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COXSACKIEVIRUSES (*PICORNAVIRIDAE*)

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History

Descriptions of a disease resembling epidemic myalgia presumably caused by viruses of the coxsackie group have been reported in different Scandinavian areas since the second half of the 19th century. The first coxsackievirus was isolated only in 1948 in New York state (USA) by Dalldorf *et al*, who were investigating an outbreak of paralytic poliomyelitis in the village of Coxsackie. They discovered a new group of viruses pathogenic for suckling mice. The observation led to the widespread use of suckling mice in the investigation of the viral etiology of poliomyelitis-like illnesses. The conditions of their isolation led some investigators to believe that the new viruses were only 'common fellow travellers of poliovirus'. But it rapidly became clear that these viruses had a full range of morbidity.

Taxonomy and Classification

Coxsackieviruses belong to the *Picornaviridae* family, genus *Enterovirus*, group of human enteroviruses. They contain a single plus-strand of RNA protected by an icosahedral capsid exclusively constituted of proteins. The viral particule contains no enzyme and no envelope. Its size is 22–30 nm. Coxsackieviruses are divided into two subgroups: coxsackievirus A (CA) with 23 serotypes 1–24 (23 is missing) and coxsackievirus B (CB) with six serotypes 1–6. This classification is based on the histopathologic lesions in suckling mice (see under Pathology and Histopathology below).

It was noticed early on that pathogenic features for suckling mice are not a definite characteristic. For instance, strains of CA23 have been recognized as being variants of echovirus 9 and some strains of CA24 have been temporarily classified as echovirus 34. Recent developments in molecular biology have contributed to the remodeling of the currently admitted classification. According to RNA analysis, four clusters have been proposed for human enteroviruses among which CA are scattered over three of them (**Table 1**).

Geographic and Seasonal Distribution

Coxsackieviruses are present worldwide, each serotype being found in a given area. More information has been gathered on CB than CA, owing to the somewhat tedious procedure for isolating the latter. Climate has a major role in the epidemiological pattern. In temperate countries, the circulation of coxsackievirus culminates in summer and autumn. In tropical regions, they occur all the year round in an endemic mode. In so-called 'Mediterranean' climates, they are distributed at a low endemic level with a summer–fall increase. They circulate together with other enteroviruses.

Host Range and Virus Propagation

Coxsackieviruses commonly cause diseases in humans. A virus very similar to CB5 has been recovered from pigs. Chimpanzees can also be infected by coxsackieviruses. They usually develop a subclinical infection, but CA7 can produce paralysis in monkeys.

Suckling mice can be experimentally infected by several parenteral routes: 1-day-old newborn mice are required for maximum pathogenic expression for CB. For CA, the susceptibility of the suckling mice extends to the first 4 days of life. Weaning mice can be infected by mutants of CB causing pancreatitis or myocarditis. Suckling merions are also susceptible.

Coxsackievirus can be grown in a number of primary tissue culture systems or continuous cell lines derived from primate (human or monkey) tissue: primary monkey kidney or embryonic human kidney, human heteroploid cells such as KB, HeLa and Hep2, or monkey kidney cell lines such as Vero and BGM. Replication results in a typical cytopathic effect with cell ballooning associated with a dendritic aspect. The virus cycle is completed within 7–8 h and takes place exclusively in the cytoplasm. Rapid cell lysis occurs.

CB induces a chronic infection in human embryonic lung fibroblasts. Some CA serotypes cannot be grown on cells, especially CA1–6. Primary or cell lines originated from mice are not susceptible *in vitro*. Cell susceptibility is linked to the presence of cell membrane receptors for the virus. It has been shown recently that the major cell receptor for CB is a 46 kDa protein that also mediates the attachment of adenoviruses 2 and 5 and has been called coxsackievirus and adenovirus receptor (CAR). The gene coding for this protein is located on chromosome 21. Some CA and CB strains are also able to bind to another receptor – the complement regulatory protein

Table 1 Classification of coxsackieviruses within the *Enterovirus* genus (according to Puli *et al* (1995) *Virology* 212: 30 and Pöyry *et al* (1996) *J. Gen. Virol.* 77: 1699

Clusters	*Coxsackieviruses*	*Other enteroviruses*
A	A2, 3, 5, 7, 8, 10, 12, 14, 16	Enterovirus 71
B	A9	Enterovirus 69
	B1, 2, 3, 4, 5, 6	All echoviruses except types 22 and 23
C	A1, 11, 13, 15, 17–22, 24	Poliovirus 1–3
D	None	Enteroviruses 68 and 70
Undetermined	A4, 6	—

Table 2 Identified cellular receptors of coxsackieviruses

Cell receptor	*Coxsackieviruses*	*Other viruses competing with the same receptor*
CAR	Coxsackieviruses B	Adenoviruses 2 and 5
DAF (CD55)	CB-3, CA-21	Echoviruses 6, 7 and 12
ICAM-1	CA-13, 15, 18, 20, 21	Major group rhinoviruses
$\alpha_v\beta_3$ integrin	CA-9	—

decay accelerating factor (DAF) – that has been shown to be the major receptor of echoviruses types 6, 7 and 12. The $\alpha_v\beta_3$ integrin (vitronectin receptor) is involved in the entry of CA9 in host cells whereas CA types 13, 15, 18, 20 and 21 use ICAM-1 as cellular receptor (**Table 2**). These data suggest that many coxsackieviruses use multiple receptor molecules for their cell entry mechanisms.

Strains of CB agglutinate human cord blood type 0 erythrocytes. The structure and location of the hemagglutinins are unknown.

Genetics

The coxsackieviruses whose full-length sequence is available (CB1, 3, 4, 5, CA9, 21) show a genomic structure similar to that of the other members of the genus *Enterovirus*, especially poliovirus, the most extensively studied. Briefly, the genome is about 7500 bases and is flanked by two nontranslating poly(A) regions (NTR) 5′ and 3′. A small protein, VPg, is linked to the 5′-NTR (**Fig. 1**). In contrast with most eukaryotic messenger RNA molecules, the 5′ NTRs of enteroviruses contain internal ribosomal entry sequences (IRES) so that ribosomes may bind in the absence of a free 5′ capped terminus. The RNA acts as an mRNA which encodes directly a high-molecular-weight polyprotein, p220, consisting of three segments coding for the three precursor proteins, namely P1, P2 and P3. A cascade of cleavages carried out by virus-encoded serine proteases (2A and 3C) leads to 11 polypeptides among which are the final structural proteins of the capsid. These structural proteins are derived from the P1 segment (mol. wt 97 000) of p220 (**Fig. 1**).

The capsid surface of the dense virion is a combination of 60 protomers of four viral proteins (VP) each, numbered from 1 to 4. In coreless particles (empty capsids), only three proteins are present: VP0, 1 and 3. The incorporation of the RNA genome to the empty capsid leads to the provirion. VP0 is the precursor protein for VP2 and 4. The autolytic cleavage of VP0 into VP2 and VP4 stabilizes the capsid structure and leads to the infectious virion. VP4 is an internal protein linked to the genome. The epitopes which bind neutralizing antibodies are mainly present on VP1. Nevertheless, minor epitopes are also present on VP2 and VP3. The structures responsible for adsorption of the virus to the cell receptor (see above)

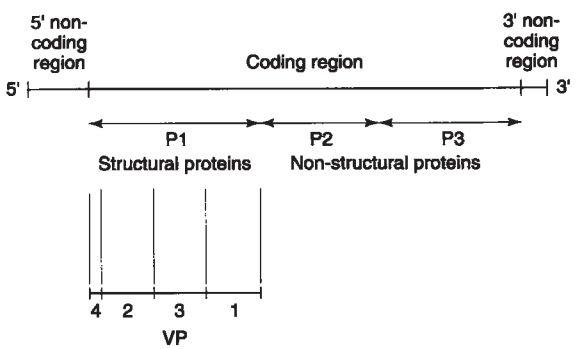

Figure 1 General organization of the genomic RNA of coxsackieviruses.

are buried within a kind of 'canyon' with VP1 on its edges. Defects in the RNA are frequent in the so-called 'defective particles'. Therefore the population of viral particles that finally burst out of the lysed cell is heterogeneous. It contains a mixture of at least the four types described above.

Evolution, Serologic Relationships and Variability

Minor antigenic drifts are currently observed in laboratory isolates and virions can exist as quasi-species in the same organism, as generally happens with other RNA viruses. Although no antigenic shifts comparable with the influenza A virus have been observed, the appearance of a variant of CA24 causing a 'new' disease consisting of a hemorrhagic conjunctivitis (see under Clinical Features of Infection below) may question this.

As a general rule, the 23 serotypes of CA and the six serotypes of CB are antigenically unrelated. The neutralization test is the reference procedure for identification of isolates: serum raised against one serotype does not crossneutralize the others. But limited neutralization sometimes occurs between related serotypes. For instance, CA3 and CA8 show antigenic crossing, as do CA13 and CA18.

Epidemiology

Coxsackieviruses demonstrate the same epidemiological pattern as the other enteroviruses. Each year, whatever the climate or geographic area, a human community experiences at least one, and often several, coxsackievirus infections.

Age is an important risk factor. Young children are the reservoir of the virus. In low socioeconomic conditions babies become infected during the first months of life. As economic development increases, the first contact with the virus may occur later during childhood or even later.

Coxsackieviruses are present in the environment. Many reports regarding isolation of CB from surface or waste waters, soils, raw vegetables and shellfish have shown that these viruses are a major public health problem. The spread of the virus from infected members in a community is due to the feces which contaminate the environment. As children with silent infections excrete the virus in their feces for several weeks, they play the role of an incessant source of virus. The poorer the sanitation, the higher the contamination level of the environment.

Transmission and Tissue Tropism

Coxsackieviruses have the same routes of transmission as polioviruses: the oral route via dirty hands and polluted water and food. Transmission by aerosol is possible when pulmonary syndromes are present, during an outbreak of conjunctivitis, or merely from subjects with subclinical infection when the virus is present in the throat during the incubation period.

Coxsackieviruses affect a wide range of susceptible tissues and organs: striated muscles, myocardium, brain and spinal cord, pancreatic islets, lung, skin and conjunctivae. Mutants with special pathogenic properties have been reported, for example cardiotropic and pancreatropic CB for mice. Much effort has been devoted to the characterization of the genetic determinants responsible for this virulence.

Pathogenicity

After entry by the oral route, the virus is thought to follow the same path as poliovirus: multiplication in the throat and small intestine and in the lymph nodes draining the primary sites of multiplication. Then, via the bloodstream or lymphatic drainage system, the virus reaches the target organs. In the lower intestine, the virus enters the Peyer patches through the M cells. Its multiplication in the absorptive cells has been shown in animal tissues *in vitro* but evidence in humans is lacking.

Most infections are clinically silent. The reason why some individuals exhibit a higher susceptibility to the virus than others is still largely a mystery. Several factors may increase susceptibility: previous immune status, immune deficiency restricted to some strains, higher pathogenic potency of mutants, genetic HLA constitution and/or hormonal interactions with viral multiplication. The last factor would explain the sex ratio of 1.5–2 males for every female when clinical expression of the infection is considered.

Clinical Features of Infection

A large panel of acute diseases are due to coxsackieviruses and there is much evidence for their causative role. The relationship between a serotype and a disease is generally not clear to the clinician, except under epidemic circumstances. Moreover it is difficult to distinguish between coxsackievirus infections and other nonpolio enterovirus infections when clinical symptoms only are considered. Another common clinical characteristic of enterovirus infections is the frequency of featureless diseases (febrile illness, resolutive rash, flu-like syndrome). During the last two decades, persistent pathologic disorders have been attributed to these viruses; their actual role in

such processes is still disputed although more and more evidence tends to accredit this concept, as is discussed below.

Much more information is available for CB than CA viruses, owing to the easier isolation of the former. For the latter, attention has been focused on those CA serotypes that multiply easily in cultured cells, such as CA7, 9, 16, 21 and 24. Coxsackieviruses induce protean clinical pictures depending on the target organ.

Nervous system

CA7 and 9 and CB1–6 have been infrequently involved in polio-like paralysis. Encephalitis, ataxis and paralysis of the cranial nerves are rare. Both CA and CB are responsible for aseptic meningitis, although echoviruses are more frequently the cause of meningitis than are coxsackieviruses.

Skin and mucosa

Herpangina is common in school age children. Clear reddish vesicles are distributed on the moderately inflamed mucosa of the throat, tonsils, soft palate and tongue. It appears as a self-limited stomatitis. CA1–6, 8, 10 and 22 have been isolated from the vesicles. Hand-foot-and-mouth disease consists of vesicles on the palms of the hand and soles of the foot, but also on the limbs and trunk, sometimes associated with stomatitis. The main serotypes involved are CA4, 5, 9, 10 and 16 and CB2 and 5. CA16 is responsible for the majority of outbreaks. Herpes-like vesicular rashes have been described, and CB1 has been isolated from the vesicular fluid. Rubella-like rashes are often associated with other more typical clinical symptoms.

Striated muscles

Pleurodynia, also known as 'epidemic myalgia' or 'Bornholm disease' (from the name of a Danish island where an outbreak gave the opportunity for a well-documented clinical description in 1933) consists of a sudden pain in the chest (due mostly to the involvement of the diaphragm) and a general malaise associated with headache, sore throat and fever. It is common in summer camps for children and teenagers.

CB are also associated with acute myositis and rhabdomyolysis. A mouse model of myositis with CB1 has been described.

Prolonged myalgia was described several decades ago in patients during convalescence from CA infections. A new concept has recently arisen, the 'post-viral fatigue syndrome' (or 'myalgic encephalomyelitis'), which consists of prolonged muscular fatigability (>6 months) often associated with other functional and/or psychological symptoms. The causative role of CB is still largely disputed and some herpetoviruses are also candidates.

Heart

CB are one of the main etiologic agents of acute myocarditis, pericarditis and myopericarditis. The association has been strengthened by the finding, by *in situ* hybridization, of viral RNA sequences in the myocardium of patients who had died from acute heart failure. In neonates, the disease is often fatal whereas the prognosis is usually milder in adolescents and adults.

Dilated cardiomyopathy (DCM), a chronic heart disease characterized by ventricular congestion leading to heart failure and for which the only remedy may be heart transplantation, has also been tentatively linked to a persistent CB infection. A mouse model using CB3 supports this hypothesis. In humans, the role of CB in DCM is suggested by high antibody titers (IgM included) against CB and by the detection of CB RNA by hybridization or polymerase chain reaction (PCR) in the myocardium of patients. The pathogenesis of the disease involves genetic, virological and autoimmune factors. The viral persistence might be related to an impaired replication of the virus illustrated by production of positive and negative RNA genomic strands in equal amount.

Alimentary tract and liver

Acute abdominal pains have been associated with CB, mimicking appendicitis in childhood. A few cases of hepatitis associated with CB have also been described, especially in neonates. Gastroenteritis is a frequent prodromic symptom of coxsackievirus infections. However, these viruses cannot be considered as main etiologic agents of viral diarrhea, owing to their low level of multiplication in the intestinal tract.

Pancreas

There are strong arguments in favor of the involvement of CB in type I insulin-dependent diabetes mellitus, including a mouse model using CB1 and 4. The disease is thought to result from several factors acting together: a genetic predisposition, the multiplication of a diabetogenic strain of CB in the islets of Langerhans and antigen mimicry between CB proteins and cell components of the pancreas, both of them resulting in cell lysis. Rather than a persistent infection, it may be the summation of repeated enteroviral infections, some of which occurred during intrauterine life, which leads to the last event responsible for the chronic insulin deficiency.

Respiratory tract

Coxsackieviruses may be responsible for infections of the respiratory tract: CA9 and 16 have been associated with pneumonia, CA21 and 24 with the common cold and CB2, 3, 4 and 5 with upper and lower respiratory tract infections. They mimic respiratory infections due to myxo-, rhino- and coronaviruses. Although occurring all through the year, the enteroviral origin of these infections may be evoked when observed during the warm season ('summer grippe').

Eye

A new nosological entity which arose in 1969–1970 and consisted of conjunctivitis was first described in South West Asia and was introduced into the Americas in 1981. A variant of CA24 was recognized as the etiologic agent of this acute conjunctivitis. It is clinically less severe than acute hemorrhagic conjunctivitis due to enterovirus 70, the subconjunctival hemorrhages being moderate. No paralysis has been reported from the areas where the main outbreaks occurred, a clinical pattern distinctive of the enterovirus 70 disease.

Coxsackievirus, pregnancy and the neonatal period

The risk of abnormalities in the fetus of pregnant women exposed to coxsackieviruses seems low, although the long-term consequences of such infections are difficult to evaluate. When the disease develops in an infant within the first 2 or 3 weeks of life, the virus is generally transmitted during or immediately after delivery. Infection in the infant ranges from mild to severe, even fatal. Meningitis, myocarditis, pneumonia, encephalitis, hepatitis and pancreatitis may be encountered. It should be noted that severe nosocomial outbreaks of coxsackieviruses have been reported in the neonatal period.

Pathology and Histopathology

The incubation period varies from 2 days (conjunctivitis) to 1 month or more, the mean time being 10–14 days. Information on the histopathological lesions comes mainly from animal models which have been extensively described (**Fig. 2**). A review of histopathological lesions observed in more than 400 isolations from patients in a UK hospital reports a wide range of lesions. The classical distinction between CA and CB is somewhat confused by the fact that a wide range of lesions can be found regardless of the subgroup. However, there are significant variations in the frequency of the main types of lesions.

With CA, polymyositis is found predominantly, as expected from previous data, with the thoracic, abdominal and thigh muscles being the most severely affected. Encephalitis and poliomyelitis are also found, as well as brown fat necrosis and myocarditis, but in a much smaller proportion than in animals infected with CB. In contrast, encephalitis, poliomyelitis, brown fat necrosis, endomyopericarditis and pancreatitis are the main lesions observed in animals infected with CB. Myositis is rare and, when present, it is much more limited. These considerations may explain the finding that the usual signs of CB infection in mice are spasticity and fine tremors rather than the flaccid paralysis commonly observed with CA infection. Wherever the sites of histological lesions are, inflammatory foci are invaded by numerous mononuclear cells.

In experimentally infected susceptible mice using a cardiotropic strain of CB3 by the intraperitoneal route, the virus can be detected in the myocardium as soon as two days postinfection; foci of necrosis associated to calcifications and to infiltrates of lymphocytic cells are present within one week. Inflammation and necrosis peak during the second week; then, these lesions decrease and are progressively replaced by fibrosis. During the acute phase of myocarditis, infectious virions can be recovered from the heart. At the end of the first month, a chronic myocarditis, characterized by an interstitial fibrosis and a mild infiltrate of mononuclear cells, can occur in some strains of mice; at this stage, no infectious virus can be cultured but RNA genome is detected by *in situ* hybridization in a small proportion of myocytes around the foci of fibrosis. Specific cytotoxic T cells and autoantibodies seem to play a leading part in the constitution of both acute and chronic lesions. Similar findings have been described in other experimental murine models using CB strains infecting peripheral muscles or pancreas.

Immune Response

Half a century of investigations has brought definite evidence that the control of and protection against viral infection is mainly related to neutralizing antibodies. Such antibodies mainly result from silent infections. They are considered to be lifelong, although the conditions for this may vary largely in space and time. Naturally acquired immunity declines when the current circulation of the virus decreases within a community.

Specific mucosal immunoglobulin A plays a protective role, especially in the gut. The presence of specific immunoglobulin M has been demonstrated by various technical approaches. However, their signifi-

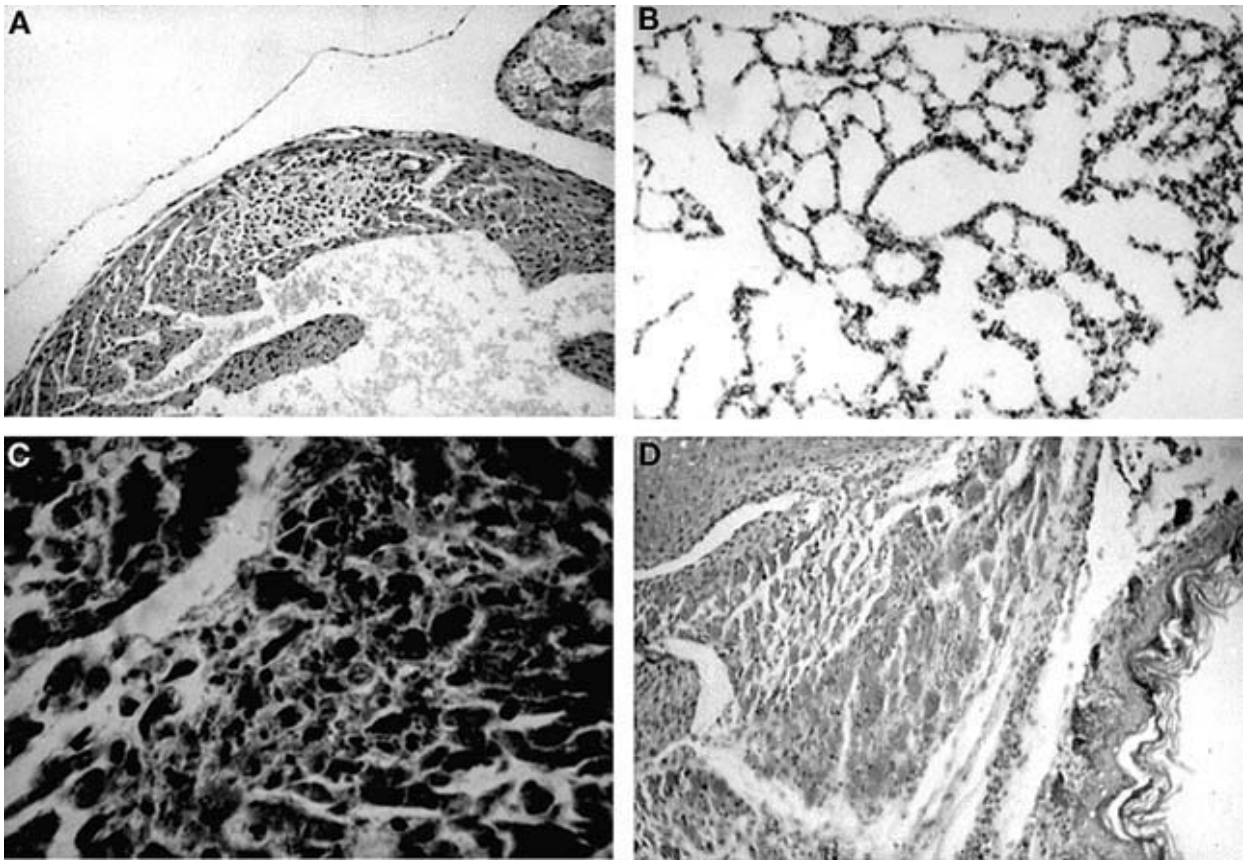

Figure 2 Tissue damage caused in baby mice by a freshly isolated strain of coxsackievirus B (**A–C**) or coxsackievirus A (**D**). (**A**) A large necrotic focus in the atrial myocardium (×100); (**B**) steatitis in the interscapular brown fat pad (×250); (**C**) a ventricular focus of necrotic cardiomyocytes (×450): note the dense mononuclear infiltrate in the infected area. (**D**) Intercostal muscles with diffuse hyalinization of myocytes and mild inflammatory infiltrate (×250).

cance as signs of a current or recent infection is still disputed, owing to the lack of data on their duration in different clinical situations.

Other aspects of immunity are still debated. The target of neutralizing antibodies is limited to epitopes on the surface of the virus. The total constitutive proteins of the capsid represent less than 50% of the total proteins encoded by the genome. Is there a host reaction against the nonstructural proteins? In the inflammatory foci seen in infected tissue in human and murine diseases, mononuclear cells are predominant; specific cytotoxic T cells probably play a key role in the control of infection. As discussed above, autoimmunity is probably involved in the genesis of tissue damage observed in chronic infections.

The immune response to coxsackieviruses is thought to be mostly type specific. However, frequent antigenic crossings of neutralizing, precipitating, complement-fixing antibodies are encountered when sera from infected patients are investigated, which evokes the possibility of common epitopes for subgroups or serotypes. Previous studies have identified a group-specific epitope located on VP1 which is common to most of the members of the enterovirus genus, including CA and CB. Similarly, T cells primed with a given serotype are able to proliferate *in vitro* in the presence of other serotypes of enterovirus.

Prevention and Control

No specific prevention by vaccines is presently available or planned for the near future. Preventive measures include a general hygiene policy, but this cannot avoid transmission through a community, especially among children. As the virus is mainly transmitted by dirty hands or contact with contaminated water or food, particular attention must be paid to individual hand washing and sanitary control of swimming pools and meals. Bathing in natural surface waters (rivers, ponds) should be avoided. Sea resorts may be at risk when waste waters are admitted into the sea at the shoreline without previous treatment. Eating raw shellfish collected in such places is a great risk.

Disinfectants have to be chosen carefully for institutions for children (hospitals, kindergartens, camps) because coxsackieviruses are resistant to many common ones, such as alcohols, quaternary ammonium compounds and chlorhexidine. They are stable at pH values ranging from 3.0 to 10.0. Chlorine is recommended for cleaning bathrooms and other surfaces. For hand disinfection, chlorinated or iodinated disinfectants should be used. For instruments, disinfectants containing aldehydes (formaldehyde or glutaraldehyde) are adequate, provided that the instruments have been cleaned before immersion in the decontaminating bath and the contact is for over 30 min.

Exposure to temperatures above 50°C destroys coxsackieviruses in a few minutes, but the presence of Ca^{2+} or Mg^{2+} can protect against heat inactivation. Viruses are highly resistant in feces, where adventitious substances reduce the efficiency of the disinfectant which does not easily penetrate the fecal material. Feces can remain at room temperature for weeks without substantial loss of infectivity.

No antiviral agent is available for therapy. γ-Globulins have proven useful in a limited number of cases.

Future Perspectives

The role of coxsackievirus has been well established in acute diseases, especially in communities of children. However, the concept of persistent infection, which is essentially related to adults, has been introduced more recently. It has been developed thanks to the tools of molecular biology. The demonstration of RNA sequences in tissues may provide a valuable argument for persistence, but will not elucidate the immunological mechanisms that allow this persistence. Increased knowledge of all facets of immunity is needed to understand these mechanisms. The wide use of PCR in the routine diagnosis of enterovirus infections will also help to identify the pathological role of those CA that cannot be cultivated in cell culture.

As development of vaccines is improbable, owing to the numerous serotypes of coxsackievirus, total control of the infection is not a realistic aim. However, if the role of CB in chronic infection of the heart was fully confirmed, a vaccine directed against the main serotypes of this subgroup could be designed. Two kinds of antiviral agents have been proposed: components acting on the initial phases of the lytic cycle (Win compounds) and protease inhibitors active against the two serine proteases 2A and 3C. They have still to prove their efficiency *in vivo/e2>*.

See also: **Echoviruses (*Picornaviridae*); Enteroviruses (*Picornaviridae*): Animal and related viruses, Human enteroviruses (serotypes 68–71); Eye infections; History of virology: General; Polio, coxsackie, echo and other enteroviruses; Persistent viral infection; Polioviruses (*Picornaviridae*): General features, Molecular biology; Quasispecies; Viral receptors.**

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Creutzfeld-Jakob Disease Virus *see* **Prions**

Cricket paralysis Virus *see* **Picornaviruses – insect**