Application of diagnostic procedures to epidemiological situations with special reference to arboviral infections

J. Brenner[(1)], M. Malkinson[(2)] & H. Yadin[(1)]

(1) Division of Virology, Ministry of Agriculture, Veterinary Services, Kimron Veterinary Institute, 52050 Beit Dagan, Israel
(2) Division of Poultry and Fish Diseases, Ministry of Agriculture, Veterinary Services, Kimron Veterinary Institute, 52050 Beit Dagan, Israel

Summary

The rationale behind the methodology employed to investigate a new disease that appears in an area hitherto unaffected is fundamentally different from that applied in an endemic disease situation. Special consideration must also be given to disease agents that appear and reappear at cyclical intervals.

The authors present three separate approaches applicable in three different epidemiological situations. Each one may be used initially to determine the identity of the causal agent and the nature of the disease. These approaches, although described in a stepwise (flowchart) manner, are not meant to be applied rigidly, but rather should serve as a guideline for investigators. Special consideration is given to situations in which disease appears intermittently, using a sentinel model. Although this latter approach is expensive and time-consuming, it can yield excellent and reliable results when applied correctly.

Keywords

Akabane virus – Arbovirus – Arthrogryposis – Diagnosis – Hydranencephaly – Israel – Nested polymerase chain reaction – Sentinel model –Real-time polymerase chain reaction.

Introduction

The primary step in planning an investigation of an outbreak is to establish a goal, and in the case of an infectious (arboviral) disease, this is to identify the causative agent. The entire investigation, planning, gathering of data and sample collection must be designed to minimise economic losses to the farmer on one hand but must not tarnish the reputation of the National Veterinary Authority on the other hand. This activity embraces a multitude of disciplines since it inherently depends on expertise in epidemiology, microbiology, pathology, entomology and meteorology (in the case of a suspected incursion of an arbovirus) among others.

The preparedness of a state system, such as a national veterinary authority and its field and diagnostic laboratories, depends very much on its expertise in mounting a campaign against a national epidemic.

A methodical approach to the investigation of an outbreak is the best way to achieve the objectives of containment or eradication of an exotic disease, the control of an endemic disease and to prevent the reappearance of an eradicated disease. This approach is further justified if the causative agent is an Office International des Épizooties (OIE) List B disease and even more importantly, if it is an OIE List A disease.

The rationale behind the methodology (Table I) employed to investigate an emerging disease (Fig. 1) is fundamentally different from that applied to an endemic disease (Fig. 2). Special consideration must also be given to pathogenic agents that appear and reappear at cyclical intervals (Fig. 3).

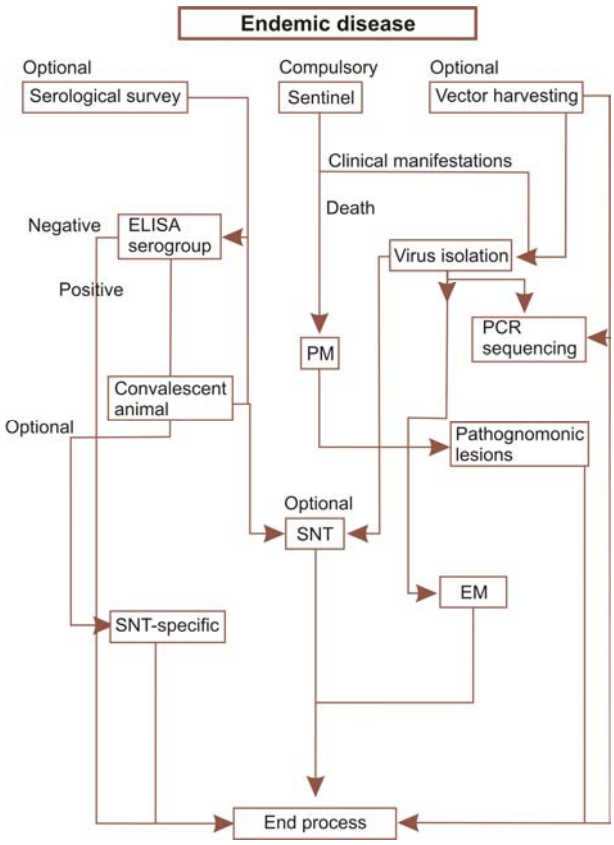
Akabane virus (AKAV) is known to cause outbreaks of abortions, stillbirths and foetal abnormalities in cattle and is designated as the congenital arthrogryposis-hydranencephaly syndrome of the musculo-skeletal and nervous systems (4). AKAV was isolated originally from mosquitoes and later

from the midge *Culicoides imicola*. *Culicoides* is now considered to be the major vector (3). AKAV is in the Simbu serogroup of the family *Bunyaviridae* (3).

Table I
Awareness and system preparedness and the types of intervention according to the epidemiological model

Awareness/ preparedness	Epidemiological presentation		
	Endemic zone	Emerging disease (epidemic zone)	Transitional (cyclic intervals)
Systems awareness	High	Low (if any)	Moderate
Intellectual and personnel preparedness	High	Low (if any)	Moderate
Laboratory and diagnostic means/ preparedness	High	Low (if any)	Moderate
Specific intervention	Almost not needed	Highly needed	Highly needed
Expected time interval between the onset and definitive diagnosis	Very short	Very long	Intermediate
Type of intervention	None	Preferably, eradication and a vaccination belt	Vaccination is weighted (cost-effective decision)

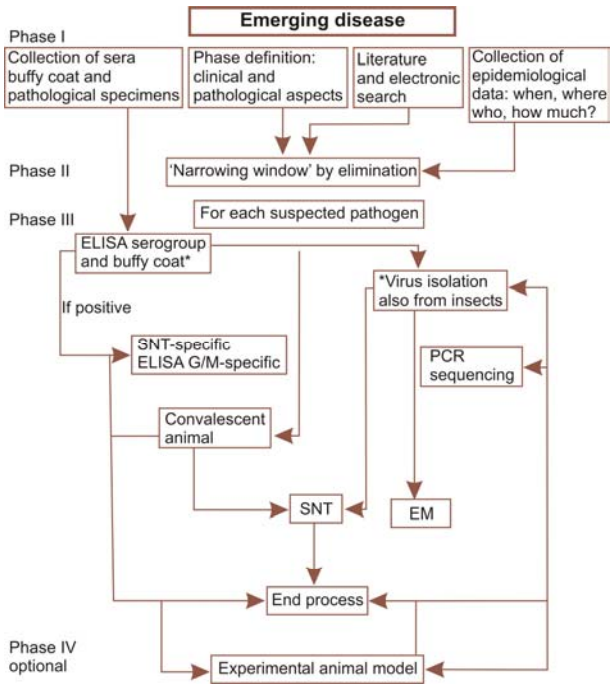
In Israel, the awareness and therefore the preparedness regarding the possible reappearance of AKAV, or another virus belonging to the Simbu serogroup, can be considered as 'dormant' since the last documented outbreak dates back to 1970 (2, 6, 7, 8, 10). Most of the investigators, veterinary practitioners, cattle and sheep breeders and other personnel who were involved in the AKAV epidemic of 1969/1970 have retired. It is therefore not surprising that the new generation of practitioners did not recognise the aetiology of the outbreak of malformed calves in 2002-2003; the blind calf syndrome (BCS) was the principal presenting feature (1). The main reason for the initial misdiagnosis was related to the unfamiliarity of most of the younger veterinary clinicians with the clinical manifestations of teratogenic arboviral infections. This was also true of the diagnostic laboratory personnel; nevertheless almost all of them were aware of the earlier epidemic. The re-establishment of the diagnostic chain from the farm to diagnostic laboratory was based on this experience and therefore took a little time. The identification of the causative agent in the new outbreak was based on the establishment of serological assays after their internal and external evaluations (1). Serology was employed to monitor the current epidemic together with the introduction of nested and real-time polymerase chain reaction (PCR) for AKAV (9), Aino virus and the Simbu serogroup. PCR was used to detect virus in a batch of trapped insects together with the serum neutralisation test (SNT) and enzyme-linked immunosorbent assay (ELISA) (5, 9).



ELISA enzyme-linked immunosorbent assay
PM post mortem
PCR polymerase chain reaction
SNT serum neutralisation
EM electron microscopy

Figure 1
Proposed applications of diagnostic procedures giving the rationale behind the methodology employed to investigate different epidemiological situations with special reference to arboviral infections: emerging disease

In this study, we present three separate approaches applicable to each epidemiological situation (Figs 1, 2 and 3). Each of these can be applied initially to determine both the identity of the causal agent and the extent of the infection. Special consideration is also given to situations where the disease appears intermittently using the sentinel model (Fig. 4). Although this latter approach is expensive and time-consuming, it yields excellent and reliable results when applied correctly.



ELISA enzyme-linked immunosorbent assay
SNT serum neutralisation
PCR polymerase chain reaction
EM electron microscopy

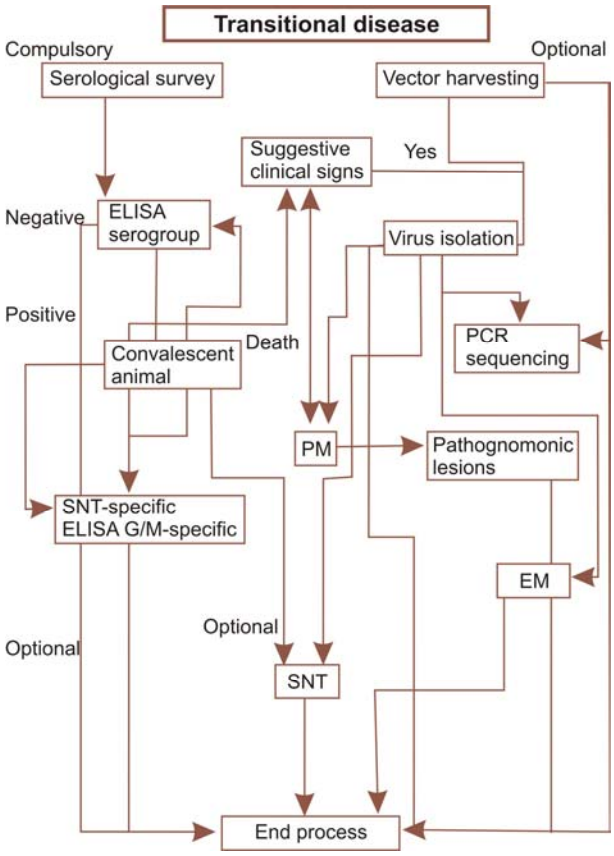
Figure 2
Proposed applications of diagnostic procedures giving the rationale behind the methodology employed to investigate different epidemiological situations with special reference to arboviral infections: endemic disease

Methodological background

Independently of the point of origin within the diagnostic chain, i.e. whether it starts with a serum sample or from diseased or dead animals or from the harvested insect vector, it always assumes the same direction (Figs 1, 2 and 3).

Serology

It is advisable that IgG/IgM-specific ELISA tests will follow group-specific ELISA screening on serum samples collected during and/or after the epidemic. The first recommended method is the group-specific ELISA test for investigating the different groups of the possible causative virus. Each serological test is directed at one group of viruses that shares the same group antigen (group cross-reactivity). After identification of the suspected 'group', it is recommended to pass to the second phase to focus on the causative agent responsible for the epidemic. For this purpose, monospecific and monoclonal antibodies are used to screen the causative agent amongst those sharing the same seroreactivity. The antibody that reacts strongest is probably directed against the causative agent of the epidemic. To obtain a definitive diagnosis, the SNT with a known pathogen or with the suspected pathogen that has been isolated from pathological samples should fulfil this aim. Convalescent sera are the best choice for the SNT. The use of precolostral serum sample taken from the affected offspring (or the aborted foetus), and a serum sample taken at the same time from the dam, is useful for providing serological evidence of *in utero* infection by viruses belonging to the Simbu serogroup (4, 10).



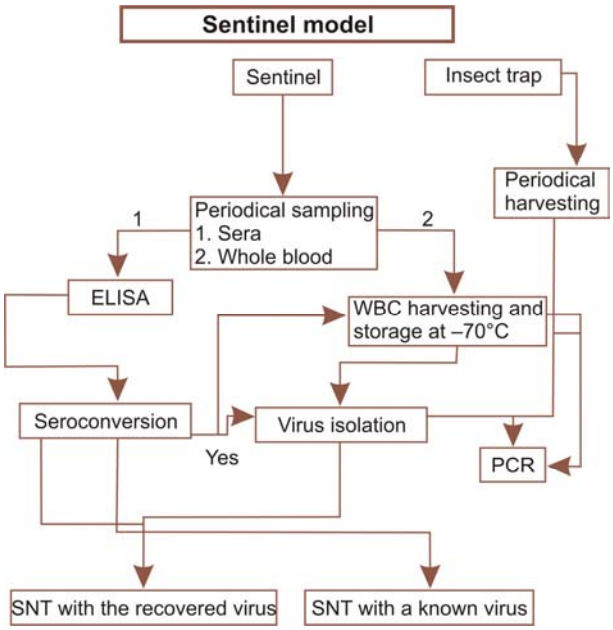
ELISA enzyme-linked immunosorbent assay
PCR polymerase chain reaction
PM post mortem
SNT serum neutralisation
EM electron microscopy

Figure 3
Proposed applications of diagnostic procedures giving the rationale behind the methodology employed to investigate different epidemiological situations with special reference to arboviral infections: transitional cyclic disease

Vector harvesting

Two informative tests can be performed with harvested insects: To save time and resources, PCR is the method of choice (assuming there is a panel of primers available), whereas virus isolation would take much more time. Both tests, when supplemented with virus genome sequencing, will provide valuable molecular epidemiological data. The isolated virus can be used in the SNT with the serum samples collected from convalescent animals during the

ongoing outbreak. This SNT will definitely confirm the identity of the causative agent and therefore is of high priority.



ELISA enzyme-linked immunosorbent assay
PCR polymerase chain reaction
SNT serum neutralisation

Figure 4
Proposed applications of diagnostic procedures giving the rationale behind the methodology employed to investigate different epidemiological situations with special reference to arboviral infections: sentinel model

Animals

Animals are the best source of serum (specific antibodies) and antigens (causative agents). Moreover, the diseased animal itself presents a spectrum of clinical signs, some of which are pathognomonic and are of incalculable diagnostic value.

The pristine sentinels are the first to be infected by the causative agent, and if infected demonstrate characteristic clinical signs. They may develop a high virus titre during the viraemic phase and produce specific IgM (primary) and IgG (secondary) antibodies if the animals enter the convalescent phase. When the sentinel and other animals die, their organs are optimal sources of antigen. The gross and histopathological lesions observed provide excellent clinical-pathological aids that help the investigator identify the causative virus.

Epidemiological data

While this is not within the scope of this presentation, epidemiological tools are essential in investigating any disease and in particular an emerging disease. We would like to mention the most important features of collecting data:

Firstly, it is crucial to determine the frequency. Thereafter, the investigator will answer 'the three W's' – when (season, year), where (the geographic and topographical area affected) and which (description of the population affected).

The discovery of even one (exotic) case might be considered as an outbreak, such as the discovery for the first time of a single case of an OIE List A disease in a previously unaffected area.

Sentinel model

There are two main types of sentinels. The first is an animal that is left unvaccinated while all others in the herd or flock are immunised routinely. For this purpose, a sentinel must be pristine and susceptible to the pathogen to be monitored, and be old enough to have no specific maternal antibodies. Sentinel animals provide valuable epidemiological data, such as whether and when the causative agent is present and whether the agent alternates its activity with periods of quiescence and the interval between these periods. Sentinels might also be a source of viable pathogens that can be used for antigenic typing and genome sequencing. This information is crucial for evaluating possible antigenic changes, which might lead to potential vaccination failure due to changes in antigenicity. Sentinels can be monitored periodically for eventual seroconversion with IgM-specific ELISA that can detect a recent infection. Sentinels scattered in different geographic or topographical areas would provide data about the extent of the infected area and the duration of each attack. Cohort animals are the second type of sentinel. They are suitable for such purposes from the time when the maternal antibodies disappear to the time of seroconversion. By testing these animals periodically it is possible to obtain information similar to that described above. In order to 'capture' the pathogenic agent, we must rely on good fortune. If bleeding is performed during the acute phase of the disease there is a good possibility of detecting viraemia. It is recommended to take blood with an anticoagulant and attempt to isolate the virus from the blood cells or perform the PCR on the cells. There is a good chance that the blood cells collected close to the seroconversion event will contain the causative agent.

During the epidemic of AKAV infection in 2002/2003, the Israeli Veterinary Services (IVS) employed most of the diagnostic tools shown in the flowchart; the chart depicts how to reach a conclusion in the case of cyclical reappearance of a disease (Fig. 3). The IVS gave a definitive diagnostic

answer as to which arbovirus of the Simbu serogroup was responsible for the epidemic within a relatively short time (1).

We succeeded in working with diagnostic tools that had been dormant since the last epidemic and succeeded in detecting the causative agent, defining its geographical boundaries and its spread from the primary focus to other geographic areas. Another achievement was the use of PCR (nested and real-time), to study the molecular properties of AKAV (9). To achieve all these goals we adopted the algorithm of a transitional zone where a disease reappears cyclically (Fig. 3).

The charts presented here might aid investigators in their pursuit of a causative agent in various epidemiological situations.

References

1. Brenner J., Tsuda T., Yadin H. & Kato T. (2004). – Serological evidence of Akabane virus infection in northern Israel in 2001. *J. Vet. Med. Sci.*, **66** (4), 441-443.
2. Kalmar E., Peleg B.A. & Savir D. (1975). – Arthrogryposis-hydranencephaly syndrome in newborn cattle, sheep and goats serological survey for antibodies against Akabane virus. *Refuah Vet.*, **32**, 47-54.
3. Kinney R.M. & Calisher C.H. (1981.). – Antigenic relationship among Simbu serogroup (*Bunyaviridae*) viruses. *Am. J. Trop. Med. Hyg.*, **130**, 1307-1318.
4. Kurogi H., Inaba Y., Takahashi E., Sato K., Satoda K., Goto, Y., Omori T. & Matumoto M. (1977). – Congenital abnormalities in newborn calves after inoculation of pregnant cows with Akabane virus. *Infec. Imm.*, **17**, 338-343.
5. Liao Y.K., Lu Y.S. Goto Y. & Inaba Y. (1996). – The isolation of Akabane virus (Iriki) from calves in Taiwan. *J. Basic Microbiol.*, **1**, 33-39.
6. Markusfeld-Nir O. & Mayer E. (1971). – An arthrogryposis/hydranencephaly syndrome in calves in Israel, 1969/70: epidemiological and clinical aspects. *Refuah Vet.*, **28**, 51-61.
7. Nobel T.A., Klopfer-Orgad U. & Neumann F. (1971). – Pathology of an arthrogryposis-hydranencephaly syndrome in domestic ruminants in Israel, 1969/70. *Refuah Vet.*, **28**, 144-151.
8. Shimshony A. (1980). – An epizootic Akabane disease in bovines, ovines and caprines in Israel, 1969-70: epidemiological assessment. *Acta Morphol. Acad. Sci. Hung.*, **28**, 197-199.
9. Stram Y., Kuznetzova L., Guini M., Rogel A., Meirom R., Chai D., Yadin H. & Brenner J. (2004). – Detection and quantitation of Akabane and Aino viruses by multiplex real-time reverse-transcriptase PCR. *J. Virol. Methods*, **116** (2), 147-154.
10. Trainin Z. (1971). – Active production of immunoglobulins in calf embryos. *Isr. J. Med. Sci.*, **7**, 627-628.