

AN ABSTRACT OF THE THESIS OF

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Serums from 270 llamas representing 21 farms throughout Oregon were obtained and assayed for antibody levels against viruses of livestock. These viral diseases included: bovine viral diarrhea (BVD), bovine herpesvirus 1 (BHV-1), parainfluenza-3 (PI-3), bovine respiratory syncytial virus (BRSV), bovine adenovirus species 3 (BA3), equine herpesvirus 1 (EHV-1), equine adenovirus (EA), equine influenza, subtypes 1 and 2 (EI-1, EI-2), equine viral arteritis (EVA), ovine progressive pneumonia (OPP), bluetongue (BT), vesicular stomatitis, New Jersey strain and Indiana strain (VSV-NJ, VSV-IN), and llama adenovirus strain 7649 (LA7649). Antibodies to *Ehrlichia risticii* (ER), the rickettsial organism causing Potomac horse fever (PHF), were also assayed.

Of the 270 llamas, 252 had antibodies to LA7649. A total of 60 llamas possessed antibodies to various viruses associated with livestock disease. Seven of these llamas had antibodies to more than one virus (excluding LA7649). Forty three exhibited antibodies to EA, 12 to BVD, and 12 to PI-3. Four had antibodies to BTV, 2 to BHV-1, and 2 to EI-1. One had antibodies to EI-2, one to EHV-1, and one to BRSV. All 270 llamas lacked antibodies to EVA, BA3, VSV-NJ, VSV-IN, OPP and ER.

Presence and type of livestock were noted on each farm. Whether a llama was born on the farm or purchased and the length of time the llama had been on the farm was also noted.

Serologic Survey of Llamas in Oregon
for Antibodies to Viral Diseases of Livestock

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SEROLOGIC SURVEY OF LLAMAS IN OREGON FOR ANTIBODIES TO VIRAL DISEASES OF LIVESTOCK

INTRODUCTION

Llamas and alpacas have become increasingly popular as companion animals and can serve to carry packs on the hiking trail, pull carts, and even carry golf club bags. The wool is a valuable commodity, especially from alpacas. Another recent function for llamas is use as guardians for flocks of sheep, because the llamas readily chase predators away from the pasture.

The fossil record indicates that the ancestors of the camelids (llamas, camels, alpacas) originated in North America 40-50 million years ago, during the Eocene epoch.¹ It is believed that, when the Asia-Alaska land bridge existed during the Pleistocene epoch, some of the camelid predecessors migrated to Asia and developed into our modern day Old World Camels. Others migrated to South America and evolved into the South American Camelids, i.e., llamas, alpacas, guanacos and vicunas. For unknown reasons, the early North American camelids became extinct.²

While the vicunas and guanacos remain as wild populations, the alpacas and llamas continue in their domesticated roles. The Andean people of 4000 B.C. decided to make use of these high mountain dwellers and the llama and alpaca soon became valued for wool, meat, packing, and fuel (dried dung).³ Pure white llamas were also used as sacrifices in religious ceremonies.²

A few lamoids were exported to North American zoos in the early 1900's. Exportation was stopped in the 1930's by the Andean countries, due to concerns that other countries would exploit the lamoids. The ban was lifted in the 1980's. Llamas and alpacas were imported from Chile after it was declared free of foot-and-mouth disease in 1984.²

Today's U.S. population of llamas (50,000 to 60,000) and alpacas (2,500 to 4000) slowly grew mainly from the early exports.⁴ Oregon's population of llamas is 15 to 20% of the U.S. population, according to current International Llama Registry records.

The taxonomic relationship of a species to other species will sometimes provide clues as to its physiology and disease susceptibility. Viruses are usually species specific, but might infect similar types of animals. (A few, such as the rabies virus, affect a multitude of species.) The most widely accepted taxonomic classification of camelids is as follows:²

Class--Mammalia

Order--Artiodactyla

Suborder--Tylopoda

Family--Camelidae

Genus--*Camelus*, Old World camelids

Species

C. dromedarius, dromedary camel

C. bactrianus, Bactrian camel

Genus--*Lama*, South American camelids

Species

L. glama, llama

L. pacos, alpaca

L. guanicoe, guanaco

Genus--*Vicugna*, South American camelid

Species

V. vicugna or *L. vicugna*, vicuna

Suborder--Ruminantia, deer, cattle, antelope, sheep, goat, gazelle

Llamas are often maintained on properties that also contain cattle, sheep, horses and goats. Llama producers, veterinarians and diagnostic laboratory personnel are concerned with the possibility that viruses which infect livestock (i.e. cattle, horses, sheep, goats) can also infect the llama. It is known that llamas can become

infected with some viruses which infect livestock. The purpose of this research was to determine the prevalence of antibodies to viruses which might possibly infect llamas and which are indigenous disease agents in cattle, sheep, horses and goats.

LITERATURE REVIEW

The objective of this research was to determine prevalence of antibodies in llamas to viruses which infect cattle, horse, sheep and goats. In this section, viruses which have been shown to infect llamas will be reviewed. In addition, a brief outline of each of the viral agents surveyed in this investigation will be offered.

Viruses In Llamas

The literature from scientists in South America contains limited information dealing with viruses which infect the llama. Fowler compiled a bibliography of articles regarding this subject.² Two investigators dominated the field during this time: H. Preston Smith during the 1930's-1950's and M. Moro Sommo in the 1960's. Both of these authors refer to general disease conditions and the clinical aspects of such conditions as rabies and brucellosis in alpacas.²

A literature search revealed very few references from researchers in North America concerning viral diseases of llamas. Torres, *et al.*,⁵ and Rebhun⁶ isolated a herpesvirus from a herd of alpacas and llamas that suffered from blindness and encephalitis. Subsequent research showed the virus to be antigenically identical to equine herpesvirus 1 (EHV-1). A rising antibody titer to EHV-1 was demonstrated in acute and convalescent serum samples from 4 alpacas. Many normal herdmates had antibodies to EHV-1. Ocular lesions are not found in horses infected with EHV-1. House, *et al.*,⁷ experimentally infected three llamas with the EHV-1 which was isolated from this outbreak. Llamas 1 and 2 developed severe neurological signs. Llama 1 became blind and llama 3 had decreased visual acuity. A virus isolated from

the thalamus of llama 2 proved to be EHV-1 by serum-virus neutralization assay. To date, EHV-1 has not been shown to be an abortifacient agent in llamas.

Williams, *et al.*, isolated and characterized a herpesvirus from a 3-year-old pregnant llama with respiratory disease.⁸ The isolate was chloroform sensitive, neutralized by bovine herpesvirus type-1 (BHV-1) specific antibody, and nearly identical to BHV-1, Cooper strain, when restriction endonuclease profiles were performed.

Underwood, *et al.*⁹, examined lung tissue from an immunosuppressed llama by transmission electron microscopy and reported observing enveloped virus particles that resembled retroviruses. The alveoli also contained *Pneumocystis carinii* cysts. A reverse transcriptase assay was conducted and was shown to be positive for retrovirus. Vogel¹⁰ challenged their findings, stating that the cell that the "virions" were shown budding from was not a mammalian cell, therefore this could not occur. He suggested these particles were budding forms of *P. carinii*.

Fowler², Rivera *et al.*¹¹, and Thedford and Johnson¹² have summarized the viral diseases that have been shown to occur in South American camelids. These diseases include contagious ecthyma, rabies, vesicular stomatitis, bovine herpes type-1, equine herpes type-1, foot-and-mouth, and rinderpest. A couple workers have isolated bovine viral diarrhea virus from feces of llamas with diarrhea.^{a,b} Other

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viruses to which llamas respond serologically but which apparently do not cause clinical disease include bluetongue, influenza A, parainfluenza-3, respiratory syncytial virus, and rotavirus.¹¹

Grouping the viruses discussed below into disease problems (respiratory, enteric, reproductive) would be convenient, but because most of the viruses affect multiple systems in the animal, a straightforward essay on each virus becomes necessary, if somewhat tedious.

Bovine Viral Diarrhea

Bovine viral diarrhea (BVD) was first recognized and described by Olafson in 1946.¹³ Ramsey and Chivers¹⁴ described a slightly different clinical syndrome and named it mucosal disease. It was finally agreed that the two diseases had the same etiologic agent, an RNA virus of the pestivirus group, family Togaviridae.¹⁵ This virus has recently been placed in the Flaviviridae family.¹⁶ The virus is shed in bodily secretions, including semen, and transmission is by direct contact. Transplacental transmission also occurs.¹⁷

Radostits and Littlejohns published a fairly recent review of bovine viral diarrhea, including vaccination recommendations.¹⁷ Ernst states that between 50 and 90% of adult cattle possess neutralizing antibodies to BVD, though far fewer have demonstrated clinical disease signs.¹⁸

Bovine viral diarrhea virus has been associated with diarrhea and enteric problems,^{13,17,18,19} immunosuppression,^{17,20,21} abortion^{18,22,23} and fetal anomalies.^{17,24,25,26}

Signs of acute disease include diarrhea, ulceration of the mucosal surface of mouth, esophagus, stomach and intestines, fever, depression, and leukopenia.

Cattle that exhibit severe signs of BVD are commonly between ages 6-24 months of age.^{17,19} It is now felt that these animals were probably infected during early gestation and therefore became immunotolerant and persistently infected with the virus.¹⁷ This early infection is believed due to a non-cytopathic biotype of the virus and, upon later infection with a cytopathic biotype, the animal develops acute disease.^{27,28,29} This has been accomplished experimentally by vaccinating persistently infected cattle.²⁹ Research indicates that immunotolerant cattle shed virus continuously, infecting their herd-mates and fetal calves.^{17,22} These immunotolerant animals may never show signs of disease, may be "poor-doers", or may succumb to BVD later in life.

Implicating BVDV in abortion cases is difficult because expulsion of a dead calf may occur months after infection. When pregnant cows are infected with BVDV up to day 125 of gestation, the calf may be aborted or become persistently infected.^{17,24,25,26} If infection occurs between days 125 and 180 of gestation, the calf may develop congenital abnormalities such as cerebellar hypoplasia, ocular lesions, musculoskeletal deformities, and alopecia. After 180 days, the calf is usually not adversely affected and may develop its own antibody response.¹⁷

Occasional researchers report isolation of BVDV from lungs of cattle with respiratory disease. Reggiardo³⁰ stated BVDV was isolated from 21% of the lungs with pneumonia in cases of shipping fever in the Texas Panhandle. Potgieter

experimentally produced respiratory disease with BVDV.³¹ Bovine viral diarrhea virus is thought to be immunosuppressive by causing a decrease in lymphocyte activity.^{18,20,32} It is surmised that this could predispose an animal to superinfection by other viruses or bacteria.³⁰ Others disagree with the mechanism of immunosuppression.¹⁸

Bovine Herpesvirus 1

When feedlot cattle in Weld County, Colorado, showed signs of an acute upper respiratory disease with necrotic areas in the respiratory mucous membranes, veterinarians felt they were dealing with a new disease and termed it necrotic rhinotracheitis.³³ Subsequently, similar outbreaks of disease in Colorado and California³⁴ initiated an investigation for the etiologic agent. An alphaherpesvirus of the family Herpesviridae was finally isolated. It is now called bovine herpesvirus type 1 (BHV-1) or infectious bovine rhinotracheitis (IBR).^{35,36,37} It is the same virus associated with infectious pustular vulvovaginitis (IPV), a mild venereal disease of cattle that has been present in Europe since 1927.³⁷ Why it started causing respiratory problems is not known.

A modified live vaccine for control of this disease was in wide use by 1957 and suspicion grew that the vaccine was causing abortions. McKercher, in 1964,³⁸ offered the first proof that this was the case, and showed that both the wild type and vaccine strains were capable of causing abortion. This was a new manifestation which was apparently not accompanied by a change in antigenic expression.³⁸

Like other herpesviruses, the IBR/IPV virus can remain latent in a dorsal root ganglion, specifically the trigeminal ganglion.³⁵ Thus, any animal with a titer to BHV-1, or known to have been infected, is a potential source of infection at any time in its life.³⁶ Strains are difficult to differentiate and there does not appear to be any correlation between strain and pathologic behavior.^{35,36} The virus can infect goats, deer, caribou and many other hoofed ruminant mammals.³⁵

Signs of IPV include reddening of the vulval mucosa with pin-point to pea size nodules which may coalesce and become ulcerous.^{36,39} In bulls, the penis and prepuce are affected similarly and the condition is termed balanoposthitis.³⁶ The virus is easily transferred during mating and effect on fertility is debated still. Failure to breed can occur due to pain. Infection does not seem to be followed by abortion.³⁹

The respiratory form of IBR is demonstrated by fever, anorexia, rapid breathing and clear to mucopurulent discharge.^{33,34,36,37} Incubation is 2-6 days,³⁵ morbidity ranges from 10-100%, but mortality is low (0-10%).³⁷ Infectious bovine rhinotracheitis is often implicated in a complex disease syndrome, commonly known as "shipping fever", which Yates reviewed.³⁵ This is a severe pneumonia, usually in newly weaned and shipped calves, that may be the result of synergism between a virus and bacteria (mainly *Pasteurella* spp), but proving the causative agents is often difficult.

In field conditions, about 25% of BHV-1-infected cows abort within 8 to 100 days.³⁶ Since the fetus remains in-utero 3-4 days, it may be autolyzed when aborted. The fetus suffers a systemic infection and lesions can be found in the liver, kidney,

spleen, brain and lymph nodes.^{36,38,40} The placenta is postulated to be the original site of infection and there the virus can remain latent. The virus may or may not spread to the fetus.⁴⁰

Bovine herpesvirus 1 has also been implicated in cases of conjunctivitis and encephalitis.^{36,37} While there is no question that encephalitis can occur, this manifestation of infection is rare.

Equine Herpesvirus 1

Viruses of the Herpesviridae family infect horses, in addition to cattle and most other species. Equine herpesvirus type 1 (EHV-1) is associated with abortion and equine herpesvirus type 4 causes febrile respiratory disease, although the two viruses are highly cross-reactive by virus neutralization tests and are occasionally isolated from atypical disease.^{41,42} Signs of the respiratory form, commonly known as rhinopneumonitis, include fever, leukopenia, anorexia, serous nasal discharge and swollen lymph nodes in the throat.

Abortion may occur 2-16 weeks post-infection with EHV-1 and the mares rarely exhibit signs of infection. Lesions in the aborted fetus include meconium discoloration of the hooves, edema of the lungs, thymic hyperplasia, and petechial hemorrhage in heart and adrenals. A foal infected close to term may be born weak and appear jaundiced.^{41,42,43}

Equine herpesvirus 1 has also been linked with neurologic disease, ranging in severity from mild ataxia to quadriplegia.⁴² As with other herpesviruses, the equine herpesviruses are thought to have latent stages of infection.

Parainfluenza 3

In 1959 Reisinger *et al.*,⁴⁴ reported the isolation of a virus from several feedlot calves that were ill with "shipping fever". This isolate (SF-4) was submitted to the National Institute of Health, Bethesda, Maryland and shown to be serologically identical to a myxovirus parainfluenza type 3 (PI-3) that had caused respiratory disease in children. Since then it has been shown that there is one serotype but many strains of PI-3.⁴⁵ Signs of disease in cattle include rapid respiration, cough, mucopurulent discharge, lacrimation, conjunctivitis, inappetence and increased temperature.^{44,46,47} Natural transmission time is 5 to 10 days with the virus being shed in the nasal secretions.⁴⁶

Virus isolation is possible from lung, trachea, larynx, turbinates, nasal secretions and tonsils.^{45,46,47} It has been recovered from aborted fetuses but failed to produce abortion in heifers with antibodies to PI-3.⁴⁸ The virus hemagglutinates red blood cells from various species. The virus induces intranuclear and intracytoplasmic eosinophilic inclusion bodies in infected cells.^{44,46}

Antibody prevalence is 48-86% in market beef cattle, depending on the state.^{46,47} Calves receive antibodies from the colostrum of serologically-converted dams,^{45,46} but these antibodies are catabolized by 6-8 months.⁴⁷ The calf then needs to be vaccinated and the best time to do so appears to be 3 weeks prior to weaning.^{46,47}

The relation of the PI-3 virus to "shipping fever" has been the subject of much research.³⁵ The original isolates were from calves diagnosed with this respiratory disease syndrome.⁴⁴ It is commonly felt, though difficult to prove, that

other factors are involved, such as infection with *Pasteurella* spp bacteria and stress (weather, dust, trauma, fatigue, dehydration, fright, excitement and crowding).^{44,47}

Bovine Respiratory Syncytial Virus

Another paramyxovirus first discovered in the early 1970's, bovine respiratory syncytial virus (BRSV), appears antigenically identical to the human respiratory syncytial virus.^{49,50,51} Bovine respiratory syncytial virus has a predilection for the lower respiratory tract and signs of the disease are similar to other respiratory diseases: fever, nasal and lacrimal discharge, cough, and inappetence.^{49,50,51} In cases where coughing is severe enough to cause lung rupture, pleural and subcutaneous emphysema may result.⁴⁹ As with BHV-1, PI-3 and BVD, BRSV is linked with bacterial infection and other stress factors that cause a secondary, and usually more severe, pneumonia and "shipping fever".^{49,51}

While colostrum-derived antibodies do not appear to confer calves with complete protection, it is felt they may help lessen the severity of the disease.^{49,51} After calves lose their colostrum antibodies (at about 4 months of age) they can be vaccinated.

Bovine respiratory syncytial virus is extremely difficult to isolate so diagnosis is usually made from fluorescent antibody tests on tissue or nasal swabs, or by serum neutralization tests on paired (acute and convalescent) serum samples.⁴⁹

Bovine Adenovirus

In the early 1950's, a new virus was isolated on several occasions from cases of acute respiratory disease in humans. Finally in 1956, a group of scientists proposed that the viruses be named adenoviruses.⁵² Since then, many serotypes of adenoviruses have been isolated from humans and domestic animals.

There are 9 recognized species of bovine adenoviruses (BA). Species 1-3 are classified as members of Subgroup 1 and share a subgroup-specific antigen.⁵³ Over 75% of adult cattle have antibodies to BA-3. Bovine adenoviruses normally infect the mucous membranes resulting in excessive nasal and lacrimal discharge, dyspnea, and cough. Subgroup 2 adenoviruses additionally causes diarrhea and may produce a viremia.^{52,53,54,55,56} Bovine adenovirus type 7 has been associated with weak calf syndrome in which calves are born weak and have subcutaneous hemorrhages over joints and develop diarrhea and polyarthritis.^{53,54} Bovine adenovirus infections range from mild to severe, depending on the virus species. The virus is shed in bodily secretions and believed to be transmitted by fomites.

Equine Adenovirus

Equine adenoviruses (EA) have not been studied extensively because, in a normal horse, the infection causes subclinical or mild clinical signs of respiratory disease. Foals with failure of passive transfer and Arabian foals with combined immunodeficiency disease develop a more serious respiratory disease.^{57,58}

Equine adenovirus type 1 has been isolated from horses with cauda equina neuritis, a polyneuritis affecting the sacral and coccygeal nerves of the cauda equina

and cranial nerves (lip and eyelid paralysis).⁵⁷ Cauda equina neuritis is thought to be a manifestation of an autoimmune disorder following infection with EA-1.⁵⁷ It is interesting to note from a comparative serologic perspective that human adenovirus type 3 is associated with encephalitis.⁵⁷

No vaccine has been developed for EA since it has not been shown to be a serious pathogen.⁵⁸

Bluetongue

Bluetongue (BT) disease is caused by an orbivirus of the Reoviridae family.⁵⁹ It has been known to infect sheep for a long time and infection was first diagnosed in cattle in South Africa in 1934 and in the USA in 1959.^{60,61} It is also known to infect many wild artiodactyls, including white-tailed deer, mule deer, elk, muntjac, pronghorn antelope, buffalo, bighorn sheep, topi, blesbok and mountain gazelle.⁵⁹ A closely related virus, epizootic hemorrhagic disease virus (EHDV), is more common in wild artiodactyls, but also is able to infect cattle. Control of BT becomes a problem as domestic and wild animal populations serve as reservoirs for each other. The vector of BT is biting midges, *Culicoides* spp.^{60,61,62}

Clinical signs of disease in sheep include lameness, fever, dyspnea, swollen tongue, ulcerous dental pad, cracked muzzle and coronitis.⁶⁰ Reports of antibody prevalence vary from 5-40%. It is difficult to reproduce disease experimentally.⁶⁰ Bluetongue can persist in a viremic state for months in cattle and it might have a latent stage.^{59,60}

Bluetongue virus has been implicated in abortion and fetal anomalies in sheep and cattle.^{61,63} A prominent calvarium (domed forehead) and crooked limbs (arthrogryposis) are the typical malformations of calves and lambs which are born to BTV-infected dams.^{60,61,63}

Equine Influenza

Influenza in all species of animals is caused by orthomyxoviruses. The two proteins on the surface of the virion, the hemagglutinin (H) and neuraminidase (N) proteins, are used to distinguish different types of the virus. There are 14 antigenically distinct hemagglutinins and 9 neuraminidases.⁶⁴

Two equine influenza viruses are known.^{64,65} Both are Type A orthomyxoviruses. The first was discovered in Czechoslovakia in 1956 and named A/equi/1/Prague/1956 (H7N7). The second was isolated from an outbreak of respiratory disease in racehorses in Miami during 1963.⁶⁵ It was designated A/equi/2/Miami/1963 (H3N8).⁶⁴ Both are present throughout the United States, with strain equi 2 being more common.

Clinical signs of disease include fever (102-108 F), nasal and lacrimal discharge, malaise and persistent cough which may last weeks to months. Many cases are mild to subclinical, but older and younger animals may be affected more severely.^{64,65} Transmission occurs through aerosol and fomites.⁶⁴

Treatment involves mainly rest and supportive care. A killed vaccine is available against both types of influenza and this serves to lessen severity of disease though not prevent infection.⁶⁴

Equine Viral Arteritis

Equine viral arteritis (EVA) was first described by Doll in 1957.⁶⁶ It is an Arterivirus in the family Togaviridae,⁶⁷ and a serious cause of abortion. Signs of disease include a stiff gait, swelling of limbs and sometimes other areas, fever, conjunctivitis, rhinitis with nasal and ocular discharge, and anorexia.^{66,67} Transmission is mainly by way of aerosol droplet, but venereal transmission can occur. A carrier state is frequently found in stallions, resulting in virus being shed constantly in semen.⁶⁷

Adult horses that become infected naturally rarely die. A mare that aborts following infection may or may not show clinical signs of EVA. The fetus may be autolyzed, in contrast to a foal aborted following EHV-1 infection, in which case the foal is almost never autolyzed.^{66,67}

Signs of disease are not distinct enough to differentiate a respiratory disease due to EVA from one due to EHV-4 or EI. A serologic assay or virus isolation is needed. In fatal cases, histopathologic examination of various organs will reveal characteristic vasculitis in smaller arteries.^{66,67}

Prevalence of infection varies greatly in different areas of the world. There is a vaccine but it should not be given to pregnant mares in their last trimester of gestation.⁶⁷

Vesicular Stomatitis

The vesicular stomatitis virus (VSV) causes vesicular lesions in horses, cattle, swine and deer, and experimentally, in guinea pigs.^{68,69,70,71} It must be differentiated

from foot-and-mouth disease (FMD) in cattle and swine vesicular disease and vesicular exanthema of swine.⁷¹ Humans can be infected by VSV, resulting in fever, chills, and muscle soreness.^{69,71}

Excess salivation or lameness are often the first signs of disease. Vesicles form on the tongue, oral mucosa, lips, coronary band, and teats.^{68,69,71} These rupture, then heal slowly. Mortality can be approximately 5 percent. Economic losses from reduced milk production and culling can be great.⁶⁹

The virus is classified as a vesiculovirus in the family Rhabdoviridae. Two strains are known: New Jersey (NJ) and Indiana (IN), with VSV-NJ causing more severe disease.⁷¹ Transmission is unclear but thought to include direct contact or arthropod vectors.^{70,71} Vaccines have been developed but are rarely used due to sporadic occurrence of VS.⁷¹

Ovine Progressive Pneumonia/Caprine Arthritis Encephalitis

Ovine progressive pneumonia (OPP) virus and caprine arthritis encephalitis (CAE) virus are two closely related lentiviruses of the family Retroviridae. Both viruses occur as latent infections of monocytes and the carrier animals are potentially constant sources of the viruses.^{72,73} Serological prevalence ranges from 1-90% for OPP in sheep and approximately 81% for CAE in goats (in a 1981 study).⁷² Clinical cases of OPP or CAE are not that high due to the insidious nature of the disease.^{72,73}

With OPP, clinical signs of disease are expressed as a chronic, progressive respiratory problems with lack of a fever and loss of general condition. A neurologic

form results in progressive ataxia of the hind limbs. The virus can also cause agalactia (not a true mastitis).⁷²

Caprine arthritis encephalitis virus most often causes a chronic inflammation with swelling of the carpal joints. However, it also is associated with afebrile, ascending paralysis, respiratory disease and agalactia.⁷³

Transmission of both OPP and CAE is via direct ingestion of milk or colostrum containing the virus. However, OPP can be transmitted by direct contact. Colostrum heated to 133 F (56 C) for 1 hour has been shown to destroy the CAE virus.⁷³ There is no treatment or vaccine and clinically ill animals are usually culled.

These two viruses are of concern to llama owners due to the practice of giving goat colostrum to crias that do not receive colostrum from their own dams.

Potomac Horse Fever

Potomac horse fever (PHF), or more correctly named, equine monocytic ehrlichiosis (EME), is caused by a rickettsia, *Ehrlichia risticii* (ER). It was included in this serologic survey because of its recognition in Oregon.

The disease was first reported in Maryland in 1979, near the Potomac River. Knowles described the disease, then known as Acute Equine Diarrhea Syndrome (AEDS) in 1983.⁷⁴ The agent was demonstrated by electron microscopy by Rikihisa^{75,76} and Holland and Ristic.⁷⁷ The indirect immunofluorescent antibody (IFA) method of testing, developed by Ristic, made the disease easier to diagnose and it was discovered the disease was present throughout the United States and Canada.⁷⁸

Clinical signs of disease include anorexia, depression, explosive diarrhea, fever, leukopenia, and occasionally laminitis. Mortality is approximately 30 percent. The disease is infectious but not contagious.^{74,75,78}

MATERIALS AND METHODS

Media and diluents. Medium used for virus dilutions, serum-virus neutralization tests, and some cell culture (African green monkey kidney, also known as Vero cells) consisted of minimal essential medium (MEM) with Earle's salts plus .05% lactalbumin hydrolysate, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 ug/ml streptomycin sulfate (MEM-E). Hanks' salts (MEM-H) were substituted for the Earle's salts for other cell cultures (bovine turbinate, llama kidney, and equine kidney cells). Bovine serum (10%) was added for cell culture and serum-virus neutralization (SN) tests. Bovine serum (5%) was added for propagation of virus pools for serology. Fetal bovine serum was substituted in some serum-virus neutralization tests (BRSV, EVA, VSV, and LA7649). Serum used in cell cultures was shown to be free of antibodies to the viruses being tested.

Diluent for PI-3 hemagglutination-inhibition (HI) tests was phosphate buffered salt solution (PBS) supplemented with 0.4% bovine serum. Diluent for equine influenza HI tests was 0.01 M phosphate buffer plus 0.2% bovine serum albumin.

For the agar gel immunodiffusion (AGID) tests, agar plates were prepared with 0.9% agarose agar in physiologic saline (0.85% NaCl in distilled/demineralized water).

Cell cultures. Bovine turbinate cells^c were used to propagate and assay BVDV, BHV-1, EHV-1, and BA3. African green monkey kidney cells (Vero)^d were

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^d National Arbovirus Laboratory, Laramie, Wyoming.

used to propagate and assay EVAV, VSV-NJ and VSV-IN. Bovine respiratory syncytial virus was propagated in primary bovine testicular cells but assayed with Vero cells. Parainfluenza 3 was propagated in primary bovine testicular cells but assayed with bovine red blood cells. Equine adenovirus was propagated and assayed in equine fetal kidney cells. Equine influenza virus 1 and 2 were propagated in eggs by inoculating into the amniotic sac. They were assayed using chicken red blood cells. Llama adenovirus strain 7649 was replicated and assayed in primary llama kidney cells. Mouse macrophage cells were used to propagate *Ehrlichia risticii*, the agent of PHF.

Virus source. The viruses were received from various sources: BVDV, BHV-1, PI-3, BRSV, EHV-1, EA, EVAV, VSV-NJ, VSV-IN, and EI-1 (A/Eq/1/Prague/56, H7N7) and EI-2 (A/Eq/2/Miami/63, H3N8)^e; BA3 5C and LA7649;^f bluetongue antigen,^g *Ehrlichia risticii*.^h

Assay for antibodies. Serum-virus neutralization (SN) tests were performed for BHV-1, BVD, BRSV, EHV-1, BA3, EVA, EA, VSV-NJ, VSV-IN, and LA7649. Serums were heat-inactivated at 56 C for 30 minutes and diluted in flat bottom 96-

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^g Veterinary Diagnostic Technology, Inc., 4890 Van Gordon Street, Suite 101, Wheatridge, Colorado 80033.

^h American Type Cell Culture, 12301 Parklawn Drive, Rockville, Maryland 20850.

well microtiter plates using 2-fold dilution steps (50ul per well). Initial serum dilution was 1:4. An equal volume of virus (50ul) containing 100 tissue culture median infectious doses (TCID₅₀) was added to the diluted serum. The plates were incubated 1 hour at 25 C after which the appropriate cells were added at a concentration of 5×10^5 cells/ml. Finally, one drop of sterile mineral oil was added to each well and the plates incubated at 37 C in a 2.5% concentration of CO₂.

The plates were examined for cell growth and presence of cytopathic effect in 5 to 7 days. Tests were performed in duplicate and the serum end-point titer was defined as the last serum dilution which inhibited cytopathic effect (CPE) of the virus.

Hemagglutination inhibition tests were performed for PI-3, EI-1 and EI-2 in round bottom microtiter plates. For the hemagglutination inhibition (HI) tests, the serums were treated with a 25% suspension of acid-washed kaolin, pH7.0. Each serum was diluted in the kaolin (0.2 ml into 0.6 ml kaolin), allowed to react 25 minutes, centrifuged 1500 x g for 10 minutes, and the supernatant poured into clean tubes. The serum was therefore at a 1:4 dilution and were diluted in the plates in 2-fold steps. An equal amount of virus (25 ul) was added. After 1 hour incubation at 25 C, 50 ul of the bovine red blood cells (0.4%) was added. The test was incubated overnight at 4 C (or 30 minutes for equine influenza). In wells without antibodies, the virus cross-linked the red bloods cells into a diffuse mat (hemagglutination). The presence of antibodies inhibits hemagglutination so the red blood cells sink to the

bottom of the well to form a "button". The titer was recorded as the last well to show inhibition.

The AGID tests were performed in glass petri dishes (60x15mm) which contained 6 ml of the agar. A template was used to cut the wells which were arranged with one well in the center (for antigen) and six wells evenly spaced around the center for the antisera. Wells were 4.0 mm in diameter and 2.4 mm from the center well. Positive control antisera was placed in every other well. The plates were incubated in moisture chambers at 25 C for 48 hours. Llama sera that contained antibody formed a precipitin line of identity with the neighboring positive control band.

The indirect fluorescent antibody test was used for PHF. *Ehrlichia risticii* was replicated in mouse macrophage cells. When 75% of the cells showed CPE, the cells were scraped from the culture flask and deposited on a 14-ring glass slide (top row only). Uninfected cells were deposited on the bottom row. The slides are were fixed in acetone and frozen at -20 C until use. The serums were diluted and applied to the slides (25 ul per well, each dilution on an infected and noninfected well). Serial 2 fold dilutions from 1:20 to 1:1280 were tested with goat anti-llama IgG conjugated with fluorescein isothiocyanate. The slides were evaluated using epifluorescent microscopy. A limited number of serums were selected for testing from areas where PHF had been documented to occur in horses through FA testing by Oregon State Veterinary Diagnostic Lab, Virology Section.

Source of serums. This survey was one aspect of a large research project involving llamas in Oregon. During the month of June 1989, jugular blood samples were obtained from 270 llamas representing 21 farms in three major regions of Oregon. Most llama farms were selected on the basis of those that responded to a query while others were contacted directly by a member of the research team. Animal numbers on the farms ranged from 13 to 250. Each farm owner was asked to select a certain number of their llamas that evenly represented six groups: males less than one year old, females less than one year old, males greater than one year old, open females, females in their first or second trimester of pregnancy, females in their last trimester of pregnancy. Based on owner histories and a detailed on-farm physical examination, the selected llamas were determined to be free of medical problems, including overt disease, lameness, and infertility. Since the above grouping did not have application to this survey, the serums were grouped by animal age.

The blood was allowed to clot at room temperature, centrifuged at 1000 x g for 15 minutes, and the clarified serum was stored at -20 C until it was tested.

RESULTS

The percentage of llamas possessing antibodies to the tested viruses is presented (Table 1). All 270 llamas lacked antibodies to EVA, BA-3, VSV-NJ, VSV-IN, and OPP. The 61 llamas tested for antibodies to *Ehrlichia risticii* (PHF) were all negative. Overall, 22% (60 out of 270 llamas) had antibodies to a virus associated with a disease of livestock. Seven llamas had antibodies to more than one virus. Llama adenovirus 7649 was excluded from this number because it is not known to occur in other livestock and 93.3% of the llamas possessed antibodies to the virus. One llama possessed antibodies to BRSV, one to EHV-1, one to EI-2. Two llamas had antibodies to BHV-1, two to EI-1. Serums from four llamas reacted positively to BTV antigen. Twelve llamas exhibited antibodies to BVDV, twelve to PI-3 and forty-three to EA. Of the 270 llamas, 252 had antibodies to LA7649.

The llamas were divided according to the region of Oregon in which they were located. Valley refers to the Willamette Valley which is between the Cascade mountain range and the Coastal mountain range, and extends from the Columbia River to Cottage Grove. East refers to llamas on ranches east of the Cascade Mountains. South refers to llamas south of Cottage Grove to the Oregon-California border.

Llamas with antibodies to viruses of livestock were divided into 6-month age groups and the number in each age group with antibodies is shown for each virus (Table 2).

Table 1. Llamas possessing antibodies to the viruses tested, shown for three regions of Oregon.

Virus	Valley (N=140)	East (N=82)	South (N=48)	Total (N=270)
BHV-1	...	2 (2.4%)	...	2 (<1%)
BVD	7 (5%)	3 (3.7%)	2 (4.2%)	12 (4.4%)
PI-3	3 (2.1)	7 (8.5%)	2 (4.2%)	12 (4.4%)
BRSV	...	1 (1.2%)	...	1 (<1%)
EHV-1	...	1 (1.2%)	...	1 (<1%)
EVA	0
BA3	0
EA	22 (15.7%)	7 (8.5%)	14 (29.2%)	43 (15.9%)
EI-1	1 (0.7%)	1 (1.2%)	...	2 (<1%)
EI-2	...	1 (1.2%)	...	1 (<1%)
VSV-NJ	0
VSV-IN	0
BT	1 (0.7%)	3 (3.7%)	...	4 (1.5%)
OPP	0
PHF	0
LA7649	137 (97.9%)	80 (97.6%)	39 (81.3%)	252 (93.3%)

- BHV-1 = bovine herpesvirus 1
 BVD = bovine virus diarrhea
 PI-3 = parainfluenza 3
 BRSV = bovine respiratory syncytial virus
 EHV-1 = equine herpesvirus 1
 EVA = equine viral arteritis
 BA3 = bovine adenovirus 3
 EA = equine adenovirus
 EI-1 = equine influenza 1
 EI-2 = equine influenza 2
 VSV-NJ = vesicular stomatitis virus-New Jersey strain
 VSV-IN = vesicular stomatitis-Indiana
 BT = bluetongue virus
 OPP = ovine progressive pneumonia
 PHF = Potomac horse fever
 LA7649 = llama adenovirus strain 7649

Table 2. Number of llamas per age group with antibodies to livestock viruses.

Livestock virus	Age in months					
	0-6	6.1-12	12.1-18	18.1-24	24.1-30	30+
BHV-1	2
BVD	1	1	1	9
PI-3	1	...	1	10
BRSV	1
EHV-1	1
EA	...	5	3	5	3	27
EI-1	1	...	1
EI-2	1
BT	...	1	3

BHV-1 = bovine herpesvirus 1
 BVD = bovine virus diarrhea
 PI-3 = parainfluenza 3
 BRSV = bovine respiratory syncytial virus
 EHV-1 = equine herpesvirus 1
 EA = equine adenovirus
 EI-1 = equine influenza 1
 EI-2 = equine influenza 2
 BT = bluetongue virus

Llamas with SN antibodies to LA7649 titers were divided into age groups of six-month increments (Figure 1). The end-point titers were separated along the x-axis of a graph and the bars represent the number of llamas in each LA7649 antibody titer category (Figure 2).

The livestock (cattle, horses, sheep, goats) present on each farm is noted along with the viruses to which llamas possessed antibodies is presented (Table 3). Several farms did not have livestock that might have served as the source of virus. Also presented are data from llamas with antibodies as to whether they were born on the farm or purchased, and, if purchased, how long they had been present on the farm. The age column applies only to those born on the farms.

Further information is provided on the seven llamas which had antibodies to more than one livestock virus (Table 4).

Rainfall, elevation, and climate conditions from representative weather stations are presented in the Appendices, along with a map of Oregon showing the locations of the weather recording stations.

Figure 1. Percent of llamas in each 6-month age group that possessed antibodies to llama adenovirus 7649 (LA7649).

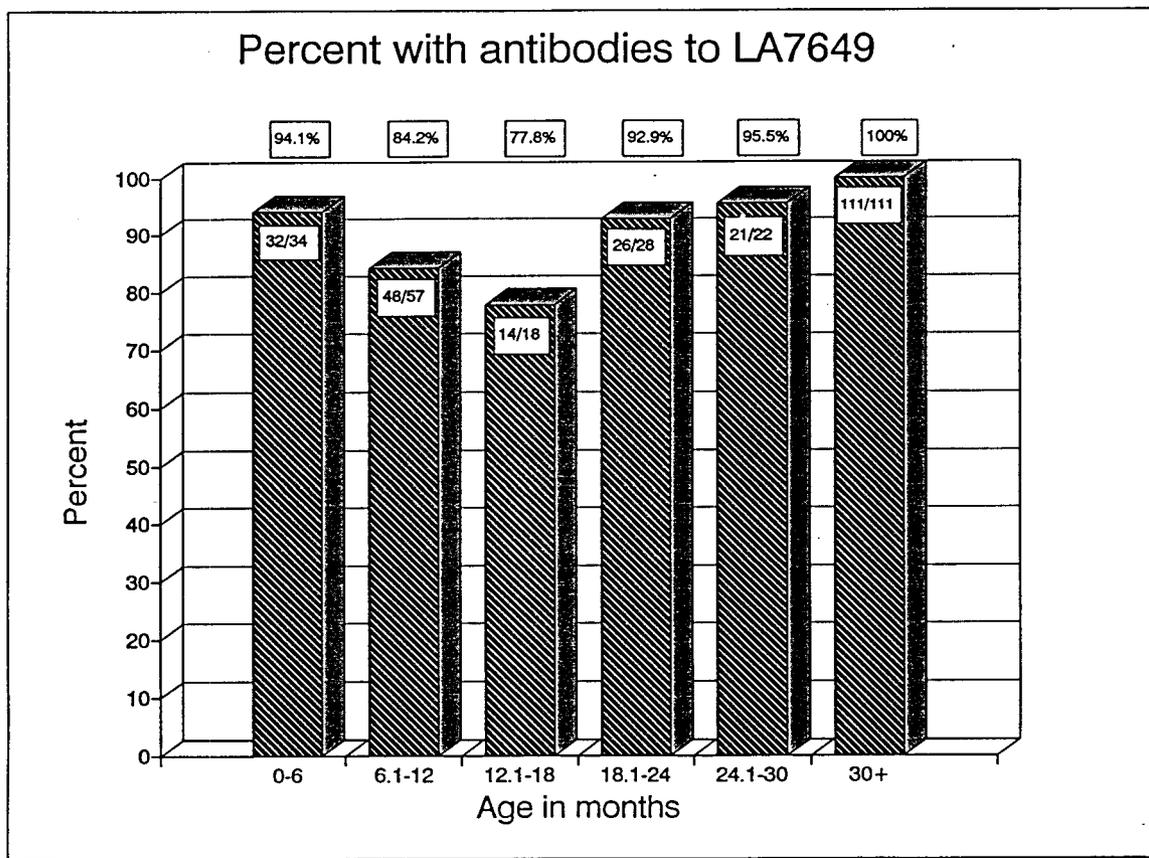


Figure 2. Number of llamas in each antibody titer level category to llama adenovirus 7649 (LA7649). Titer is defined as the reciprocal of the highest dilution exhibiting inhibition of cytopathic effect.

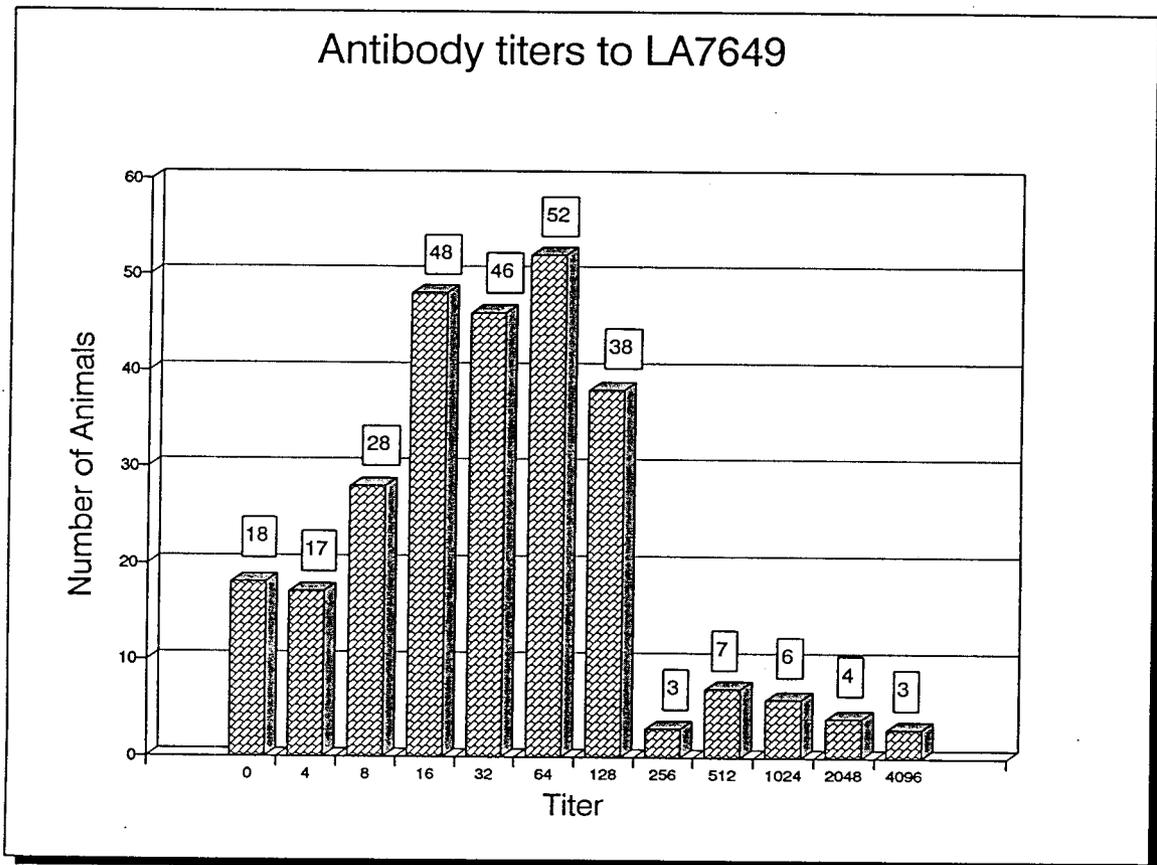


Table 3. Summary of llamas possessing antibodies to livestock viruses and their previous contact with livestock.

Farm	Livestock	Antibodies	Llamas born on farm	Range of age (months)	Llamas bought	Time on farm (months)
1	none	EA BT	2	7.6-11.3	0	0
2	none	none	0	0	0	0
3	B E	PI-3 BRSV EHV-1	1	1.1	4	17-44
4	none	EA	1	35.2	0	0
5	none	PI-3 BT	0	0	1	60
6	E	BHV-1 BVD PI-3 EA EI-1 EI-2	0	0	4	2-18
7	B E	BHV-1 PI-3 EA BT	0	0	3	34-48
8	E	BVD EA BT	1	20.8	2	33-53
9	B E	none	0	0	0	0
10	E	none	0	0	0	0
11	none	EA	0	0	2	28-33
12	B E	EA	0	0	5	2-18
13	none	EA EI-1	0	0	2	4-11
14	B E	none	0	0	0	0
15	none	EA	1	17.3	1	28
16	E	EA	0	0	1	31
17	B	BVD PI-3 EA	2	0.3-24.2	5	13-34
18	none	EA	5	6.5-13.4	4	33-36
19	B E	BVD PI-3 EA	0	0	9	7-43
20	B E	PI-3 EA	0	0	3	10-30
22	B E O C	PI-3 EA	0	0	4	2-82

B= bovine, E= equine, O= ovine, C= caprine

Table 4. Llamas with antibodies to more than one virus (excluding LA7649).

Llama number	Virus	Livestock	Born or purchased	Time on farm (mos)
1073	BHV-1 BVD PI-3 EA EI-1 EI-2	E	purchased	18
1216	BVD PI-3	B	born	0.3
1250	BVD EA	B E	purchased	19
1254	BVD EA	B E	purchased	31
1259	BVD EA	B E	purchased	43
1265	PI-3 EA	B E	purchased	10
1278	PI-3 EA	B E O C	purchased	11

BHV-1 = bovine herpesvirus 1
 BVD = bovine virus diarrhea
 PI-3 = parainfluenza 3
 EA = equine adenovirus
 EI 1&2 = equine influenza types 1 and 2

B= bovine, E= equine, O= ovine, C= caprine

DISCUSSION

While the farm and animal selection process was not strictly random, the llama population in Oregon was well represented in this study. One of the purposes of this study was to demonstrate presence of antibodies to a variety of diseases that these llamas are exposed to through their contact with other llamas and/or livestock. None of the llamas had been vaccinated for the viruses in question as far as the present owners knew. Where a measurable antibody titer was shown, it was concluded that llamas become infected by that virus. For the viruses with no measurable antibody response, the possibility of infectivity is not ruled out. A prevalence survey by definition deals with a single point in time, inferring no conclusion as to when exposure to the virus occurred. Conceivably, animals could have been infected with the virus in question immediately prior to time of sampling or sometime in the past. In either case, the antibody level may have been too low for detection. It should also be pointed out that serological tests conducted with a single serum from an animal should not imply that a disease state exists. The best serological diagnostic procedure is to obtain two blood samples from the animal about two weeks apart, preferably during the acute and convalescent phases of the illness. If the titer has increased by a four-fold or greater factor, one can conclude the animal recently underwent an infection with the agent.

There is the temptation to attribute an unusually high antibody titer from a single serum sample to a recent or current infectious state. While long time field experience occasionally lends credence to this practice, one must be careful about

basing any major conclusions or management decisions on this assumption. In the case of this survey, the warning is especially emphasized for two reasons: 1) no attempt was made to specifically examine or sample each llama for disease diagnosis, and 2) the ranges of antibody titers and their association with infection or disease needs further research in llamas, and 3) the presence of antibody in relation to time after infection in llamas is not known.

None of the 270 llamas tested demonstrated titers to OPP, BA3, EVA, or VSV. Only 61 were tested against ER, but none of those showed an antibody titer. None of these disease organisms has reportedly been isolated from a llama and data in this investigation suggest that the llama may not become infected with these specific viruses or rickettsia. Infection with BA3 in cattle and OPP in sheep is quite common in Oregon. Equine viral arteritis and VSV are rare in horses in Oregon, and likewise, seroprevalence studies with ER in Oregon indicate infection rate varies from 1 to 5 percent.ⁱ

One llama possessed antibodies to BRSV (1:4). The animal in question was a 1.1 month-old cria, and there is a chance that antibodies might have resulted from passive transfer (colostrum). The dam was not sampled. Antibodies to BRSV have previously been documented in llamas.¹¹

The only llama with antibody titer to EHV-1 (1:4) was imported from Bolivia in 1987 and had been on the farm in Oregon 17 months. There were horses in

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contact with this llama and llamas on this farm had not previously shown signs attributed to EHV-1 infection, i.e., pyrexia, encephalitis and blindness.

One llama had antibodies to both EI-1 and EI-2. It had resided on the farm for 18 months and was in contact with horses. Another llama had antibodies to EI-1 only. It was not in contact with horses on the farm but had been purchased only four months prior to sampling. The previous owners indicated that there was potential for interacting with horses on their farm.

Two llamas had antibodies to BHV-1. One was a female in the third trimester of gestation (1:4). She later delivered a normal cria. There were cattle on the farm. The other llama was a male who was not known to have been in contact with cattle on the farm where it was sampled (time on farm had been 18 months), nor where it was previously (12 months). Cattle and horses were associated with this llama prior to this time. Its antibody level was 1:32, which is a relatively high titer in cattle. This llama did not have a previous history of respiratory disease. Duration of antibody levels requires further investigation. Also, the possibility of recrudescence of herpesviruses can not be ruled out.

Llamas can become infected by the bluetongue virus and/or epizootic hemorrhagic disease, as is evidenced by the four positive results with the AGID test. Again, isolation of the virus from the llama was not attempted and the llama did not show signs of disease. One of the llamas with an antibody titer to BTV was only 8 months old and was born on the farm in the Willamette Valley. Since BTV is not believed to occur in the Valley region, either the result was a false positive or

represented antibodies to EHD which are cross-reactive with BTV with the AGID test. The llama had no history of association with cattle or sheep. Since BT/EHD is spread by bites from *Culicoides* spp, any animal capable of propagating this virus may have served as a source of virus, i.e. deer.

Twelve of the 270 llamas (4.4%) possessed antibodies to BVDV. Four were pregnant females in various stages of gestation. While three of these llamas later delivered normal crias, the fourth dam apparently aborted. She has been considered a problem breeder, which can be attributed to a number of factors including infection with BVDV. She was born on her farm and had contact with cattle. A 10-day old cria had an antibody titer to BVDV of 1:64, which was the same titer as her dam. Nine of the 12 llamas with antibodies to BVDV were on farms with cattle; seven of the 9 were on the same farm. The 3 llamas on farms without cattle had been on their respective farms for 18 to 33 months. Two had exposure to cattle prior to that and one did not have exposure. It is interesting to note that, in contrast, seven of the farms with cattle did not have llamas with BVD antibody titers. None of the llama owners that also had cattle felt that BVDV was a problem on their farm.

Thirty-five percent of alpacas in a South American study had antibody titers to PI-3.¹¹ In this present survey (Oregon llamas), 12 of 270 llamas had antibody titers to PI-3 (4.4%). A 10-day old cria had a titer of 1:8, but the dam was negative. The other 11 llamas had been on their respective farms from 7 to 60 months. Most farms reported contact between llamas and cattle or sheep. Two farms lacked cattle and sheep, and the 3 llamas with a PI-3 antibody titer had been on these farms for 18, 18

and 60 months respectively. Again, it is not known how long a llama maintains an antibody titer. Infection with PI-3 was believed to be subclinical in all cases as the animals in question did not have a history of respiratory disease.

Forty-three llamas (15.9%) had antibody titers to EA. Of the 15 farms these llamas represented, 7 farms (20 llamas total) reported no horses present. Eight of these 20 llamas were born on their farms and had not left to breed. This raises the questions: Is the virus a true equine virus or a llama adenovirus that shares antigens with standard EA? Are the llamas shedding the virus, thereby acting as the source of infection in place of horses?

The great number of llamas (252) with antibody titers to LA7649 suggests that this is a very common virus among llamas and appears to be infectious. Most adenoviruses are shed in bodily secretions, particularly nasal and feces. Llama adenovirus has been isolated from young llamas with enteritis and pneumonitis but most infections are believed to be mild or subclinical.

Experimental animal infectivity and immunologic studies have not been conducted with llama adenoviruses. Nothing is known about the significance of these viruses and how long antibody titers are retained after infection. Thirty-two of the llamas with antibodies were crias under 6 months of age. By this age most calves have lost their colostral antibodies. Thirteen of the 34 crias had dams that were sampled also. All thirteen cria/dam pairs possessed antibodies. Adenoviruses of other animals (cattle and sheep) are usually transmitted by aerosol infection but in-utero infection can occur. One cria sampled was 6 days old and had a titer to LA7649 of

1:1024. The dam had a titer of 1:4096. This is suggestive of a recent infection in the dam, with subsequent colostral transfer to the cria, but the normal range of LA7649 antibody titers is not known.

From Figure 1, it can be observed that the number of llamas without LA7649 titers was greatest in the 6 month to 18 month range. It is generally accepted that calves and foals lose their passively-acquired antibodies by age 6 months unless these animals become infected themselves. Because a random animal selection method was not used for this study, statistical significance can not be determined from this data.

Questions are raised regarding the source of infection with recognized viruses of cattle, horses, sheep and goats for llamas, such as whether direct contact with livestock is needed or can the llamas transmit livestock viruses to each other. Ten of the 21 farms did not have the livestock species present that would be the source of virus which might have caused the antibody response in llamas on that farm. Six of these 10 had at least one llama (sampled in the survey) that had been born on the farm. This data suggests that llamas can become infected and transmit the virus to herd mates. It is possible that the llamas become infected at shows or breeding farms. Obviously, further study is needed in this area. Only one farm had no livestock and no antibodies to test viruses (excluding LA7649) in the llamas sampled. Presence of livestock is not a guarantee of viral transmission as evidenced by the fact that 3 of the 21 farms had livestock but no antibody titers in the llamas sampled.

Seven llamas had antibodies to more than one virus (excluding LA7649). One of these was a 9 day-old cria (BVD 1:64, PI-3 1:8). Its dam had an antibody titer to BVDV of 1:64, but no antibodies to PI-3. Cattle were present on the farm. The other 6 were purchased, with time on their farms ranging from 10 to 43 months. Five of these had contact with livestock that could account for their antibodies. The sixth llama had antibody titers to BHV-1, BVDV, PI-3, EA, EI-1, and EI-2. It had been on its farm 18 months with exposure to horses but not cattle. The previous owners had it for 12 months with no cattle/horse contact during this time. They purchased the llama from a trader who reportedly possessed horses and cattle.

BIBLIOGRAPHY

1. Webb SD. Pleistocene Mammals of Florida. Gainesville: University of Florida Press, 1974.
2. Fowler ME. Medicine and Surgery of South American Camelids. Ames, IA: Iowa State University Press, 1989;102-108.
3. Novoa C, Wheeler JC. Llama and alpaca. In: Mason IL, ed. Evolution of Domestic Animals. London: Longman, 1984.
4. Smith BB. Major infectious and non-infectious diseases of the llama and alpaca. Vet Hum Toxicol. (in press)
5. Torres A, Dubovi EJ, Rebhun WC, King JM. Isolation of a herpesvirus associated with an outbreak of blindness and encephalitis in a herd of alpacas and llamas. Abstracts, Annual Meeting of the Conference of Research Workers in Animal Disease, 1985, 50.
6. Rebhun WC, Jenkins DH, Riis RC, Dill SG, Dubovi EJ, Torres A. An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus 1 in a herd of alpacas and llamas. J Am Vet Med Assoc 1988;192:953-956.
7. House JA, Gregg DA, Lubroth J, Dubovi EJ, Torres A. Experimental equine herpesvirus-1 infection in llamas (*Lama glama*). J Vet Diagn Invest 1991;3:137-143.
8. Williams JR, Evermann JF, Beede RF, Scott ES, Dilbeck PM, Whetstone CA, Stone DM. Association of bovine herpesvirus type 1 in a llama with broncho-pneumonia. J Vet Diagn Invest 1991;3:258-260.
9. Underwood WJ, Morin DE, Mirsky ML, Haschek WM, Zuckermann FA, Petersen GC, Scherba G. Apparent retrovirus-induced immunosuppression in a yearling llama. J Am Vet Med Assoc 1992;200:358-362.
10. Vogel P. Retroviral basis for immunosuppression remains to be proven (letter). J Am Vet Med Assoc 1992;201:1318.
11. Rivera H, Madewell BR, Ameghino E. Serologic survey of viral antibodies in the Peruvian alpaca (*Lama pacos*). Am J Vet Res 1987;48:189-191.

12. Thedford TR, Johnson LW. Infectious Diseases of New-World Camelids. *Vet Clin of NA: Food An Prac* 1989;5:145-157.
13. Olafson P, MacCullum AD, Fox FH. An apparently new transmissible disease of cattle. *Cornell Vet* 1946;36:205-213.
14. Ramsey FK, Chivers WH. Mucosal disease of cattle. *North Am Vet* 1953;34:629-633.
15. Andrewes C, Pereira HG, Wildy P. *Viruses of the Vertebrates*, 4th ed. London: Bailliere Tindall, 1978.
16. Francki RIB, Fauquet CM, Knudson DL, Brown F. Classification and nomenclature of viruses: Fifth report of the international committee on taxonomy of viruses. *Arch Virol Supplement* 2. 1991.
17. Radostits OM, Littlejohns IR. New concepts in the pathogenesis, diagnosis and control of diseases caused by bovine viral diarrhea virus. *Can Vet J* 1988;29:513-528.
18. Ernst PB, Baird JD, Butler DG. Bovine viral diarrhea: an update. *The Comp Cont Ed* 1983;5:S581-S589.
19. Kahrs RF. The differential diagnosis of bovine viral diarrhea-mucosal disease. *J Am Vet Med Assoc* 1971;159:1383-1386.
20. Johnson DW, Mucoplat CC. Immunologic abnormalities in calves with chronic bovine viral diarrhea. *Am J Vet Res* 1973;34:1139-1141.
21. Markham RJF, Ramnaraine ML. Release of immunosuppressive substances from tissue culture cell infected with bovine viral diarrhea virus. *Am J Vet Res* 1985;46:879-883.
22. McClurkin AW, Coria MF, Cutlip RC. Reproductive performance of apparently healthy cattle persistently infected with bovine viral diarrhea virus. *J Am Vet Med Assoc* 1979;174:1116-1119.
23. Kahrs RF. The relationship of bovine viral diarrhea-mucosal disease to abortion in cattle. *J Am Vet Med Assoc* 1968;153:1652-1655.
24. Casaro APE, Kendrick JW, Kennedy PW. Response of the bovine fetus to bovine viral diarrhea-mucosal disease virus. *Am J Vet Res* 1971;32:1543-1561.

25. Kahrs RF. Effects of bovine viral diarrhea on the developing fetus. *J Am Vet Med Assoc* 1973;163:877-878.
26. Scott FW, Kahrs RF, de Lahunta A, Brown TT, McEntee K, Gillespie JH. Virus induced congenital anomalies of the bovine fetus. I. Cerebellar degeneration (hypoplasia), ocular lesions and fetal mummification following experimental infections with bovine viral diarrhea-mucosal disease virus. *Cornell Vet* 1973;63:536-560.
27. Bolin SR, McClurkin AW, Cutlip RC, Coria MF. Severe clinical disease induced in cattle persistently infected with the non-cytopathic bovine viral diarrhea virus by superinfection with cytopathic bovine viral diarrhea virus. *Am J Vet Res* 1985;46:573-576.
28. McClurkin AW, Littledike ET, Cutlip RC, Frank GH, Coria MF, Bolin SR. Production of cattle immunotolerant to bovine viral diarrhea virus. *Can J Comp Med* 1984;48:156-161.
29. Bolin SR, McClurkin AW, Cutlip RC, Coria MF. Response of cattle persistently infected with noncytopathic bovine viral diarrhea virus to vaccination for bovine viral diarrhea and to subsequent challenge exposure with cytopathic bovine viral diarrhea virus. *Am J Vet Res* 1985;46:2467-2470.
30. Reggiardo C. Role of BVD virus in shipping fever of feedlot cattle. *Proceedings. Am Assoc Vet Lab Diagn* 1979;315-320.
31. Potgieter LND, McCracken MD, Hopkins FM, Walker RD. Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. *Am J Vet Res* 1984;45:1582-1585.
32. Reggiardo C, Kaeberle ML. Detection of bacteremia in cattle inoculated with bovine viral diarrhea virus. *Am J Vet Res* 1981;42:218-221.
33. Miller NJ. Infectious necrotic rhinotracheitis of cattle. *J Am Vet Med Assoc* 1955;126:463-467.
34. Schroeder RJ, Moys MD. An acute upper respiratory infection of dairy cattle. *J Am Vet Med Assoc* 1954;125:471-472.
35. Yates WDG. A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. *Can J Comp Med* 1982;46:225-262.
36. Kahrs RF. Infectious bovine rhinotracheitis: A review and update. *J Am Vet Med Assoc* 1977;171:1055-1064.

37. Rosner SF. Infectious bovine rhinotracheitis: Clinical review, immunity and control. *J Am Vet Med Assoc* 1968;153:1631-1638.
38. McKercher DG, Wada EM. The virus of infectious bovine rhinotracheitis as a cause of abortion in cattle. *J Am Vet Med Assoc* 1964;144:136-142.
39. Kendrick JW, Gillespie JH, McEntee K. Infectious pustular vulvovaginitis of cattle. *Cornell Vet* 1958;48:458-495.
40. Kendrick JW. Effects of the infectious bovine rhinotracheitis virus on the fetus. *J Am Vet Med Assoc* 1973;163:852-854.
41. Gerber H. Infectious diseases of the respiratory tract. In: Wintzer HJ ed. *Equine Diseases*. Berlin: Springer-Verlag, 1986;31-34.
42. Timoney PJ. Rhinopneumonitis and viral abortion. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;173-177.
43. Doll ER. Rhinopneumonitis. In: Catcott EJ, Smithcors JF eds, *Equine Medicine and Surgery*, 2nd ed. Wheaton, IL: American Veterinary Publications, 1972;28-35.
44. Reisinger RC, Heddleston KI, Manthei CA. A myxovirus (SF-4) associated with shipping fever of cattle. *J Am Vet Med Assoc* 1959;135:147-152.
45. Frank GH, Marshall RG. Parainfluenza-3 virus infection of cattle. *J Am Vet Med Assoc* 1973;163:858-860.
46. Woods GT. The natural history of bovine myxovirus parainfluenza-3. *J Am Vet Med Assoc* 1968;152:771-779.
47. Sweat RL. Bovine myxovirus parainfluenza-3 and its role in bovine respiratory disease. *J Am Vet Med Assoc* 1968;153:1639-1644.
48. Swift BL, Trueblood MS. Failure to induce fetal infection by inoculation of pregnant immune heifers with bovine parainfluenza-3 virus. *J Infect Dis* 1973;127:713-737.
49. Baker JC. Bovine Respiratory Syncytial Virus. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;85-88.

50. Eddington N, Jacobs JW. Respiratory syncytial virus in cattle. *Vet Rec* 1970;87:762.
51. Lehmkuhl HD, Gough PM, Reed DE. Characterization and identification of a bovine respiratory syncytial virus isolated from young calves. *Am J Vet Res* 1979;40:124-126.
52. Enders JF, Bell JA, Dingle JH, Frances T, Hilleman MR, Huebner RJ, Payne AMM. Adenoviruses: Group name proposed for new respiratory tract viruses. *Science* 1956;124:119-120.
53. Mattson DE. Adenoviruses. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;70-72.
54. Cutlip RC, McClurkin AW. Lesions and pathogenesis in young calves experimentally induced by a bovine adenovirus type 5 isolated from a calf with weak calf syndrome. *Am J Vet Res* 1975;36:1095-1098.
55. Mattson DE. Adenovirus infection in calves. *J Am Vet Med Assoc* 1973;163:894-896.
56. Mattson DE. Naturally occurring infection of calves with a bovine adenovirus. *Am J Vet Res* 1973;34:623-629.
57. Edington N, Wright JA, Patel JR, Edwards GB, Griffiths L. Equine adenovirus 1 isolated from cauda equina neuritis. *Res Vet Sci* 1984;37:252-254.
58. Crawford TB. Adenovirus. In: Castro AE, Heuschele WP eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;155-156.
59. Hoff GL, Trainer DO. Bluetongue and Epizootic Hemorrhagic Disease Viruses: Their Relationship to Wildlife Species. *Advances in Vet Sci and Comp Med*, Vol 22. San Diego, CA: Academic Press, 1978.
60. Bowne JG. Is bluetongue an important disease of cattle? *J Am Vet Med Assoc* 1973;163:911-914.
61. Luedke AJ, Jochim MM, Jones RH. Bluetongue in cattle: Effects of *Culicoides variipennis* transmitted bluetongue virus on pregnant heifers and their calves. *Am J Vet Res* 1977;38:1687-1695.
62. Luedke AJ, Jochim MM, Jones RH. Bluetongue, epizootic hemorrhagic disease and ibaraki. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;76-79.

63. McKercher DG, Saito JK, Singh KV. Serologic evidence of an etiologic role for bluetongue virus in hydranencephaly of calves. *J Am Vet Med Assoc* 1970;156:1044-1047.
64. Easterday BC. Influenza. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;169-171.
65. Wadell GH, Teigland MB, Sigel MM. A new influenza virus associated with equine respiratory disease. *J Am Vet Med Assoc* 1963;143:587-590.
66. Doll ER, Bryans JT, McCollum WH, Crowe MEW. Isolation of a filterable agent causing arteritis of horses and abortion by mares. *Cornell Vet* 1957;47:3-41.
67. Timoney PJ. Equine Viral Arteritis. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;166-169.
68. Cotton WE. The causal agent of vesicular stomatitis proved to be a filter-passing virus. *J Am Vet Med Assoc* 1926;70:168-181.
69. Ellis EM, Kendall HE. The public health and economic effects of vesicular stomatitis in a herd of dairy cattle. *J Am Vet Med Assoc* 1964;144:377-380.
70. Jenney EW, Hayes FA, Brown CL. Survey for vesicular stomatitis virus neutralizing antibodies in serum of white-tailed deer (*Odocoileus virginianus*) of the southeastern United States. *J Wildl Dis* 1970;6:488-493.
71. Erickson GA. Vesicular Stomatitis. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;131-133.
72. Knowles DP, McGuire TC, Cheevers WP. Ovine Progressive Pneumonia (Visna/Maedi). In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;209-212.
73. Knowles DP, McGuire TC, Cheevers WP. Caprine Arthritis Encephalitis. In: Castro AE, Heuschele WP, eds. *Veterinary Diagnostic Virology*. St. Louis, MO: Mosby-Year Book, Inc., 1992;202-205.
74. Knowles RC, Anderson CW, Shipley WD, Whitlock RH, Perry BD, Davidson JP. Acute equine diarrhea syndrome (AEDS): A preliminary report. *Proc Am Assoc Eq Pract* 1983;353-357.
75. Rikihisa Y, Perry BD, Cordes D. Rickettsial link with acute equine diarrhoea. *Vet Rec* 1984;115:390.

76. Rikihisa Y, Perry BD. Causative agent of Potomac horse fever. *Vet Rec* 1984;115:554.

77. Holland CJ, Ristic M, Goetz T, *et al.* Causative agent of Potomac horse fever. *Vet Rec* 1984;115:554-555.

78. Ristic M, Holland CJ, Dawson JE, Sessions J, Palmer J. Diagnosis of equine monocytic ehrlichiosis (Potomac horse fever) by indirect immunofluorescence. *J Am Vet Med Assoc* 1986;189:39-46.

APPENDICES

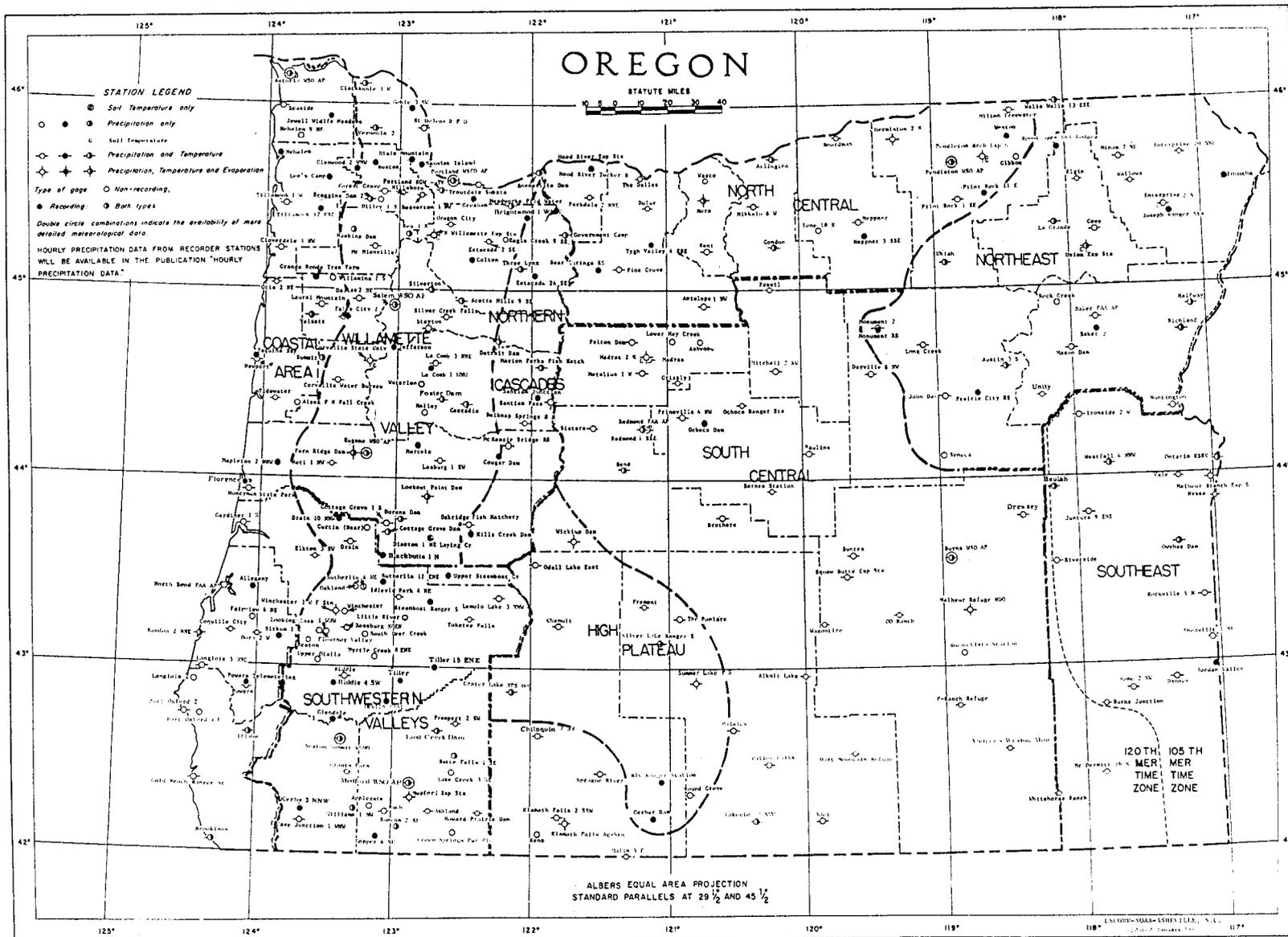


Figure 3. Map of Oregon showing weather recording stations.

Table 5. Climate data. Weather recording stations were selected that best represented the respective farms. Data provided by George H. Taylor, State Climatologist, Oregon State University, Corvallis, Oregon.

Portland, Oregon Elevation 20 feet (Farms 12, 19, 20, 22)														
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year	
Mean temperature (F)	Maximum	45.4	50.9	56.1	60.5	67.2	73.8	79.7	80.1	74.5	64.1	52.7	45.6	62.6
	Minimum	33.8	36.0	338.6	41.4	46.9	52.8	56.4	56.8	51.8	45.0	39.5	38.4	44.5
Precipitation (inches)														
Monthly mean	5.35	3.68	3.54	2.39	2.06	1.48	.63	1.09	1.75	2.66	5.34	6.13	107.0	

North Willamette Exp. Strn., Aurora, Oregon Elevation 150 feet (Farm 13)														
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year	
Mean temperature (F)	Maximum	46.0	51.0	55.5	59.9	66.7	73.2	79.7	80.2	74.6	64.0	52.8	46.1	62.5
	Minimum	32.4	34.3	36.6	39.4	44.2	49.7	52.5	52.3	48.5	41.4	37.4	33.1	41.8
Precipitation (inches)														
Monthly mean	6.17	4.39	3.99	2.64	2.17	1.73	.70	.94	1.84	3.11	6.03	7.09	40.80	

Salem, Oregon Elevation 200 feet (Farms 11, 14)														
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year	
Mean temperature (F)	Maximum	46.2	51.3	55.8	60.4	67.0	74.4	81.6	81.9	76.0	64.3	52.5	46.2	63.1
	Minimum	32.6	34.2	35.7	37.7	42.3	48.1	50.7	51.2	46.9	41.0	37.3	33.6	40.9
Precipitation (inches)														
Monthly mean	5.91	4.50	4.17	2.42	1.88	1.34	.56	.76	1.55	2.98	6.28	6.80	39.16	

Dallas, Oregon Elevation 290 feet (Farm 15)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	46.0	51.0	55.8	62.3	68.4	75.7	82.3	82.9	77.1	65.4	52.2	45.6	63.7
Minimum	32.8	34.9	36.2	37.8	42.1	46.9	48.9	48.8	46.2	41.2	37.1	33.4	40.5
Precipitation (inches)													
Monthly mean	7.83	6.17	5.68	2.71	2.01	1.24	.50	.72	1.55	3.33	7.56	9.15	49.07

Corvallis, Oregon Elevation 225 feet (Farms 1, 2, 10)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	45.5	50.4	54.9	59.5	66.1	73.1	80.2	81.1	75.4	64.3	52.3	45.6	62.4
Minimum	33.0	35.1	37.0	39.2	43.1	48.6	51.0	51.3	47.8	41.7	38.0	33.9	41.6
Precipitation (inches)													
Monthly mean	6.82	5.04	4.55	2.56	1.95	1.23	.52	.87	1.51	3.11	6.82	7.72	42.71

Bend, Oregon Elevation 3,650 feet (Farms 3, 4, 5, 6, 7)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	41.6	46.3	51.1	57.5	65.1	73.6	81.5	80.9	73.1	63.1	48.5	41.7	60.3
Minimum	21.9	24.5	25.9	29.1	34.5	41.1	45.0	44.6	37.4	31.2	27.1	22.4	32.1
Precipitation (inches)													
Monthly mean	1.83	.97	.92	.60	.77	.86	.49	.58	.47	.65	1.57	1.99	11.70

Prineville, Oregon Elevation 2840 feet (Farms 8, 9)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	42.9	49.2	54.7	61.5	69.5	78.1	86.6	85.6	77.3	66.3	50.7	43.0	63.7
Minimum	21.7	24.8	25.7	28.0	34.5	40.7	43.1	42.2	35.3	29.5	26.6	21.7	31.1
Precipitation (inches)													
Monthly mean	1.17	.86	.81	.72	.92	.91	.45	.60	.47	.76	1.42	1.41	10.37

Roseburg, Oregon Elevation 470 feet (Farm 18)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	48.8	53.2	57.9	62.9	69.6	76.6	83.5	84.3	77.9	67.1	54.5	47.9	65.2
Minimum	34.6	35.9	37.6	39.2	44.3	50.3	53.6	53.9	49.3	43.5	39.5	34.9	42.9
Precipitation (inches)													
Monthly mean	5.13	3.70	3.56	2.24	1.43	.83	.43	.73	1.24	2.23	5.36	5.47	32.44

Medford Experiment Station, Oregon Elevation 1460 feet (Farms 16, 17)													
Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Year
Mean temperature (F)													
Maximum	46.2	53.6	58.6	65.0	73.0	81.4	88.8	88.3	81.5	68.3	52.6	45.1	66.7
Minimum	30.1	32.0	34.3	36.5	41.3	47.7	50.6	50.7	44.2	37.7	34.6	30.9	39.2
Precipitation (inches)													
Monthly mean	2.87	2.05	2.09	1.38	1.11	.77	.29	.61	1.03	1.68	3.34	3.64	21.22