OXOID™

Volume 10 No 2 September 1989

culture

Published as a service to microbiology
by Oxoid Limited Oxoid is a registered trade mark.

Modern media and methods in food mycology

Dr John I. Pitt and Dr Ailsa D. Hocking, CSIRO Division of Food Processing, North Ryde, NSW, 2113 Australia.

Methods used in food mycology were largely developed from those used in food bacteriology. Enumeration by the dilution plate count, incubation at high temperatures, and reliance on a single general purpose medium, to provide a general 'yeast and mould' count, are all legacies of the influence of food bacteriology. However, food borne filamentous fungi have growth patterns quite different from those of bacteria, and grow under a much wider range of environmental conditions. They produce quite different kinds of toxins, and must be identified by quite different techniques. The challenge facing those studying food borne fungi has been to draw on the expertise of the bacteriologist, mycologist, plant pathologist and microbial physiologist to develop enumeration methods, media, isolation techniques and identification schemes specific for the fungi in foods. In the past decade, this challenge has been met with increasing effectiveness. This paper describes some of the ideas and techniques, the methods and the media, which are being developed specifically in the study of fungi in foods.

Improved enumeration techniques

Unlike most bacteria, fungi grow as filaments. This has three consequences of importance in the development of enumeration techniques: first, growth and the formation of particles (i.e. spores) which can readily be counted are two separate events, and not always directly related; second, fungal colonies spread and, if unchecked, rapidly become very difficult to enumerate; and third, fungi can penetrate solid foods. Techniques which enumerate fungi only from the surfaces of solid foods may be ineffective and misleading. The basic reproductive structures in fungi, the spores, are almost inert biologically, so enumeration of spores, especially from the surfaces of solid or particulate foods, may give little or no information about growth or toxin production within the food.

Developments in the enumeration of food borne fungi have come from many sources. The resulting lack of standardisation makes comparison of results from different laboratories very difficult, because of the plethora of techniques and methods used.

At a specialist workshop held in Boston in 1984[1] it was agreed that for powdered or liquid foods, certain of the techniques employed by bacteriologists were perfectly adequate. These included dilution techniques with appropriate diluents, spread plating, which is superior to pour plates for filamentous fungi, plates incubated upright, and incubation almost always at 25°C. However, for particulate or solid foods, for example nuts or grains, the workshop also recommended the technique known as direct plating[1,2]. This is a quite radical departure from bacteriological techniques.

In direct plating, samples of particulate foods are surface disinfected in a chlorine solution, then placed on the surface of appropriate solidified media and incubated for a suitable time. Plates are then examined visually, usually with a stereomicroscope, and the types of fungal growth can be counted and recognised. Selected areas of fungal growth may then be picked off for examination with the compound microscope or inoculated onto suitable isolation or identification media (**Figure 1**).

For particulate foods such as nuts or grains, direct plating provides an estimate of the extent of infection of a commodity, and is usually expressed as a percentage[3]. Surface sterilisation is an essential step in this process[4]. It is an effective method for monitoring invasion of commodities by toxigenic fungi. For solid foods, where sampling involves cutting pieces of food for plating, direct plating is essentially a qualitative technique. Despite this, it is the only satisfactory isolation method for certain fastidious xerophiles discussed below.

Modern media

General purpose media

Fungal enumeration and isolation media should not only restrict fungal spreading but must prevent bacterial growth also. Decades ago, soil mycologists found that rose-bengal and ox gall reduced both spreading growth and bacteria. However, food microbiologists enumerating or isolating fungi still relied until much later on low pH media, acidified potato dextrose agar being the most popular.[5] Mossel *et al*[6] showed that neutral pH media, with oxytetracycline added to control bacteria, were more effective for fungal enumeration than formulations which relied on acidity for bacterial suppression, because low pH often adversely affected moribund cells or spores.

The problem of spreading colonies remained. Jarvis[7] devised rose-bengal chlortetracycline agar (RBC) which proved to be an effective general purpose enumeration medium. King *et al*[8] added dichloran[a] to produce dichloran-rose-bengal-chlortetracycline agar (DRBC), and greatly improved the suppression of spreading fungi such as *Rhizopus* and *Mucor* species. Put and Conway[11] reported that chloramphenicol was a superior antibiotic to chlortetracycline: both RBC and DRBC are now customarily formulated with chloramphenicol[3]. Both media have found wide acceptance among food mycologists[1], and both are in commercial production.

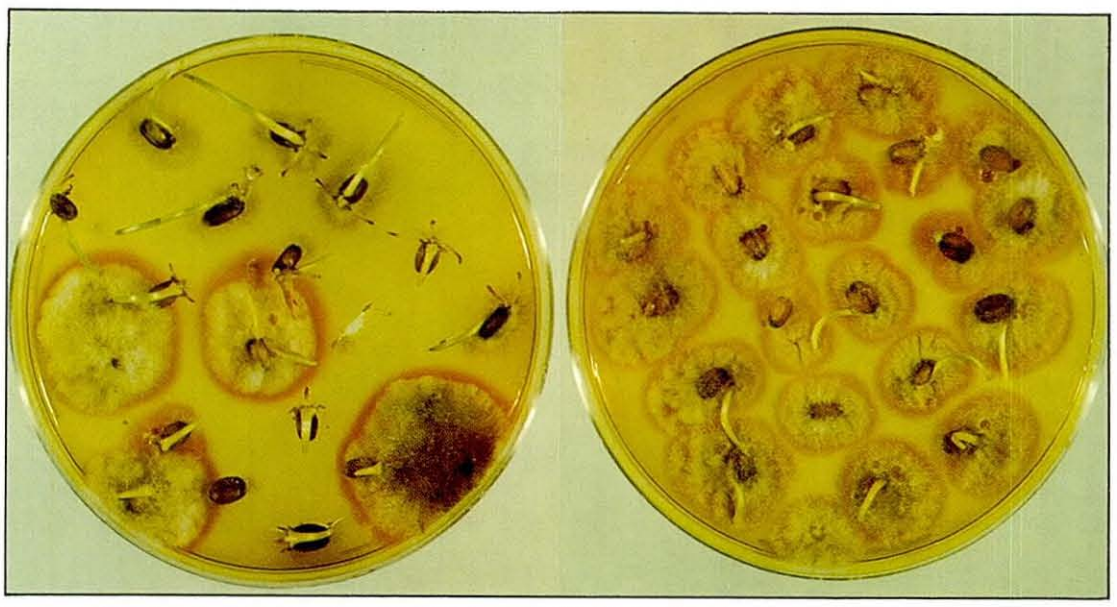


Figure 1: Wheat, surface sterilised and direct-plated onto *Aspergillus flavus* and *parasiticus* agar (AFPA) for detection of the mycotoxigenic fungi *Aspergillus flavus* and *A.parasiticus*. The sample on the left hand Petri dish has a low level of infection with *A.parasiticus*, and the sample on the right hand plate has a 100% infection rate. *A.flavus* and *A.parasiticus* form colonies with a bright orange reverse pigment on AFPA.

Figure 2: A food sample dilution-plated onto DRBC agar, showing restriction of fungal colony size. The colony at the top centre is *Rhizopus stolonifer*. The food sample also contains *Aspergillus flavus*, a *Penicillium* species, and *A.niger*.

Media for xerophilic fungi

Xerophilic fungi are defined as being able to grow under conditions of greatly reduced water activity. Such fungi are of great importance in the spoilage of dried and concentrated foods and bulk commodities. Many marginally xerophilic fungi, such as *Aspergillus* and *Penicillium* species. have optimal a_w for growth very close to 1.0, and can be effectively enumerated on general purpose media. However, other quite commonly occurring xerophilic species, including *A. penicilloides, Wallemia sebi* and many *Eurotium* species, have lower growth optima and hence germinate and grow poorly if at all on commonly used high a_w media. For enumerating these fungi, dichloran-18 per cent glycerol agar (DG18)[12] was devised. Counts of fungi on dried foods such as pepper, chillies or dried fish can be up to 5 log cycles higher on DG18 than on DRBC.[13]

Enumerating extreme xerophiles such as *Xeromyces bisporus* or the xerophilic *Chrysosporium* species is a special challenge. *Xeromyces* will not grow above about 0.95 a_w, so will be missed if any normal isolation or enumeration medium is used, even if visual inspection of mouldy food indicates vigorous growth. *C. fastidium* and *C. inops* are little better. Also, experience has shown that these species are very difficult to isolate by dilution plating, even when low a_w diluents are used. The recommended technique for enumeration or isolation of these fungi is direct plating, either of particulate foods, or by cutting pieces from solid foods such as dried fruit or licorice. When xeromyces or the chrysosporia are present in pure culture, and they often are, direct plating onto malt yeast 50 per cent glucose agar (MY50G)[3] is effective. If other extreme xerophiles such as *Eurotium* species are also present, recourse must be made to malt yeast 70 per cent glucose fructose agar (MY70GF).[3] On this medium, of 0.76 a_w, xeromyces will outgrow any other fungus.

Isolating and enumerating halophilic xerophiles from salty foods, particularly salted, dried seafoods, again requires special media. For

(a.) 2,6-dichloro-4-nitroaniline: also spelled 'dicloran'[9], but the authoritative CRC Handbook of Chemistry and Physics[10] uses 'dichloran'.

Polypaecilum pisce, malt-yeast-5 per cent sodium chloride-12 per cent glucose (MY5-12; 0.96 a_w)[3] is recommended, while for the more xerophilic *Basipetospora halophila*, the medium of choice is malt-yeast-10 per cent sodium chloride-12 per cent glucose (MY10-12; 0.93 a_w).[3]

Media for toxigenic fungi

The first notable attempt to formulate a selective medium for toxigenic food borne fungi was by Bothast and Fennell[14]. They produced a medium on which *Aspergillus flavus* and *A. parasiticus*, the aflatoxin producers, were readily distinguished from other fungi by an orange colour reaction. However, the medium lacked selectivity. The addition of antibiotics and dichloran, refinements to the formula and altered incubation conditions, resulted in Aspergillus flavus and parasiticus agar (AFPA)[15]. AFPA permits enumeration of these two species in 48 hours or less and is very easy to use. The medium has found wide application and is available commercially.

A medium which allows differentiation of *Penicillium viridicatum* and *P. verrucosum*, the major ochratoxin producer, from other fungi is pentachloronitrobenzene-rose-bengal-yeast extract agar (PRYS)[16] Dichloran-chloramphenicol-peptone agar (DCPA)[17] was developed for the isolation of *Fusarium* species and dematiaceous hyphomycetes which occur in grain. Many more developments of this kind are needed.

Methods for yeast

Preservative resistant yeasts

Most yeasts important in foods can be enumerated and isolated by bacteriological techniques. However, one group of spoilage yeasts deserves mention here, the preservative resistant yeasts. Some of these cause spoilage by forming films on the surfaces of acetic acid preserves such as pickles, olives and sauces. A few species, however, are able to ferment preserved liquid foods such as fruit juices to sufficiently high carbon dioxide pressures as to cause explosive spoilage.[3] The major species involved is *Saccharomyces bailii* Lindner (synonym *Zygosaccharomyces bailii* (Lindner) Guilliermond). This yeast has the ability to adapt to high levels of preservatives in processing plants, shedding cells into the lines, then into containers at filling. Growth to levels of 10^6 or more may then occur, so that container distortion and sometimes explosion may result. *S. bailii* isolates exist which can grow in fruit based cordials of pH 2.8, 45° Brix, and preserved with 800mg/kg of sorbic or benzoic acids.[3] Only a very few cells per container are needed to cause eventual spoilage. Food losses around the world from *S. bailii* amount to millions of dollars per annum, particularly in tomato sauce, mayonnaise, fruit juices, concentrates and drinks, ciders and wines.

S. bailii and other preservative resistant yeasts can be detected on a selective medium, acetic-malt agar. This consists of standard malt extract agar to which 0.5 per cent acetic acid is added just before pouring.[3] Only preservative-resistant yeasts can grow on this medium, allowing them to be differentiated from the large variety of inocuous yeasts which may contaminate raw materials and processing lines.

Detection of low numbers of yeasts

Perhaps the simplest way to detect small numbers of yeasts in liquid products is by membrane filtration for clear liquids, and enrichment techniques for products which cannot be filtered. A simple enrichment medium is the product itself, diluted 1:1 with sterile water. This increases the a_w of juice concentrates, syrups and similar products to a level which will allow growth of potential spoilage yeasts without causing lethal osmotic shock to cells. In products containing preservatives, it dilutes the preservative concentration, again allowing faster growth of potential spoilage yeasts.[3]

Small numbers of xerophilic yeasts in solid and liquid foods can also be detected by a simple presence-absence test using direct homogenisation of the sample in yeast extract-50 per cent glucose broth with incubation at 30°C from 2-30 days.[18] The direct epifluorescence filter technique (DEFT) has also been used to detect yeasts in numbers as low as 1/g in creme fondant.[19]

New methods

Various indirect methods of measuring fungal growth in foods have been proposed or are being investigated, to replace the traditional enumeration methods. Older methods include chemical estimation of chitin and ergosterol. Newer methods, such as impediometry and estimation of fungal ATP, are also being evaluated but are not yet in general use.

Perhaps the most promising new techniques for detecting and quantifying mould growth in foods is the ELISA technique, using antigens specific for common food-borne fungi such as *Aspergillus, Penicillium* and *Cladosporium* species.[20] The technique appears to be very specific, and possibly more sensitive than colony counting methods.[21] However, ELISA techniques will need to be rigorously tested against the traditional methodologies before the results can be compared with our current concepts of acceptable and unacceptable levels of moulds in foods.

Conclusion

It has taken a relatively few years for food mycology to emerge from its origin in food bacteriology, with interaction from several other fields, as a discrete discipline. Much, however, remains to be done.

Better measures of fungal growth are urgently needed, both microbiological and chemical. Measures of growth, regardless of type, need to be linked mathematically to more fundamental properties, such as biomass production, and phenomena of more practical significance, such as sporulation, spoilage and toxigenesis.

Better selective media are urgently needed, especially for toxigenic fungi. Ideally a medium should select for one or more related species, and also develop some distinct colour or other reaction to allow differentiation from extraneous organisms, so that non-specialists can enumerate or isolate the desired species. Such methods need to be as rapid as possible, so they can be of value in quality control. Greater standardisation of existing methodology is also an urgent requirement.

References

1. King, A.D., Pitt, J.I., Beuchat, L.R., Corry, J.E.L., (eds) (1986). *Methods for the Mycological Examination of Food*. Plenum Press. New York.
2. Mislivec, P.B., and Bruce, V.R. (1977) *J. Assoc. Off. Anal. Chem.*, **60:** 741-743.
3. Pitt, J.I., and Hocking, A.D. (1985). *Fungi and Food Spoilage*. Academic Press, Sydney.
4. Andrews, S. (1986). In *Methods for the Mycological Examination of Food*. King, A.D., Pitt, J.I., Beuchat, L.R., Corry, J.E.L., (eds) Plenum Press, New York pp. 28-32.
5. Sharf, J.M. (ed.) (1966) *Recommended Methods for the Microbiological Examination of Foods*. 2nd edn. American Public Health Association, New York.
6. Mossel, D.A.A., Visser, M., and Mengerink, W.H.J. (1962). *Lab. Prac.*, **11:** 109-112.
7. Jarvis, B. (1973). *J. Appl. Bacteriol.*, **36:** 723-727.
8. King, A.D., Hocking, A.D., and Pitt, J.I. (1979). *Appl. Environ. Microbiol.*, **37:** 959-964.
9. Abildgren, M.P., *et al* (1987). *Lett. Appl. Microbiol.*, **5:** 83-86.
10. Weast, R.C., (ed.) (1986). *CRC Handbook of Chemistry and Physics*. 67th edn. CRC Press, Boca Raton, Florida.
11. Put, M.C., and Conway, C.C. (1974) *Arch. Lebensmittelhygiene*, **25:** 73-83.
12. Hocking, A.D., and Pitt, J.I. (1980). *Appl. Environ. Microbiol.*, **39:** 488-492.
13. Hocking, A.D. (1981). *CSIRO Food Res Quart.*, **41:** 7-11.
14. Bothast, L.R., and Fennell, D.I. (1974) *Mycologia* **66:** 365-369.
15. Pitt, J.I., Hocking, A.D., and Glenn, D.R. (1983). *J. Appl. Bacteriol.*, **54:** 109-114.
16. Frisvad, J.C. (1983). *J. Appl. Bacteriol.*, **54:** 409-416.
17. Andrews, S., and Pitt, J.I. (1986). *Appl. Environ. Microbiol.* **51:** 1235-1238.
18. Jermini, M.C., Geiges, O., and Schmidt-Lorenz, W. (1987). *J. Food Prot.*, **50:** 468-472.
19. Pettipher, G.L. (1987). *Lett. Appl. Microbiol.*, **4:** 95-98.
20. Notermans, S., *et al* (1986). *J. Food Prot.*, **49:** 786-791.
21. Notermans, S., Dufrenne, J., and Soentoro, P.S. (1988). *J. Food Sci.*, **53:** 1831-1833.

OXOID NEWSLINES

Oxoid Expand Range of Toxin Detection Kits

The recently launched Reversed Passive Latex AgglutinatTion Test for the detection of Bacillus cereus enterotoxin joins a rapidly expanding range of simple, fast and reliable toxin tests from Oxoid. The Oxoid product range brings routine testing for aflatoxins and the enterotoxins of Staphylococcus aureus, Vibrio cholerae, Escherichia coli, Clostridium perfringens and Bacillus cereus within the reach of all types of laboratory. The various applications provide many benefits including improved quality control of foods, increased detection capabilities in the investigation of food poisoning outbreaks and more rapid clinical diagnosis.

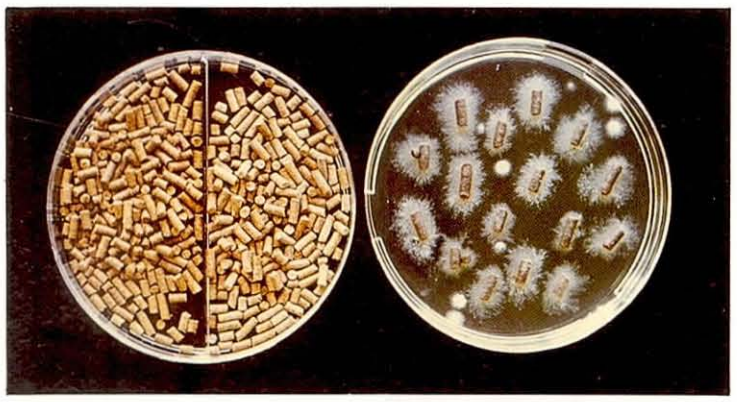


Figure 3: Isolation of *Xeromyces bisporus* from pelleted animal feed vitamin supplement by direct plating of the sample onto a low water activity (a_w) medium, MY50G. the left hand Petri dish contains an uncontaminated sample (left) and a mouldy sample (right). When plated onto MY50G, the mouldy sample yielded an almost pure growth of the xerophilic fungus *X.bisporus*. This species will not grow on high a_w media such as DRBC.

OXOID NEWSLINES

New Listeria Media from Oxoid

Oxoid's range of media for the detection of Listeria monocytogenes has been extended to include Listeria Selective Enrichment Broths for use in the food and dairy industries. The Listeria Selective Enrichment Medium (UVM formulations), Oxoid Code CM863 (broth base) with selective supplements SR142 and SR143, are a modification of the original description by Donnelly and Baigent and form the basis of a two-step selective enrichment method (USDA-FSIS method). Listeria Enrichment Broth Base CM862 with selective supplement SR141 is for use in the FDA method. Listeria monocytogenes is widely distributed in the environment and may be transmitted to man through contamination of foodstuffs at any point from source to kitchen. Milk and dairy products, meat, poultry, vegetables, salads and seafoods have all been found to be contaminated. These new media form part of a comprehensive range of media available from Oxoid for the detection of Listeria monocytogenes that includes McBride Medium (CM819), and Listeria Selective Medium (Oxford formulation) CM856 and SR140.

Lyme disease—a perspective

Sally J. Cutler, MSc, Medical Microbiology Department, Charing Cross Hospital, London W6 8RF, UK.

Figure 1: Nymph stage of *I.ricinus* tick.

Lyme disease is a recently recognised disease affecting both man and animals and is caused by the spirochaete *Borrelia burgdorferi*. The disease is transmitted by the bite of ixodid ticks, *Ixodes ricinus* being the principal European vector (**Figure 1**) and *Ixodes dammini* the main vector in NE America. Early symptoms include the formation of a characteristic skin rash at the site of the tick bite, flu-like symptoms and lymphadenopathy. Later manifestations of Lyme disease effect the central nervous system, heart and may be associated with chronic skin disease and arthritis. Although Lyme disease responds well to antibiotic therapy, prevention gives rise to problems. Clinical and laboratory criteria for diagnosis are poorly defined with atypical presentations sometimes complicating the picture.

History

Signs now known to be synonymous with Lyme disease have been described since 1909, when Afzelius described a skin rash, erythema migrans, which was associated with the bite of a tick. In 1922, Garin-Bujadoux recorded the neurological complication of meningopolyneuritis which was associated with tick bites.[1] By 1951, penicillin was shown to aid the resolution of erythema migrans and associated meningitis. Human to human transmission of erythema migrans was demonstrated in 1960, further supporting an infective cause. Sixteen years later, in NE America a cluster of cases of suspected juvenile rheumatoid arthritis were observed in Old Lyme, Connecticut. This resulted in 'Lyme disease' being reported to the Yale University by two concerned mothers. The subsequent investigation by Allan Steere showed that 39 children and 12 adults from a population of 12,000, had what became known as Lyme arthritis. Twenty-five per cent of cases also described a skin rash weeks before the onset of arthritis. Epidemiological analysis of cases incriminated an arthropod vector which was later found to be *I.dammini*.[2] A later prospective analysis of patients revealed that arthritis was only one of the sequelae of this disease. Patients also developed a variety of neurological and cardiac complications; as a consequence of this the condition was renamed Lyme disease. The parallel was drawn between the skin rash of Lyme disease and erythema migrans previously seen in Europe. It was not until the early 1980's when Dr Willy Burgdorfer at the National Rocky Mountain Fever Laboratory in Montana, while examining ticks from Shelter Island for rickettsia, observed spirochaetes in the mid gut of an adult female *I.ricinus*. These spirochaetes (later termed *Borrelia burgdorferi*) reacted with sera from patients with Lyme disease when tested by an indirect immunofluorescence technique and produced skin lesions resembling erythema migrans after intradermal inoculation in rabbits.[3] DNA homology studies confirmed that these spirochaetes were a genetically distinct *Borrelia* species.

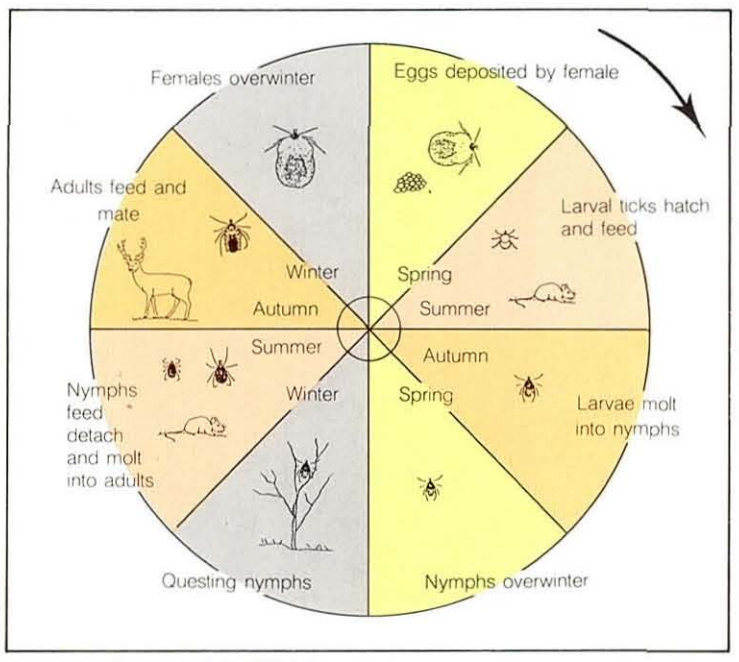


Figure 2: Life cycle of ixodid ticks.

Epidemiology

The true incidence and exact prevalence of Lyme disease in the United Kingdom, and indeed the rest of the world, is difficult to determine. The medical and veterinary professions are only just becoming aware of the diverse spectrum of presenting features of Lyme disease; diagnostic tests, although improving, are not sufficiently sensitive and specific to detect all cases, and there is accumulating evidence of asymptomatic cases of Lyme disease. All of these features have so far prevented the exact incidence of the disease from being known. Between 1986 and 1988, our laboratory has serologically diagnosed over 400 cases in the UK and, as mentioned above, this underestimates the true incidence of the disease. Distribution between sexes appears equal with a peak incidence in the 30 to 50 age group. Other studies have found a predominance of cases amongst males, possibly as a result of increased exposure to ticks through work or recreation.

Geographical distribution

Almost all European countries have reported indigenous Lyme disease cases, including Great Britain, France, Germany, Austria, Belgium, Switzerland, Sweden, Italy and European Russia. The southern European limit is above the 32°C isotherm. Cases have also been reported from as far afield as Canada, Japan, China and Australia. In America, Lyme disease has been reported in at least 32 states, however, most cases have been concentrated in the Northeast (Connecticut, Massachusetts and New York); the West (California, Oregon and Utah); and the Northern midwest (Wisconsin and Minnesota).

The vector

Currently five species of Ixodid ticks have been incriminated as vectors of Lyme disease. These are *I.ricinus* in Europe (**Figure 1**); *I.dammini* in NE United States; *I.pacificus* on the West coast of the United States and *I.scapularis* in the south. *I.persulcatus* is postulated as the vector in Japan. *B.burgdorferi* has been detected in all three life stages of *Ixodes* ticks, however, the nymphal and adult stages harbour *B.burgdorferi* to a much greater extent than the early larval form. The life cycle requires three hosts and can be completed within two years (**Figure 2**). Although transovarial passage of *B.burgdorferi* has been experimentally demonstrated, it occurs at a low frequency (1.9%).[4] Larval ticks tend not to transmit *B.burgdorferi*, but acquire the infection by feeding on infected reservoir hosts such as mice. Mice may harbour spirochaetes in their circulation for up to three months following infection and therefore serve as a good source of infective material.[5] Once the tick is infected the spirochaete persists in the mid gut of the tick throughout its life stages. The seasonal variations in infection reflect the tick ecology, with high relative humidity and temperatures above 13°C required for the tick to quest, explaining the spring and autumnal peaks of the disease. Although other ticks, and indeed other haematophagus arthropods may harbour *B.burgdorferi*, their ability to act as vectors for Lyme disease appears to be minimal.[6]

Animal reservoirs

A large number of animals and birds have been suggested as reservoirs for *B.burgdorferi*. Immature *I.ricinus* ticks commonly parasitise rodents such as mice, squirrels, rabbits, voles and birds. Adult ticks show a predilection for larger mammals, especially sheep and deer. Mice are believed to be the major reservoir for *B.burgdorferi*, with infected mice harbouring the spirochaete for up to three months thereby providing ample opportunity to infect immature ticks.[5,7] Infected ticks are then able to transmit the infection to larger mammals. Birds may have an important role for dissemination of Lyme disease and following migrations may lead to the establishment of new disease foci.[8] Deer have a different role in the epidemiology of Lyme disease, providing a host able to support large numbers of adult ticks so permitting completion of the tick's life cycle (**Figure 2**). A wide variety of other animal species including domestic dogs[9] and cats[10] have now been identified as having circulating antibody against *B.burgdorferi*. *B.burgdorferi* has also been cultured from these animals, which rarely slow symptomatic infection.

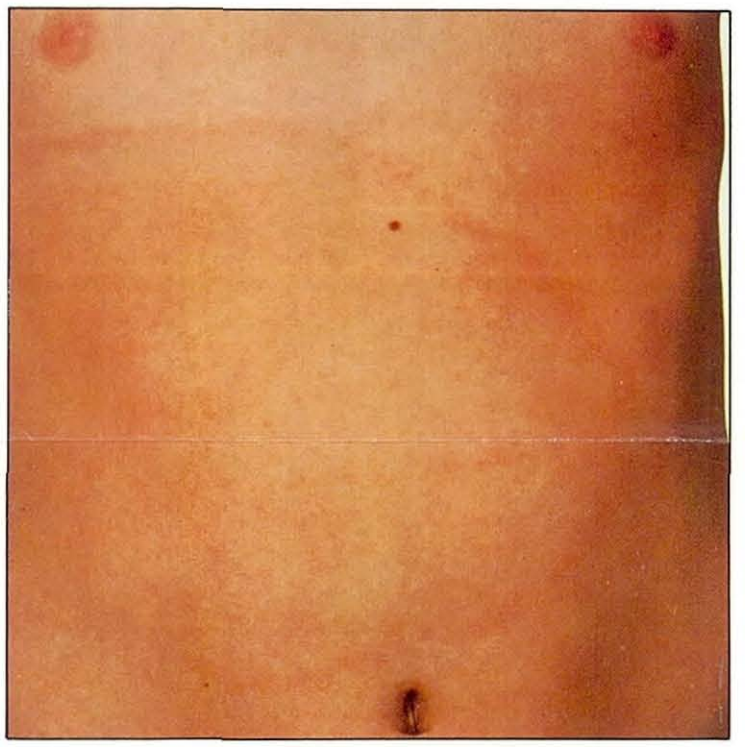


Figure 3: Erythema migrans: lesion showing expanding red border and central clearing.

Control measures

Several methods of control have been investigated including the destruction of vegetation in endemic areas by burning. Although ticks are reduced for the subsequent year, they return. Chemicals are prohibitively expensive for treating vegetation but do have a role as repellents to protect against tick bites. Some success has been achieved by applying pesticides to cotton wool which is then utilized by mice as nest material. Elimination of deer has been followed by a reduction of larvae in the following year and gradual decline in nymphs, however, adult ticks were abundant and in search of alternative hosts.[11] Biological controls are being investigated with an entomophagous wasp showing promise. This wasp parasitises the nymphal stage by laying eggs in the tick. The wasp eggs hatch once the nymph tick has engorged on a suitable host, at the tick's expense.[12] The individual exposed to ticks should take precautions to prevent tick bites, for example tucking trousers into socks or wearing boots. Examination for any attached ticks following exposure is advisable and prompt removal of any ticks reduces the probability of infection.

Symptoms

There has been great temptation to divide the symptoms of Lyme disease into three stages by analogy with syphilis. Stage one comprises of the non-specific systemic changes and the characteristic skin rash erythema migrans (formerly termed erythema chronicum migrans), the second stage is characterised by neurological and cardiac sequelae whilst stage three includes the chronic skin, neurological and arthritic symptoms. These stages can overlap and may even be absent, so that it is preferable to classify disease symptoms into those representing either early or late disease.

Early symptoms

One to two weeks following the introduction of the spirochaete by the bite of a tick, non-specific symptoms of fatigue, malaise, headache, fever, chills, arthralgia, myalgia, regional

Table 1:
Neurological complications of Lyme disease.

Symptoms Associated with Lyme Disease
Acute transverse myelitis
Ataxia
Cranial nerve palsies
Encephalitis
Hemiparesis
Meningitis
Optic neuritis
Peripheral neuropathy
Radiculitis
Spastic paraparesis

lymphadenopathy and stiff neck may be experienced. A skin rash, erythema migrans may develop at the site of the bite (**Figure 3**). This characteristic rash is the hallmark of Lyme disease and occurs in approximately 70% of cases two to three weeks following a tick bite. The rash slowly expands with central clearing to form an annular lesion with a bright red border.[13]

Late complications of Lyme disease

A small percentage of people will progress to later complications of Lyme disease. Approximately 15% of patients develop neurological manifestations, especially cranial nerve palsies, meningoradiculitis and encephalitis.[13] Lyme meningitis is typified by a lymphocytic pleocytosis with approximately 100 cells per mm^3, the protein slightly raised and glucose normal or slightly reduced. A range of neurological symptoms which have been associated with Lyme disease are listed in the accompanying table. Chronic skin eosinophilic fasciitis lesions, lymphadenosis benigna cutis, acrodermatitis chronica atrophicans (ACA) may develop within six months but may be delayed for many years after initial infection. ACA starts with an inflammatory phase followed by cutaneous atrophy rather like morphoea and occurs in up to 6% of Swedish and central European cases.[13] These complications although found in the UK seem to be less common. Cardiac complications occur weeks to months after infection with an incidence of 8% in American patients, however, this is seen less often in European countries. Symptoms of Lyme carditis include pericarditis and fluctuating heart block particularly involving the atrioventricular node.[13,14] Lyme arthritis was initially thought to be a common complication occurring in 60% of American cases. More recent studies have shown the true incidence of rheumatological complications to be nearer 18%. Patients experience migratory episodes of joint pain and a minority may subsequently develop episodes of arthritis weeks to years after the onset of Lyme disease. Arthritic attacks may recur over several years and may lead to chronic synovitis with erosion of the joint.[13]

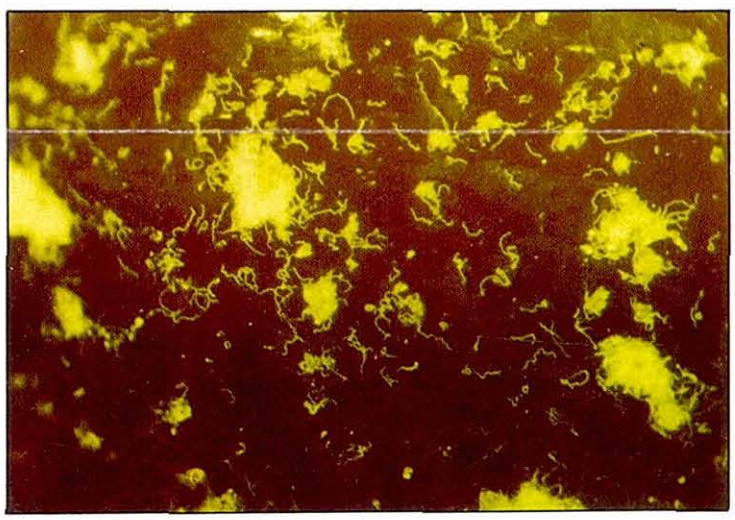


Figure 4: Immunofluorescence stain of spirochaetes ×50 (UK strain). Note tendency of spirochaetes to clump.

Treatment

Early disease responds well to treatment which both shortens the duration of symptoms and reduces the incidence of later complications. Late disease responds less well, especially neurological complications. Tetracycline is generally considered the most appropriate treatment for uncomplicated Lyme disease. Penicillin or erythromycin may be used as an alternative, however, treatment failures have been reported. Late Lyme disease is more refractory to treatment. Parenteral therapy with third generation bactericidal cephalosporins such as ceftriaxone or cefotaxime are more effective.[13] Jarisch-Herxheimer reactions may occur at the commencement of treatment, but are rarely as life threatening as seen in the relapsing fever borreliosis.

Animal symptoms

Veterinary case reports have described symptoms of arthritis in dogs,[15] encephalitis in a horse[16] and a wasting disease and arthritis in a cow.[17] There have also been reports of kidney failure followed by death and symptoms of heart block involving the atrioventricular node in dogs.[18]

Laboratory diagnosis

Microscopy

Microscopic examination of material for *B.burgdorferi* can be achieved using a variety of different methods including dark field microscopy, silver stains and fluorescent staining with either monoclonal or polyclonal antibodies. None of these methods are sensitive and all require lengthy examination of material for spirochaetes.

Dark field examination requires the presence of a large number of spirochaetes and thus is probably best restricted to the examination of cultured borreliae rather than direct examination of clinical material. False positive results may be observed with clinical material, for example, collagen fibres resembling spirochaetes. This has been reported when examining material for leptospires. A variety of stains can be used to visualise spirochaetes most of which are variations on silver staining methods. None of these are particularly sensitive or specific. The Warthin-Starry and Dieterle staining techniques are the two most popular methods. Spirochaetes can also be stained with acridine orange, however, an immunofluorescence method is preferable giving extra specificity (**Figure 4**). Immunofluorescence with either polyclonal or monoclonal antisera specific for *B.burgdorferi*, will simultaneously visualise and identify spirochaetes present. Electron microscopy is not a practical method for detection of spirochaetes in clinical material but has a role in confirming that the spirochaetal forms seen on dark field are genuine. Electron microscopy also distinguishes borrelia from treponema by the number of periplasmic flagella present (**Figure 5**).

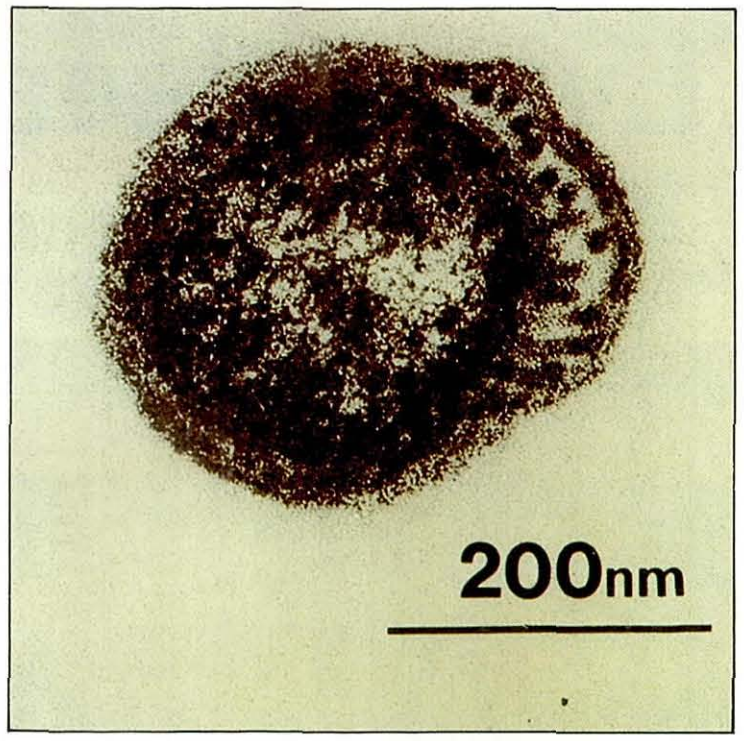


Figure 5: Electron microscopy showing cross section of *B.burgdorferi* (UK strain).

Culture

Borreliae have been cultured from a variety of specimens including ticks, skin biopsies, CSF, blood and synovial fluid. Culture is a low yield procedure with microscopy positive material not infrequently failing to yield growth of borreliae. The medium used is modified Barbour-Stoenner-Kelly medium (BSK).[19] It is a highly nutritious complex medium but suffers from batch to batch variation and has a short shelf life. The medium can be made selective by the addition of antibiotics such as kanamycin, 5-fluorouracil, phosphomycin, rifampicin and colistin sulphate.

Serology

As a result of the problems with microscopy and culture, serology remains the only practical method for the diagnosis of Lyme disease.[20] The two principal methods used are indirect immunofluorescence (**Figure 4**) and enzyme-linked immunosorbent assay methods (ELISA). Immunoblotting can be used to confirm serology results. Serology can also be used to analyse other body fluids such as CSF and synovial fliud to assist the identification of areas of disease activity.

During early disease serology is less useful with patients with erythema migrans not producing significantly elevated levels of antibodies. The IgM response is often not usually detectable during the first two weeks and peaks between the third and sixth weeks. The IgG response is not usually detectable until the fourth week following infection and may remain elevated years after clinical remission. Prompt antibiotic treatment may prevent significantly elevated antibody levels. Although serodiagnosis is now readily available, it is not without shortcomings. Sensitivity of the tests is poor during early disease. Serological tests for Lyme disease are not very specific, patients with other spirochaetal diseases and active collagen disorders may give false positive results. Subjectivity is an additional problem encountered with IFA and immunoblotting methods. The problems of serodiagnosis of Lyme disease have recently been reviewed.[20]

Concluding remarks

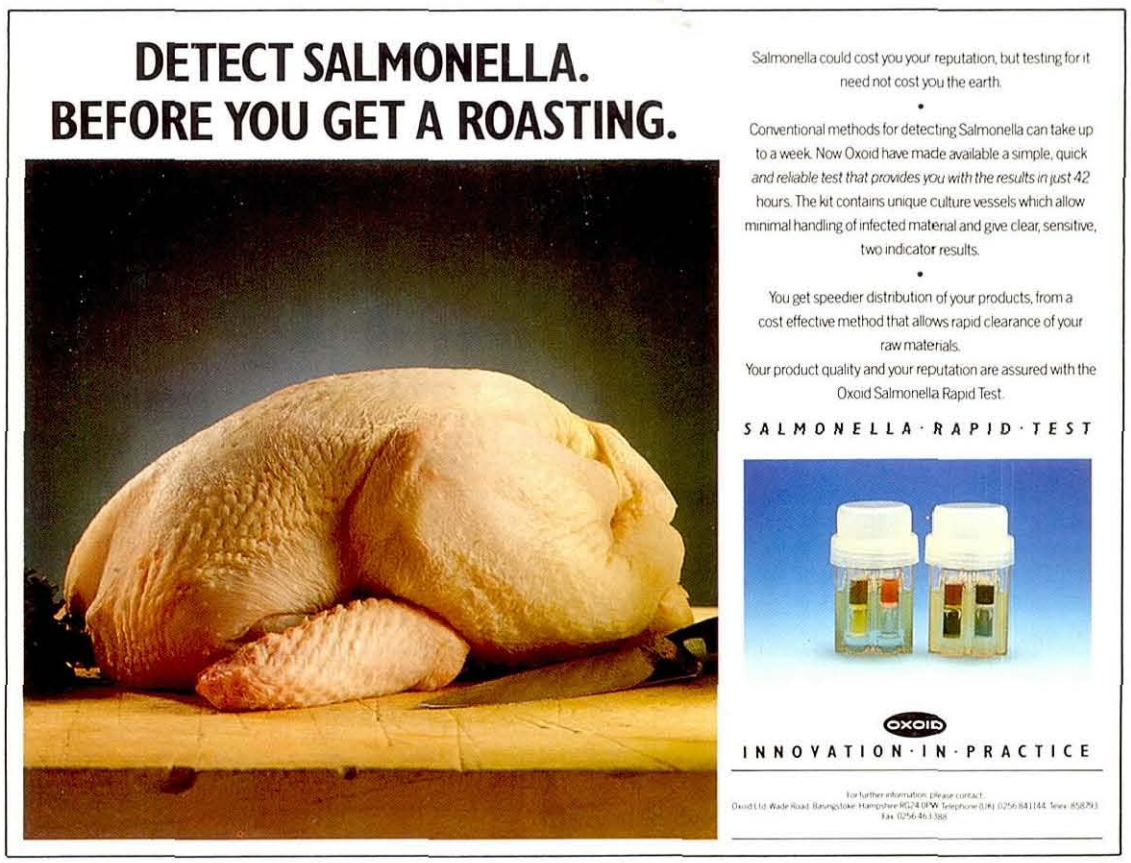
As a result of the large number of animal species which serve as hosts, it is unlikely that a control programme could be implemented in the near future. Avoidance and prompt removal of ticks and treatment of clinical symptoms as they occur will help to reduce the risk of late complications of Lyme disease. Although serodiagnostic tests are now available for the diagnosis of Lyme disease, they should not be used in isolation from the clinical symptoms.

Acknowledgements

I thank Dr DJM Wright, Medical Microbiology, Charing Cross Hospital, for his help.

References

1. Dammin, G.J. (1989). *Rev. Infect. Dis.*, **11:** 142 – 151.
2. Steere, A.C. *et al.* (1986). *Zentralbl. Bakteriol. Hyg.* (A), **263:** 3 – 6.
3. Burgdorfer, W. *et al.* (1982). *Science* **216:** 1317 – 1319.
4. Magnarelli, L.A., Anderson, J.F. and Fish, D. (1987). *J. Infect. Dis.*, **156:** 234 – 236.
5. Donahue, J.G., Piesman, J. and Spielman, A. (1987). *J. Trop. Med. Hyg.*, **36:** 92 – 96.
6. Magnarelli, L.A. and Anderson, J.F. (1988). *J. Clin. Microbiol.*, **26:** 1482 – 1486.
7. Levine, J.F., Wilson, M.L. and Spielman, A. (1985). *Am. J. Trop. Med. Hyg.*, **34:** 355 – 360.
8. Anderson, J.F. and Magnarelli, L.A. (1984). *Yale J. Biol. Med.*, **57:** 627 – 641.
9. Eng, T.R. *et al.* (1988). *J. Infect. Dis.*, **158:** 1410 – 1411.
10. Curran, K.L. and Fish, D. (1989). *New Eng. J. Med.*, **320:** 183.
11. Wilson, M.L. *et al.* (1988). *J. Med. Entomol.*, **25:** 224 – 228.
12. Hyland, K.E., Amr, Z.S. and Hu, R.H. (1988). *Rhode Isl. Med. J.*, **71:** 475 – 484.
13. O'Neill, P.M. and Wright, D.J.M. (1988). *Brit. J. Hosp. Med.*, **40:** 284 – 289.
14. Steere, A.C. *et al.* (1980). *Annal. Intern. Med.*, **93:** 8 – 16.
15. Magnarelli, L.A. *et al.* (1987). *J. Am. Vet. Med. Assoc.*, **191:** 1089 – 1094.
16. Burgess, E.C. and Mattison, M. (1987). *J. Am. Vet. Med. Assoc.*, **191:** 1457 – 1458.
17. Burgess, E.C., Gendron-Fitzpatrick, A. and Wright, W.O. (1987). *J. Am Vet. Med. Assoc.*, **191:** 1468 – 1470.
18. Levy, S.A. and Duray, P.H. (1988). *J. Vet. Intern. Med.*, **2:** 138 – 144.
19. Barbour, A.G. (1984). *Yale J. Biol. Med.*, **57:** 521 – 525.
20. Cutler, S.J. and Wright, D.J.M. (1988). *Serodiag. Immunother. Infect. Dis.*, **2:** 4 – 6.



Printed in Great Britain by Flexiprint Limited.