, however, where enough data have been collected to allow mathematical modelling to provide interesting possibilities to guide experimental and observational follow-ups on some of these questions.

Much of the current linkage of the environment to human health seems to occur through the concept of “epigenesis”. The overarching concept underlying epigenetics is that environmental toxins or conditions can have a heritable effect on the health and biology of organisms without effecting changes in the genome, in DNA sequences (Drake and Liu [in press]; Hanson and Gluckman [2008]). There are critical windows of development in which epigenetic re-modelling can have dramatic consequences for individuals; *e.g.*, during exposure to environmental intoxicants *in utero* (Szyf et al. [2008]). For humans and most mammals, at least, the environment includes the social environment as well as the natural one (Neigh et al. [2009]; Szyf [2009]).

Epigenetics encompasses a wide range of research. At one end, there is research into the basic mechanisms, including the specific genes that are the targets of epigenetic modification as the result of environmental conditions. Further along, there are the studies of the mechanisms involved in the generation of diseases of complex etiology, such as cancer. These kinds of diseases are, of course, multifactorial in causation, with epigenetic modification of gene expression contributing to an unknown degree. One of the most startling examples is the effects of environmental estrogen disruptors and their contribution to birth abnormalities among both females and males, as well as striking and increasing disparities in birth ratios favouring females (Godmann et al. [2009]; Masse et al. [2009]; Rittler and Castilla [2002]). At the other end, there is research on how epigenetic mechanisms operate at the population level, for instance, on the epidemiology of exposures and association with diseases (Foley et al. [2009]). As with other areas of epidemiology, these investigations could almost certainly benefit from implementation of appropriate mathematical models.

Table 2 also suggests that the humanities (sociology, political science) may be an important component of medicine at higher levels of the biological hierarchy. One area which has attracted much research to date is that of “fear appeals” (Witte and Allen [2000]) aimed at modifying human behaviour. Many environmental concerns like climate change, deleterious changes in water and air quality, and so-on, have obvious implications for human health - and would be mediated by changes in the behaviour of human populations. Yet, these are very controversial and difficult issues, as illustrated by the case of global warming, raised interestingly in Schellnhuber [2008].

Public health policy is “clearly tied up with broader trends in the ... economy, public attitudes to the welfare state, business pressure to lower taxation levels in the face of globalization”, as described by Haddow [1999] for the situation in Canada. Realities like this seem to have brought authors like Mechanic [2003] to pose the question: “Who Shall Lead: Is There a Future for Population Health”. He writes that “there is much concern about disparities and meeting improved population health objectives, but interest waxes and wanes with scientific developments and especially with dominant political alignments and ideologies ... Population health faces many issues in seeking to become legitimized as both a unique field of study and as a significant force in public policy”. As mathematical models

already play a significant role in socio-economic life, so they may increasingly contribute to environment-oriented questions with human health implications.

4. MODELLING FOR THE ENVIRONMENT'S SAKE?

Modelling is an active partner in scientific endeavors. While much modelling occurs “for the environment’s sake”, it is of value to keep in mind that the environmental constituency is relatively small compare to that concerned with human health. The objective of this study is to argue that if one is guided by the hierarchy of life, then a number of human activities which are currently considered externalities do in fact have direct impacts on human health. This is notably so for levels above the population level.

To put these higher levels in the context of current medical research and practice, it may be useful to reflect upon what is considered in the medical community as the broad definition of health ... “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” (Constitution of WHO, 1948). Note that even in this broad definition the focus remains on the individual. By placing “social” alongside “physical” and “mental”, the definition seems to relegate all levels above the individual to externalities. In this definition, there is no explicit reference to the health of the society, even though it is well recognized in Public Health that encouraging healthy attitudes in society is beneficial to the individual. In this definition, other species like bacteria and viruses are not envisaged to be in dynamic relationship with humans - but simply as externalities affecting normal human behaviour. Our assertion is that looking at medicine in relation to the hierarchy of life can be lead to a better understanding of the factors affecting human health.

This assertion has major implications for the organization of Contemporary Medicine, albeit it is only one of a number of emerging challenges. Evolutionary Medicine is a relatively new concept, first discusses in a book edited by Trevathan et al. [1999]. Recent papers by Swynghedauw [2004] and Swynghedauw [2009] discuss the implications of the view that “it’s clear, and nearly self-evident, that” the major causes of mortality - heart failure and cancer - “result from conflicts between our modern lifestyle and our genetic endowment”. The range of therapies has also expanded dramatically in recent decades (*e.g.*, Anonymous [1996]), following up the dramatic increase in the use of complementary and alternative medicine (CAM) in the developed world (Rao [2006]). Most significantly, an interdisciplinary field that studies the relationships between human and animal health, and with environmental conditions, is emerging. An early book, under the name of Conservation Medicine was published by Aguirre et al. [2002]: “The environmental causes of health problems are complex, global, and poorly understood - practitioners in this area form multidisciplinary teams to tackle these issues, and can include physicians, veterinarians, researchers and clinicians in many disciplines including microbiologists, pathologists, landscape analysts, marine biologists, toxicologists, epidemiologists, climate biologists, anthropologists, economists, and political scientists. Clinical areas include HIV, Lyme disease, Severe Acute Respiratory Syndrome (SARS), avian influenza, West Nile virus, Nipah virus, and other emerging infectious diseases.”

“By looking at the environment and health as a continuum, conservation medicine has the potential to rapidly change public perspectives on many high profile societal issues, making the distant and ill-defined, local and pressing. For instance, global warming may have vaguely defined long-term impacts, but when an immediate effect is a relatively slight rise in air temperature, which in turn raises the flight ceiling for temperature-sensitive mosquitoes, allowing them to infect higher flying birds, and so forth, the issue becomes more real. Likewise, the broad topic of suburban sprawl is made more relevant when seen in terms of the immediate imbalance it brings to rural ecosystems, which leads to population increases of, and forces humans into closer contact with, certain animal populations (like rodents), introducing the risk of hosts of new cross-species diseases. Seemingly common sense scenarios like these lie at the heart of conservation medicine. When tied to actual cases (like SARS or HIV/AIDS), this holistic outlook seems likely to resonate more powerfully with the public than the more abstract explanations of environmental and health issues that we are currently used to.” (http://www.wordiq.com/definition/Conservation_medicine)

Conservation medicine is now more frequently referred to under the aegis of “One Health” or “One World, One Health”. In fact, the Public Health Agency of Canada recently hosted an expert consultation on the subject in Winnipeg, Manitoba (captured in a document “Report of the Expert Consultation OneWorld One Health: From Ideas To Action; <http://www.phac-aspc.gc.ca/publicat/2009/er-rc/index-eng.php>).

It is clear that Contemporary Medicine is undergoing rapid change. New opportunities are being created. Many of the participants in this conference are dealing with models/ information as contributors to population biology, sociology, resource management, geography/geomancy, ecology, remote sensing, political science, and so on. Many of the sessions of this meeting can be interpreted in terms of human health. For example, the investigation of socio-ecological and socio-environmental systems are of obvious importance to human well-being. Progress on modelling water supply (alternatively: environmental fluid mechanics, water quality, urban water, regional water resource systems, integrated wastewater systems) directly affect human health at the organism and population levels, and indirectly at the community, biome, and biosphere levels. Work on modelling forest fires and the northern environment link directly to the biome level. Studies concerned with energy, material and water flows, or air quality and climate change are ecosystem-and-above level studies and linkage with human health modelling should be directed here. Concern with issues like stakeholders, sustainability, and environmental monitoring is central to progress in One World, One Health; and tools and techniques (Decision Support Systems, Environmental Information Systems, Integrated Modelling, Data Mining, and concern with the role of uncertainty and model diagnostics) provide the refinement of the field of environmental modelling.

5. CONCLUSIONS AND RECOMMENDATIONS

Human health includes the dimensions of individual health as well as community health, and cannot be separated from the health of social groups and the physical environment. Any disease presentation or health outcome is a result of, or depends on, the complex interaction of the individual (including internal systems such as the endocrine system and those studied under the discipline of immunology) with many environmental and social factors (external to the individual). The potential interactions, both subtle and profound, that affect homeostasis and disease are extremely complex. Modelling will facilitate a deep understanding of the issues and provide directions for new research to fill in existing data gaps. Many environmental models will have significant health implications for humans and where they do, the constituency of interest in the findings can be huge. The time seems right to make such contributions to human health.

6. REFERENCES

- Abdul-Raouf, U.M., L.R. Beuchat, and M.S. Ammar, *Survival and growth of Escherichia coli O157:H7 on salad vegetables*. Applied and Environmental Microbiology 59(7),1999-2006, 1993.
- Aguirre, A.A., R.S. Ostfeld, G.M. Tabor, C. House, and M.C. Pearl, eds. *Conservation medicine: ecological health in practice*. Oxford University Press, Oxford, 2002.
- Anonymous, *Complementary medicine is booming worldwide*. British Medical Journal 313(7050),131-133, 1996.
- Anonymous, *From the Centers for Disease Control and Prevention. Outbreaks of Escherichia coli O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider--Connecticut and New York, October 1996*. JAMA : the journal of the American Medical Association 277(10),781-782, 1997.
- Asakura, H., S. Makino, T. Shirahata, T. Tsukamoto, H. Kurazono, T. Ikeda, and K. Takeshi, *Detection and genetical characterization of Shiga toxin-producing Escherichia coli from wild deer*. Microbiology and immunology 42(12),815-822, 1998.

- Astin, J.A., *Use of alternative medicine by women with breast cancer*. N Engl J Med 341(15),1156; author reply 1156-1157, 1999.
- Belongia, E.A., P.H. Chyou, R.T. Greenlee, G. Perez-Perez, W.F. Bibb, and E.O. DeVries, *Diarrhea incidence and farm-related risk factors for Escherichia coli O157:H7 and Campylobacter jejuni antibodies among rural children*. J Infect Dis 187(9),1460-1468, 2003.
- Beutin, L., D. Geier, H. Steinruck, S. Zimmermann, and F. Scheutz, *Prevalence and some properties of verotoxin (Shiga-like toxin)-producing Escherichia coli in seven different species of healthy domestic animals*. Journal of clinical microbiology 31(9),2483-2488, 1993.
- Blanch, A.R., C. Garcia-Aljaro, M. Muniesa, and J. Jofre, *Detection, enumeration and isolation of strains carrying the stx2 gene from urban sewage*. Water Sci Technol 47(3),109-116, 2003.
- Drake, A.J., and L. Liu, *Intergenerational transmission of programmed effects: public health consequences*. Cell, in press.
- Foley, D.L., J.M. Craig, R. Morley, C.A. Olsson, T. Dwyer, K. Smith, and R. Saffery, *Prospects for epigenetic epidemiology*. Am J Epidemiol 169(4),389-400, 2009.
- Garcia-Aljaro, C., M. Muniesa, J.E. Blanco, M. Blanco, J. Blanco, J. Jofre, and A.R. Blanch, *Characterization of Shiga toxin-producing Escherichia coli isolated from aquatic environments*. FEMS microbiology letters 246(1),55-65, 2005.
- Gibbs, D.S., G.L. Anderson, L.R. Beuchat, L.K. Carta, and P.L. Williams, *Potential role of Diploscapter sp. strain LKC25, a bacterivorous nematode from soil, as a vector of food-borne pathogenic bacteria to preharvest fruits and vegetables*. Appl Environ Microbiol 71(5),2433-2437, 2005.
- Godmann, M., R. Lambrot, and S. Kimmins, *The dynamic epigenetic program in male germ cells: Its role in spermatogenesis, testis cancer, and its response to the environment*. Microsc Res Tech 72(8),603-619, 2009.
- Haddow, R., *Interest representation and the Canadian state*. In: Canadian Politics, 3rd ed. J. Bickerton and A. Gergon, eds. Broadview Press, Peterborough. pp 501-522, 1999.
- Hanson, M.A., and P.D. Gluckman, *Developmental origins of health and disease: new insights*. Basic Clin Pharmacol Toxicol 102(2),90-93, 2008.
- Heijnen, L., and G. Medema, *Quantitative detection of E. coli, E. coli O157 and other shiga toxin producing E. coli in water samples using a culture method combined with real-time PCR*. J Water Health 4(4),487-498, 2006.
- Heuvelink, A.E., F.L. van den Biggelaar, E. de Boer, R.G. Herbes, W.J. Melchers, J.H. Huis in 't Veld, and L.A. Monnens, *Isolation and characterization of verocytotoxin-producing Escherichia coli O157 strains from Dutch cattle and sheep*. Journal of clinical microbiology 36(4),878-882, 1998.
- Higgins, J.A., K.T. Belt, J.S. Karns, J. Russell-Anelli, and D.R. Shelton, *tir- and stx-positive Escherichia coli in stream waters in a metropolitan area*. Appl Environ Microbiol 71(5),2511-2519, 2005.
- Holler, C., S. Koschinsky, and D. Witthuhn, *Isolation of enterohaemorrhagic Escherichia coli from municipal sewage*. Lancet 353(9169),2039, 1999.
- Johnson, J.Y., J.E. Thomas, T.A. Graham, I. Townshend, J. Byrne, L.B. Selinger, and V.P. Gannon, *Prevalence of Escherichia coli O157:H7 and Salmonella spp. in surface*

- waters of southern Alberta and its relation to manure sources. *Can J Microbiol* 49(5),326-335, 2003.
- Karch, H., H. Russmann, H. Schmidt, A. Schwarzkopf, and J. Heesemann, *Long-term shedding and clonal turnover of enterohemorrhagic Escherichia coli O157 in diarrheal diseases*. *J Clin Microbiol* 33(6),1602-1605, 1995.
- Kenney, S.J., G.L. Anderson, P.L. Williams, P.D. Millner, and L.R. Beuchat, *Persistence of Escherichia coli O157:H7, Salmonella Newport, and Salmonella Poona in the gut of a free-living nematode, Caenorhabditis elegans, and transmission to progeny and uninfected nematodes*. *Int J Food Microbiol* 101(2),227-236, 2005.
- Kudva, I.T., P.G. Hatfield, and C.J. Hovde, *Characterization of Escherichia coli O157:H7 and other Shiga toxin-producing E. coli serotypes isolated from sheep*. *J Clin Microbiol* 35(4),892-899, 1997.
- Kurokawa, K., K. Tani, M. Ogawa, and M. Nasu, *Abundance and distribution of bacteria carrying stxII gene in natural river water*. *Lett Appl Microbiol* 28(5),405-410, 1999.
- Makino, S., H. Kobori, H. Asakura, M. Watarai, T. Shirahata, T. Ikeda, K. Takeshi, and T. Tsukamoto, *Detection and characterization of Shiga toxin-producing Escherichia coli from seagulls*. *Epidemiol Infect* 125(1),55-61, 2000.
- Masse, J., T. Watrin, A. Laurent, S. Deschamps, D. Guerrier, and I. Pellerin, *The developing female genital tract: from genetics to epigenetics*. *Int J Dev Biol* 53(2-3),411-424, 2009.
- Mechanic, D., *Who shall lead: is there a future for population health?* *J Health Polit Policy Law* 28(2-3),421-442, 2003.
- Muniesa, M., and J. Jofre, *Abundance in sewage of bacteriophages that infect Escherichia coli O157:H7 and that carry the Shiga toxin 2 gene*. *Appl Environ Microbiol* 64(7),2443-2448, 1998.
- Neigh, G.N., C.F. Gillespie, and C.B. Nemeroff, *The neurobiological toll of child abuse and neglect*. *Trauma Violence Abuse* 10(4),389-410, 2009.
- Orr, P., D. Milley, D. Colby, and M. Fost, *Prolonged fecal excretion of verotoxin-producing Escherichia coli following diarrheal illness*. *Clin Infect Dis* 19(4),796-797, 1994.
- Rao, D., *Choice of medicine and hierarchy of resort to different health alternatives among Asian Indian migrants in a metropolitan city in the USA*. *Ethn Health* 11(2),153-167, 2006.
- Rittler, M., and E.E. Castilla, *Endocrine disruptors and congenital anomalies*. *Cad Saude Publica* 18(2),421-428, 2002.
- Saracci, R., *Introducing the history of epidemiology*. In: *Teaching Epidemiology*, 2nd ed. S. R. Olsen J, Trichopoulos D, ed. Oxford University Press, Oxford. pp 1-19, 2001.
- Schellnhuber, H.J., *Global warming: stop worrying, start panicking?* *Proc Natl Acad Sci U S A* 105(38),14239-14240, 2008.
- Sproston, E.L., M. Macrae, I.D. Ogden, M.J. Wilson, and N.J. Strachan, *Slugs: potential novel vectors of Escherichia coli O157*. *Appl Environ Microbiol* 72(1),144-149, 2006.

- Steele, B.T., N. Murphy, G.S. Arbus, and C.P. Rance, *An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice*. J Pediatr 101(6),963-965, 1982.
- Steinberg, K.M., and B.R. Levin, *Grazing protozoa and the evolution of the Escherichia coli O157:H7 Shiga toxin-encoding prophage*. Proc Biol Sci 274(1621),1921-1929, 2007.
- Swynghedauw, B., *Evolutionary Medicine*. Acta chirurgica Belgica 104(132-139), 2004.
- Swynghedauw, B., *Evolutionary medicine an introduction. Evolutionary biology, a missing element in medical teaching | [Une introduction à la médecine évolutionniste. L'évolution biologique, grande absente de l'enseignement médical]* Bulletin de l'Academie Nationale de Medecine 193(5),1147-1164, 2009.
- Szyf, M., *The early life environment and the epigenome*. Biochim Biophys Acta 1790(9),878-885, 2009.
- Szyf, M., P. McGowan, and M.J. Meaney, *The social environment and the epigenome*. Environ Mol Mutagen 49(1),46-60, 2008.
- Tadjbakhsh, H., *History of Medicine and Veterinary Medicine in Iran*. Fondation Merieux, 508 pp., Lyon, 2003.
- Trevathan, W.R., E.O. Smith, and J.J. McKenna, eds. *Evolutionary Medicine*. Oxford University Press, New York, 1999.
- Vernozy-Rozand, C., M.P. Montet, Y. Bertin, F. Trably, J.P. Girardeau, C. Martin, V. Livrelli, and L. Beutin, *Serotyping, stx2 subtyping, and characterization of the locus of enterocyte effacement island of shiga toxin-producing Escherichia coli and E. coli O157:H7 strains isolated from the environment in France*. Appl Environ Microbiol 70(4),2556-2559, 2004.
- Witte, K., and M. Allen, *A meta-analysis of fear appeals: implications for effective public health campaigns*. Health Educ Behav 27(5),591-615, 2000.