Antiviral chemotherapy in veterinary medicine: current applications and perspectives

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Summary

The current situation in the use of antiviral drugs in veterinary medicine is characterised by a novel and optimistic approach. Viruses of veterinary importance are still used as animal models in the development of human therapeutics, but there is growing interest in many of these viruses in the identification of antiviral molecules for use in both livestock and companion animals. The use of antiviral drugs in livestock animals is envisaged for the treatment or control of disease on a large scale (mass treatment), whereas in companion animals an individual approach is favoured. An overview of the most recent examples of research in the use of antivirals in veterinary medicine is presented, with particular emphasis on their *in vivo* applications.

Keywords

Animal model – Antiviral therapy – Chemotherapy – Companion animals – Disease control – Drugs – Immunomodulators – Livestock animals – Veterinary medicine.

Introduction

The use of antiviral drugs in human and veterinary medicine is limited in comparison with the use of antimicrobial agents. Viruses are obligate intracellular pathogens that utilise the biochemical machinery of the host cell during their replication. The most severe constraint in the development of antiviral drugs has been the identification of specific viral targets with increased selectivity and reduced side effects. However, in recent years a more rational approach has characterised the search for new antiviral drugs, thanks to a better understanding of the molecular replication machinery of viruses and use of computational methods for modelling protein structure, together with the use of RNA interference (RNAi) technology for sequence-specific inhibition of viral nucleic acids. Efforts are being made in numerous research networks to provide information on viral structural proteins, replication mechanisms and potential drug targets (1).

At present, only one antiviral compound has been licensed for use in veterinary medicine: feline interferon-omega (IFN-ω), which has a yet undefined mechanism of activity that most probably combines immunostimulatory activity with antiviral activity (2). Despite this, several antivirals licensed for use in human medicine are currently used with the cascade principle in therapy for animal diseases (3); for example, idoxuridine, trifluoridine and aciclovir in cats with feline herpesvirus 1 (FeHV-1) ocular infection (4) or zidovudine against feline immunodeficiency virus (FIV) (5). At the beginning of the 1990s, state-of-the-art reviews on the use and perspectives of antiviral chemotherapy in veterinary medicine (6, 7) cited several reasons for the low use of these agents in veterinary medicine:

– the high cost of development of new chemical compounds, particularly for use in food species

– use restricted to a single virus and a specific animal species

– difficulties encountered in development of broad-spectrum antivirals with low cytotoxicity

– absence of rapid diagnostic techniques allowing prompt use of a specific antiviral agent in the course of an acute infection (6, 7).

Regardless of these arguments, animal viruses were used as models in the development of antivirals for human medicine. Examples include bovine viral diarrhoea virus (BVDV), which is considered a valuable surrogate for hepatitis C virus in antiviral drug studies (8, 9), and cottontail rabbit papillomavirus, which has been used as an effective model for assessment of anti-papillomavirus activity, in particular in experiments for determining dosing schedules and treatment regimens *in vivo* (10). Although FIV infection in cats differs from human immunodeficiency virus in humans for the target lymphocyte cell population, this animal model has been used in exploratory studies to test the antiviral activity of selected compounds before their subsequent use in non-human primates (11).

Several factors have a role in the changing scenario characterised by more favourable conditions for use of antiviral drugs in veterinary medicine. The successful use of antiviral chemotherapy in some human viral diseases has increased confidence and awareness of the existence of efficient antiviral drugs that can also be used in veterinary medicine. In addition, veterinary internal medicine has undergone refinement and progress with diagnostics and treatment, allowing the use of sophisticated and expensive protocols. The relationship between humans and their companion animals is often characterised by an emotional bond that justifies recourse to therapeutic means not previously envisaged. Furthermore, despite the efforts in development of efficient vaccines, control of important animal viral diseases by vaccination has limitations, caused for example by the high genetic and antigenic variability of the virus. Culling susceptible animals is

policy in the World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code* and is recommended to prevent the spread of highly contagious diseases of livestock animals in cases of epidemics occurring in disease-free countries. However, measures to stamp out disease imply serious direct and indirect economic consequences related to the loss of valuable animals, loss of productivity, compensation for the owners of the animals and the costs of disposing of the animals and their carcasses. The impact on the environment and the emotional effects are further serious outcomes of this measure. The culling of millions of animals during the foot and mouth disease (FMD) outbreak in the United Kingdom in 2001 was the topic of heated debate, because of controversies around its effectiveness, the economic and ethical consequences, and the impact on public opinion (12). The use of efficient antivirals and other control measures such as vaccination has been proposed as an alternative approach to culling in control of highly contagious diseases of livestock (7, 13, 14).

In both the academic and the private sector, interest is growing in research on the use of antivirals in animal health. The following paragraphs present an overview of the most recent examples, with particular emphasis on *in vivo* research applications.

Viral infections of livestock animals

Foot and mouth disease virus

Foot and mouth disease virus (FMDV) is a highly contagious pathogen of cloven-hoofed mammals and one of the biggest concerns for veterinary authorities. The control measures to be used in an outbreak vary according to the disease-free or enzootic disease status of the affected area. Vaccination requires identification of the involved viral serotype and subtype, immunity is limited to six months and there is an immunity gap of four to seven days to trigger the immune response (15). The use of anti-FMD drugs has been discussed as an alternative or supplementary method to be used in previously FMD-free countries or zones (13). Use of such antiviral treatment in a vaccinated zone could protect against viral dissemination and fill the

time gap between vaccination and the development of protective immunity.

Apart from broad-spectrum antiviral agents such as ribavirin, specific anti-FMDV molecules have been identified *in vitro* (13, 16). Among them, the ribonucleoside analogue 2'-C-methylcytidine, able to interfere with intracellular viral RNA synthesis (13), was recently used successfully to protect severe combined immunodeficient mice from FMDV infection (17). Successful use has been reported of the pyrazinecarboxamide compound T-1105 mixed into the feed of pigs after challenge with FMDV (18): the treated animals did not show any clinical signs of the disease or viraemia, and did not excrete the virus via the nasal route, thus reducing the risk of transmission to susceptible animals (18). This preliminary *in vivo* result and the possibility of treating large numbers of animals in the feed suggest that this molecule may be a promising tool to control FMDV, especially in FMD-free countries. The viral target of T-1105 has been suggested as the RNA-dependent RNA polymerase (19), and further efforts should be made to clearly identify the mechanism of action. Large-scale experiments will be required before the development of T-1105 as an effective alternative control tool.

Immunomodulators such as IFN have been investigated for their anti-FMDV properties. Porcine type I IFN (po IFN-α) delivered by a replication-defective human adenovirus vector rapidly protected pigs against FMDV challenge 24 h post-inoculation (20). The pigs did not show clinical signs, viraemia was not detected and low levels of neutralising antibodies were found (20). However, treatment with po IFN-α delivered with the same adenovirus system did not induce the same protection in cattle, which displayed a delayed and mild form of disease after FMDV challenge (21). Nevertheless, immediate and prolonged protection against FMDV was obtained by combining the po IFN-α with an FMD subunit vaccine in both swine (22) and bovines (23). The benefit of this approach is the early onset of protection induced by the antiviral properties of the type I IFN and the long-lasting immunity obtained by the subunit vaccine. However, despite these encouraging results, this approach has limitations, such

as the high doses of treatment that are required, the intramuscular route of administration hampering the treatment of large numbers of animals, the absence of field trials in animals having concomitant infections and the inclusion of only one FMDV serotype in the vaccine. In a recent study, the efficacy of po IFN-α combined with FMD subunit vaccine against a challenge with different serotypes was proved, supporting the continuance of investigations on IFN as an anti-FMDV agent (24).

As an alternative anti-FMDV strategy, RNAi technology has been explored. Several viral proteins have been investigated *in vitro* as potential targets to be used with RNAi, although only a few experiments have been performed during clinical trials in swine or in animal models (25, 26, 27). In addition to the viral targets selected in the course of these experiments, RNAi has shown high specificity and selectivity, but applicability in the field is remote because of constraints around the use of genetically engineered materials for therapeutic or prophylactic use.

Classical swine fever virus

In the European Union and in non-European contexts, the presence of endemically infected wild boar or trade in infected animal products can lead to sporadic outbreaks of classical swine fever (CSF). The OIE policy on CSF is similar to that for FMD: the prophylactic vaccination of domestic pigs is banned in countries free of the disease and, instead, stamping-out measures (slaughter and burial or incineration of carcasses) are required to control disease outbreaks. Recourse to inhibitors of viral replication was recently proposed as an alternative or additional approach in controlling the disease. Various scenarios have been analysed in a model where CSF epidemics were simulated in an area of dense pig population (14) and the findings support the use of antiviral therapy in control of the infection.

Within a novel class of imidazopyridines, the compound 5-[(4-bromophenyl)methyl]-2-phenyl-5H-imidazo[4,5-c]-pyridine (BPIP), targeting the viral RNA-dependent RNA-polymerase NS5B (28), has been shown to have potent *in vitro* and *in vivo* activity towards CSF

virus (CSFV) (28, 29). The compound was formulated for oral administration in feed as a rapid way to deliver the drug to large numbers of animals. Outcomes of treatment were a significant reduction of viraemia, absence of viable virus in the tonsils of challenged pigs four weeks post-infection and absence of adverse effects in healthy animals after treatment for 15 consecutive days (29). The effect of BPIP treatment on transmission of the virus from infected to naïve pigs was subsequently tested (30), and reduced transmission was demonstrated in naïve pigs in contact with BPIP-treated animals compared with the positive control group, although a low and transient viraemia was detected (29). All together, these studies highlight the need for further experiments in larger numbers of animals in order to gain more significant data on the efficacy of BPIP treatment. Moreover, development of new imidazopyridine molecules could identify more potent inhibitors of this virus, resulting in greater reduction or complete suppression of viral transmission.

Following the recent examples of successful combination of immunostimulating cytokines such as type I IFN with subunit vaccines for rapid and long-lasting protection against FMDV, similar efforts have made for CSF. Indeed, the immunogenicity of a vaccine candidate based on E2-CSFV was increased by the co-administration, in an oil-based vehicle, of human recombinant type I IFN (31). This approach prevented the appearance of clinical signs and viraemia in pigs challenged seven days post-vaccination (31). The minimum interval of time required to confer full protection should be evaluated in order to use this co-formulation as an early strategy for control of CSFV.

Other viral infections of livestock

Bovine viral diarrhoea virus (BVDV) is of veterinary importance and the focus of research on identification of new antiviral agents. Nevertheless, clinical application of putative candidates and promising molecules for use in control of the disease remains remote. The virus is used *in vitro* as a surrogate model of human hepatitis C virus in antiviral assays (8, 9), as the two viruses have similarities in their

replication cycles, genetic organisation and function of their gene products. In comparison with other pestiviruses, BVDV offers many advantages for *in vitro* screening and characterisation of the mechanisms of action of selected antiviral compounds (8). However, despite the numerous efforts in the synthesis and further screening of anti-BVDV drugs (32) and the major problems caused by this virus in bovine production (33), there have been no clinical trials to develop an antiviral therapy suitable for treatment of acute infections and control of the disease in an infected herd.

Bluetongue virus is a potential target for development of antiviral drugs. The virus has multiple serotypes, there is no universal vaccine, and the direct and indirect economic consequences in the course of epizootics are dramatic. Control measures are limited to vaccination with serotype-specific vaccine, restrictions of animal movement, control of the vectors and animal culling (34). No *in vivo* antiviral studies on use of specific inhibitors of the virus are known, observations being limited to *in vitro* assays (35). However, the virus has been used in development of a high-throughput antiviral screening method (36), which could increase the library of compounds tested against this virus and select potential candidates for future *in vivo* applications. Some thiophene derivatives have been further characterised *in vitro* and showed potent and selective activity against the virus, with a proposed virostatic mechanism of action (37).

Acyclic nucleoside phosphonates have broad-spectrum antiviral activity against many DNA viruses. In particular, cidofovir is officially licensed for treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome (AIDS), but it also has proven activity against adenoviruses and pox, herpes and papilloma viruses (38). Cidofovir has been used in several *in vivo* trials against caprine herpesvirus 1, an alphaherpesvirus infecting goats which has several biological similarities to human herpesvirus 2 (HHV-2). Topical application of 1% cidofovir cream proved effective in reducing clinical signs and viral shedding in goats infected with the caprine virus (39) but not in preventing viral latency or viral recurrence, thus reducing interest in its possible application in control

of the disease in an infected herd (40). Nevertheless, these studies highlighted the use of the caprine virus as a model in evaluation of new therapeutic protocols for HHV-2, which causes (recurrent) genital herpes in humans (39, 40).

Orf virus is the causative agent of contagious ecthyma, a neglected zoonotic disease of sheep and goats but with severe economic consequences for animal production. After *in vitro* testing of the antiviral activity of several acyclic nucleoside phosphonates and prodrugs against orf virus (41, 42), cidofovir was selected as the best candidate for subsequent clinical trials. Topical administration of 1% cidofovir cream in lambs four days post-infection proved to be the best therapeutic protocol (43). To improve administration of the drug to large numbers of animals, an alternative formulation based on a cidofovir/sucralfate gel combination in spray was tested on infected lambs (44). In order to mimic field conditions, the therapeutic paint was sprayed onto lesions as soon as they were visible (lesions three to four days old). Treatment resulted in rapid resolution of the lesions, with scabs containing significantly lower amounts of viable virus compared with lesions treated with sucralfate alone (44). Such treatment would have therapeutic and prophylactic consequences, reducing both viral shedding in the environment and the possibility of re-infections.

Viral infections of companion animals

Feline herpesvirus 1

At present, the use of antivirals in veterinary medicine is mostly limited to treatment of cats with acute ocular disease caused by FeHV-1. Despite the availability of many antiviral agents for topical or systemic administration adapted from treatment of human herpesvirus 1 (HHV-1) ocular keratitis, none has been developed specifically for FeHV-1 infection in cats. Following initial *in vitro* investigations, clinical trials have defined the *in vivo* efficacy and toxicity of these drugs, highlighting remarkable differences in their pharmacodynamics and pharmacokinetics in cats and humans (45).

Aciclovir, ganciclovir and penciclovir are acyclic nucleoside analogues and, after three phosphorylation steps, act as competitive inhibitors of the normal substrates (deoxynucleoside triphosphates or dNTPs) by interaction with viral DNA polymerase (46). Aciclovir has lower *in vitro* antiviral activity against FeHV-1 than against HHV-1 (47, 48) and poor bioavailability after oral administration in cats (49). Nevertheless, frequent administration of 0.5% aciclovir ophthalmic ointment was shown effective in resolving ocular FeHV-1 lesions in cats (50). Valaciclovir, the prodrug of aciclovir and characterised by higher oral bioavailability in cats, caused fatal hepatic and renal necrosis, as well as bone marrow suppression associated with a lack of protection towards FeHV-1 infection (51).

Cidofovir is an acyclic nucleotide analogue that needs only two phosphorylation steps to reach the active metabolite stage and is active against a broad range of DNA viruses (46). Cidofovir, in addition to a high *in vitro* antiviral activity (47, 48), also showed potent *in vivo* activity when administered twice daily as a 0.5% ophthalmic solution in experimentally infected cats (52). However, its use in the field should be cautious, as data supporting its long-term safety as a topical agent in cats are insufficient at present.

Penciclovir has shown *in vitro* anti-FeHV-1 activity similar to that of cidofovir (47). An ophthalmic formulation of penciclovir is lacking, but the safety of its prodrug famciclovir in pharmacokinetic studies of oral administration in cats (53) supported use of the latter in a recent study in experimentally infected cats (54). After treatment with famciclovir, the cats showed no detectable adverse clinical effects, their body weight continued to increase and the total clinical score was significantly lower than in the placebo-treated group of cats. Nevertheless, despite a reduction in viral shedding, the corneal ulcers were not significantly reduced in the famciclovir-treated cats, implying that topical mucinomimetics and antibiotics should be used simultaneously (54).

Idoxuridine and trifluoridine are both toxic if given systemically, but because of their high *in vitro* anti-FeHV-1 activity they are

recommended for topical use in cats with ocular herpesvirus disease (4).

Next to the use of synthetic specific antiviral compounds, oral administration of the aminoacid L-lysine as a diet supplement has been proposed as a safe treatment in FeHV-1 infected cats, although the mechanism of action is not precisely defined. *In vitro*, cell culture media enriched with L-lysine and deprived of L-arginine induced reduction of FeHV-1 growth, suggesting a combined effect of the two aminoacids on viral replication (55). Contradictory *in vivo* results have shown a potential antiviral effect of L-lysine in primary and latent infections in some experimental conditions (56, 57), but in the course of natural infections a lack of activity and sometimes an increase in disease severity has been observed (58, 59). Although use of L-lysine does not represent a threat to animals, as it appears safe, its administration as an antiviral cannot be recommended, as evidence of its efficacy is uncertain.

In a recent clinical trial in cats with naturally acquired viral keratoconjunctivitis, topical administration of recombinant feline IFN-ω did not improve the outcome of the infection when compared with recombinant human IFN-α (60).

Feline retrovirus infections

In contrast to feline leukaemia virus (FeLV), no vaccine is currently available in Europe to protect cats against FIV. The inactivated vaccine available in the United States of America did not show any protection of cats during challenge with a virulent European strain (61). Although many drugs and immunomodulators have been reported active against both these retroviruses, only a few successful clinical trials have been reported (62). The first and most commonly used anti-retroviral in veterinary medicine is zidovudine, a nucleoside analogue that blocks viral reverse transcriptase. This antiviral can effectively inhibit FeLV and FIV *in vitro* and *in vivo*, thus reducing the virus load in plasma and improving immunological and clinical conditions. Reversible anaemia is the most common adverse effect and requires reduction of the dose or interruption of treatment (63).

Fozivudine, a thioether lipid conjugate of zidovudine with a lower toxic potential than its parent compound, has been tested recently in a preliminary *in vivo* experiment in treatment of acute FIV and showed ability to reduce plasma viraemia and maintain unaltered haematocrit parameters (64). Further clinical trials and pharmacokinetic studies are necessary before fozivudine can be used in routine clinical practice.

The use of type I IFN in FeLV and FIV infections is controversial because, although the results of some studies have been encouraging, others have failed to confirm previous observations (65, 66). However, feline IFN-ω was recently licensed for veterinary use in Europe and Japan (5, 67). In a controlled study in symptomatic cats with FeLV infection or FeLV/FIV co-infection (68), a moderate positive effect was observed in IFN-treated cats, where milder clinical scores and higher survival rates were measured. No virological parameters, such as viraemia or virus shedding, were monitored (68).

Canine viral infections

The treatment of ocular lesions associated with canine herpesvirus 1 infection with topical idoxuridine and trifluoridine has been reported (69) but, as for the feline herpesvirus, no specific antiviral agents have been developed.

Canine parvovirus type 2 (CPV-2) causes a lethal infectious enteritis of dogs, with the highest susceptibility in puppies during the weeks between the decline of maternal antibodies and development of active immunity following vaccination. Symptomatic treatment includes administration of intravenous fluids, anti-emetics, antibiotics and analgesics; normally no specific antiviral therapy is used. The therapeutic potential of feline IFN-ω against CPV-2 has been assessed under experimental (70) and field conditions (71) and has demonstrated the possibility of reducing the clinical severity of the disease and numbers of deaths in comparison with dogs treated for symptoms only. Unfortunately, these two studies did not investigate the potential of IFN-ω treatment in reducing viral shedding, which would undoubtedly be relevant in preventing re-infection and

environmental contamination in infected kennels. In Europe, feline IFN-ω is licensed for use in dogs with CPV-2 clinical infection.

Equine herpesvirus 1

Equine herpesvirus 1 is a widespread alphaherpesvirus of horses and causes abortion and myeloencephalopathy. Vaccination is poorly protective against abortion and not effective against myeloencephalopathy. The virus is an attractive target for antiviral chemotherapy because of the animal health and economic consequences of outbreaks affecting race competitions and in the context of horse breeding.

The activity of aciclovir against this virus has been tested *in vitro*, followed by *in vivo* pharmacokinetic experiments after intravenous administration in horses (72). However, repeated daily intravenous administration of aciclovir is not a treatment of choice in horses. In order to simplify administration of this drug, the pharmacokinetics of valaciclovir, the oral prodrug of aciclovir, have been studied in order to determine the dose that ensures adequate plasma concentrations of aciclovir (73). Valaciclovir was subsequently used in ponies experimentally infected with the virus, but despite the high plasma and mucosal levels of aciclovir, no effect was observed on development of clinical signs, viral shedding or levels of viraemia (74). Although this initial study was unsuccessful, this virus infection in horses is an ideal candidate and a current challenge for identification of new antiviral compounds.

Influenza A virus infection in animals

Influenza A virus is a pathogen of livestock (swine, poultry) and companion animals (horses, dogs, cats). Although there is potential to treat influenza A virus infection in affected animals, the zoonotic character of this virus hampers the use of antivirals. Indeed, extensive use of anti-influenza molecules against the infection in swine and poultry could promote the emergence of resistant strains and impair the use of these drugs in humans. In pig and poultry farms, influenza infections should be controlled with prophylactic measures such as

vaccination, biosecurity, hygiene and reduction of contact between domestic and wild animal populations. However, the use of anti-influenza drugs could be envisaged in the treatment of protected species such as tigers (75).

Animal models are extensively used for demonstrating the efficacy of antiviral drugs against influenza virus. Despite the absence of clinical signs following infection with seasonal influenza, mice are frequently used as a first animal model to test the efficacy of new anti-influenza candidates (76). Isolates of highly pathogenic H5N1 virus were found to be lethal in mice and were used in assays on the efficacy of molecules such as oseltamivir *in vivo* (77). Cotton rats, guinea pigs and especially ferrets are used for more advanced studies (78). Ferrets develop clinical signs very similar to those in human infection, but the reduced availability of reagents for this species in comparison with mice, together with the higher cost of experiments, hamper their more extensive use *in vivo* (76). As an example study, ferrets have been used to test the efficacy of oseltamivir, zanamivir and other developmental molecules against susceptible and resistant influenza A virus strains (79).

Discussion

The availability of potent and specific antiviral drugs for use in veterinary medicine presents interesting perspectives for both companion and livestock animals.

Feline IFN-ω is currently licensed in Europe for treatment of FIV and FeLV infections in cats and CPV infections in dogs, and its efficacy has also been tested against feline calicivirus infections in clinical studies (80). However, the extensive use of feline IFN-ω in treatment of other diseases not covered by the licence should be cautious and await evidence from further clinical trials. At present, apart from treatment of feline retrovirus and CPV infections with feline IFN-ω, use of antiviral therapy in veterinary practice mostly concerns the treatment of FeHV-1 keratitis with molecules developed in research in humans. Nonetheless, the current situation, which is characterised by the convergence of numerous research efforts on the development of

new and specific molecules for use in veterinary medicine, can prepare the field for more rationale use of antivirals in companion and livestock animals in the future. Furthermore, many of the highlighted constraints (6, 7) have been overcome, and great advances have been made in diagnosis of diseases and in understanding the machinery of viral replication. However, the selection of drug-resistant variants has to be considered an emerging threat in the use of antivirals. This problem has been already observed in human medicine for RNA and DNA viruses, and it represents the major obstacle to use of antiviral therapy against the influenza virus in animal populations. Recently, intra-host variability of the gene for protein NS5B of CSFV was shown to increase when antiviral pressure was applied *in vitro* (81). The potential emergence of resistant viruses needs to be considered during evaluation of antiviral therapy for future veterinary medicine.

Antiviral therapy for companion animals can be envisaged for treatment of infected individual animals in order to improve their health and quality of life. For infected animals in kennels or cat shelters, treatment could be for both therapeutic and prophylactic purposes by reducing viral shedding and consequently the potential infection of susceptible animals.

In livestock animals, potential applications of antiviral drugs have been the subject of several considerations, especially for highly contagious and economically devastating diseases such as FMD and CSF (13, 29, 30). In particular, strategic use of antivirals in areas free of these two diseases has been discussed, with the aim of treating infected animals and reducing viral shedding, and consequently protecting other susceptible groups of animals. This strategy has been envisaged either alone or in association with the use of vaccines stimulating long-lasting immunity during the first days of disease emergence. The main efforts in development of an efficient antiviral strategy in livestock animals have been towards highly pathogenic RNA viruses, which are characterised by genetic variability and are more difficult to control. Nevertheless, there are some examples of DNA virus infections in livestock animals that show promise in the future use of antivirals.

The treatment of livestock animals requires easy administration of drugs, such as with oral preparations, and also requires relatively inexpensive drugs, as there is a need to treat large numbers of animals. The use of antiviral drugs in livestock animals also requires specific regulation on residues in animal products and in meat.

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