relating to the development of virus-shedding inapparent infections or those resulting in infertility are among other factors relevant to maintenance of virus in an environment and the relative importance of a virus. A virus which has strong stability to heat, in a wide pH range, is readily released from host cells, can invade mucosal cells as well as lymphoid aggregates, and which does not cause death of very many host animals is likely to become a widespread infection under most circumstances. Furthermore, if latent infections with intermittent viral shedding states are a part of the circumstances as they are in many herpesvirus infections, the disease will very likely have great durability. The management of large numbers of animals together in a relatively small space tends to favor the maintenance of respiratory and enteric viral infections. The most important viral diseases of dairy cattle seem to reflect this observation.

References

Bovine Lymphosarcoma

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Introduction

Bovine lymphosarcoma (leukosis, malignant lymphoma) is a malignant neoplastic disease of the lymphoid tissues of cattle. The condition is sometimes referred to as bovine leukemia, but this term is somewhat inappropriate, because many of the affected animals do not have an elevated circulating white blood cell count; so leukemia is not part of the disease. Lymphosarcoma is one of the most frequently diagnosed tumors of cattle, resulting in condensation of 14 carcasses per 100,000 cattle slaughtered in federally inspected plants in 1967 (27).

Age

Age is an important factor in development of the disease, as most tumors are diagnosed among cattle more than 3 years old (16, 22). In contrast, young calves account for a disproportionate share of the tumors among immature cattle. Because of the uniformity of lesions in these calves, the lower incidence, and the random distribution, it has been suggested that the juvenile form of lymphosarcoma represents a distinct disease entity (11, 22, 23, 25).

The adult form of the disease, with which we are concerned in this discussion, is perhaps more frequently diagnosed among dairy cattle because of usual husbandry practices. Both beef and dairy cattle are affected, however, and there is no evidence of specific breed susceptibility to lymphosarcoma. The lymph nodes are most commonly involved in the neoplastic process, but the heart, kidneys, spleen, and uterus are also frequently invaded by neoplastic tissue (3, 22). The superficial lymph nodes are often enlarged and can be observed as subcutaneous masses or readily palpated (Fig. 1). Involvement of orbital tissues sometimes causes exophthalmos (Fig. 2). Tumors consist of either discrete masses of solid neoplastic tissue or diffuse infiltrations of lymphoid cells among normal tissue structures of nonlymphoid organs.

Clinical Evidence

The clinical features of the disease vary according to the sites of localization, rate of tumor growth, and dissemination of the neoplastic process. Affected cattle are usually sick for several weeks or months; they become progressively weaker and more emaciated and death invariably follows (16). This typical course, however, varies considerably and cattle in apparently good health have died suddenly from lymphoid neoplasms in the heart (22). Clinical signs suggestive of circulatory, digestive, respiratory, reproductive, urinary, or other disorders may occur because of invasion of specific organs by tumor tissue and subsequent interference with normal functions.
clustering of cases within certain high-incidence lymphosarcoma groups, however, may sometimes result in significant losses within a given herd. An important economic factor is the possible loss of export markets for animals and animal products, if the current programs to eliminate lymphosarcoma from cattle in European countries are successful (1, 21).

**Causes**

The cause or causes of bovine lymphosarcoma remain obscure. However, inclusion of this discussion in a symposium on viral diseases of cattle indicates contemporary views. This viral causation hypothesis is not without sound basis. Since 1908 (7), it has been recognized that lymphoid tumors of chickens were caused by viral agents and in 1951 (8) viruses were identified as the cause of mouse leukemia. These discoveries have been important in stimulating interest and investigations into the role of viruses in lymphoid malignancies of other animals and man. More recent success in finding viral agents associated with leukemia of cats and guinea pigs has provided added impetus for viral studies (12, 19).

**Transmissibility**

Epidemiologic observations have provided some of the best evidence that a transmissible agent is involved in lymphosarcoma. It had long been recognized that bovine lymphosarcoma is not randomly distributed among the cattle population but is frequently aggregated in multiple-incidence herds. In some herds, the disease developed in the descendants of certain cows while in others the disease appeared most frequently in the offspring of a given sire. It was, of course, recognized that genetic factors could explain many of these observations, but it was also suggested that these clusters of bovine leukemia cases within cow families or sire groups could result from the vertical transmission of an infectious agent, perhaps to the fetus in utero or to the newborn calf by way of the milk (2). In other studies (13), the disease appeared among animals born during a specific time period, and it was suggested that the horizontal transmission of an infectious agent among a group of newborn calves could account for these neonatal cluster groups. Possibly the infection of fetuses or neonatal animals, which are not immunologically competent, provides circumstances in which foreign antigenic components of viruses or tumor cells are not recognized and suppressed and, therefore, favors the development of the disease.
Evidence of the transmissibility of lymphosarcoma was presented in a report from Sweden (18) concerning the use of a whole blood product, prepared from calves, for the prevention of piroplasmosis. Inoculation of this material was followed by the occurrence of a number of cases of lymphosarcoma among cattle in an isolated area, which had previously been free of the disease. It was suggested that the calves used in the preparation of the immunizing material were infected with a viral agent which caused lymphosarcoma in the inoculated animals.

In spite of the available evidence which indicates the presence of a transmissible agent in lymphosarcoma, results of attempts to transmit the disease experimentally to calves have been disappointing. Only a few animals have developed lymphosarcoma after experimental inoculation and the long (2- to 14-yr) incubation periods have made it difficult to prove the relationship between the inoculation and development of the disease (10, 20, 24).

Attempts to visualize virus-like particles in tissues of lymphosarcomatous animals have met with some success. Particles resembling the known leukemia viruses of other species have been demonstrated by electron microscopy in the milk (5) and tissues (4, 22) of both normal and lymphosarcoma-affected animals. This occurrence of virus-like particles in the tissues of apparently normal animals is not difficult to explain if a situation exists, as has been suggested (9), in which the virus is widely distributed among the cattle population, but infection only infrequently results in development of malignant neoplasms. Unfortunately, no biological activity has been observed when particles demonstrated by electron microscopy were inoculated into cell cultures or experimental animals; therefore, their true significance in the disease remains unknown.

Virus-like particles have also been seen in cell cultures prepared from tissues of lymphosarcomatous cattle (17, 26). The formation of syncytia, abnormal nuclei, and resistance of cells to superinfection with vesicular stomatitis virus also suggest that viruses may have been present in some of these cultures (6, 26). Attempts to pass serially these viral agents or to transmit the infection to other cell types have usually failed.

Isolation

In our laboratory (14), we have isolated a bovine syncytial virus from normal and lymphosarcomatous cattle. The virus seems to be closely associated with the leukocytes of infected cattle and has been isolated from both blood and milk. It can be serially propagated in cell cultures and transferred to various cell systems. Infection of bovine embryonic spleen cell cultures causes the formation of large syncytial masses in otherwise normal cell sheets (Fig. 3). Infectivity seems to be cell-associated and only small quantities of infectious material are present in the supernatant fluids from infected cell cultures.

Electron micrographs of infected tissue culture cells reveal virus particles approximately 100 to 200 mμ in diameter which have a central spherical nucleoid 30 to 40 mμ in diameter. The virus particles seem to mature at the cell surface by budding in a manner similar to that observed with leukemia viruses (Fig. 4). The size, structure, and method of formation of the nucleoid seem to differ from leukemia viruses, however. The surface projections or spikes would suggest a possible similarity to mouse mammary tumor virus or cat leukemia virus.

Infected cattle carry the virus for a long time, in spite of an antibody response. The antibodies produced by carrier animals were conjugated with fluorescein isothiocyanate and used to stain infected cells. Antigens associated with viral infection were present both in the cytoplasm and in the nuclei of syncytia.
FIG. 4. Electron micrograph of a bovine embryonic spleen cell culture infected with bovine syncytial virus. Virus particles with surface projections are budding from the cell membrane; × 88,000.

(Fig. 5). Immunofluorescent tests with this conjugate, and bovine viral diarrhea and para-influenza-3 viruses and conjugates, have shown that this bovine syncytial virus is not antigenically related to either of these two agents which are widely distributed among cattle.

Distribution

An agar gel diffusion test has been devised with antigen prepared from cell cultures infected with bovine syncytial virus. By this test the virus seems to be widely distributed in the cattle population among both multiple-incidence lymphosarcoma herds and apparently normal cattle (Table 1). Some lymphosarcoma-affected cattle seem never to have had contact with the virus; consequently, its etiologic significance must be questioned. Calves can be readily infected with the virus and become carriers for long periods, but no evidence of development of lymphosarcoma has been detected. Much additional research will be required to elucidate what role, if any, this virus has in bovine lymphosarcoma. Meanwhile, it can only be regarded as a previously undescribed agent which is widely distributed in the cattle population and has some structural similarities to known oncopgenic viruses.

Milk

Quite apart from its role in bovine lymphosarcoma, the demonstration of a virus of this type in milk has aroused some interest in the heat stability of the agent. Preliminary studies indicate that infectivity is lost when infectious cell culture fluids are exposed to 56 C for 20 minutes. The virus would, therefore, seem quite heat sensitive and is probably rapidly in-

Table 1. Results of immunodiffusion tests of bovine sera against bovine syncytial virus antigen.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number tested</th>
<th>Number positive</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphosarcomatous cattle</td>
<td>37</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Cattle from multiple-incidence</td>
<td>160</td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td>lymphosarcoma herds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparently normal cattle</td>
<td>390</td>
<td>95</td>
<td>24</td>
</tr>
</tbody>
</table>
activated at pasteurization temperatures. Further studies of infected leukocytes suspended in milk are in progress to gain additional information regarding heat stability.

Lymphosarcoma control and eradication programs are in effect in Denmark and Germany. They are based on the herd history of clinical lymphosarcoma cases and the use of leukocytes to detect infected cattle. There is no general agreement (15) regarding the association of persistent lymphocytosis and lymphosarcoma of cattle in the United States, and no control or eradication programs exist.

Conclusions

In conclusion, it is recognized that research on bovine lymphosarcoma is still in its early stages. There is evidence supporting the viral causation hypothesis, but no agent has been isolated which will reproduce the disease when inoculated into calves. Additional studies of immunologic and hereditary factors are needed, for they may play key roles in the development of the disease. The clarification of the interaction of these viral, immunologic, and genetic factors would yield information of benefit not only to the livestock industry but would provide clearer insights regarding similar diseases of other animal species and man.

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Bovine Virus Diarrhea: Infectious Bovine Rhinotracheitis Complex

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Bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) are two “new” diseases appearing within the last 25 years (6,10,12). There is serologic and clinical evidence that they may be considerably older in other forms or infecting other species (7,12). Bovine virus diarrhea has also been called mucosal disease, but this is the more severe form and the use of two names only confuses the issue. Infectious bovine rhinotracheitis has also been called infectious pustular vulvovaginitis (IPV) or coital vesicular exanthema when the vulvovaginitis form of the disease occurred, and the respiratory form is often called “red-nose.” The term IBR seems to be solidly established, even though the term coital vesicular exanthema is much older and probably was the first form of the disease (7).

As the names suggest, BVD virus was first observed as a pathogen of the digestive tract, and IBR virus was most often observed as an infectious agent of the upper respiratory tract. These respective manifestations of disease are probably still the most common forms observed, but BVD virus can be involved in disease of the respiratory tract and IBR virus has been isolated from the alimentary tract of sick cattle (2). It is easy to see that the clinical disease involving one or occasionally both of these viruses can be similar to the disease caused by either virus alone in some instances. This is why there is a tendency to speak of the BVD-IBR complex. It is these virus infections that resemble each other and may also be confused with “shipping fever” which cause the greatest diagnostic problems. Both IBR and BVD virus infections can resemble shipping fever, since both can be involved in respiratory disease either alone or with other pathogens. In addition, similarly to shipping fever both IBR and BVD may occur after exposure to cattle from other sources. Bovine virus diarrhea and IBR viruses are not related to each other and in many infections will not create any confusion. Bovine virus diarrhea is related to hog cholera virus and IBR virus is related to many viruses, including equine rhinopneumonitis virus and Herpesvirus hominis, or the cold-sore virus of man. Serologic findings indicate that BVD virus can also infect

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