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Could a zoonosis cause some cases of anencephaly? *Mycobacterium avium* subspecies *paratuberculosis* inhaled from aerosolized dairy cow manure and the Washington State rural anencephaly cluster

Ellen S Pierce*
Spokane Valley, Washington, USA

Abstract

The rural Yakima Valley community in central Washington in the United States has suffered from a long-running tragedy of a cluster of babies with anencephaly, with an initial reported rate of over 60 times the national average. The mothers of the majority of affected babies lived in an area where an aggregation of massive dairy herds known as concentrated animal feeding operations (CAFOs) or factory farms is located. *Mycobacterium avium subspecies paratuberculosis* (MAP), the cause of a chronic gastrointestinal disease in domestic livestock, is endemic in United States dairy cattle, affecting 100% of herds with greater than 200 cows. MAP is recognized as a probable zoonosis, involved in the pathogenesis of Crohn's disease, other autoimmune disorders and neurologic diseases including autism, multiple sclerosis and Parkinson's disease. MAP is present in an infected dairy cow's feces at the rate of over one million organisms in a few drops of manure, and the average adult cow produces 12-14 gallons of manure per day. Manure from dairy CAFOs is stored in manure lagoons the size of football fields and applied to agricultural fields at the rate of 1000 gallons per acre. MAP persists in manure and the environment for an indefinite time and is readily aerosolized. The inhalation of pathogen-contaminated feces or manure is a major route of transmission of zoonotic pathogens from infected animals to humans. The inhalation of aerosolized MAP-contaminated manure by women in the first four weeks of pregnancy, and intrauterine transmission to the embryo, may be responsible for the development of anencephaly in the fetus.

Keywords

Anencephaly clusters; Mycobacterium avium subspecies paratuberculosis; Dairy feces; Pregnancy outcomes; Industrial farming

Introduction

The Yakima Valley anencephaly tragedy

Barron first reported a cluster of four babies with anencephaly to the Washington State Department of Health in 2012 [1-3]. Two of the four babies were born at the small hospital in Prosser, Washington where she worked, a hospital which had 300 births per year, constituting a rate "over 60 times the national average"[3]. Coverage followed in the lay media [4-6]. The cluster, now at 48 babies and counting [7], dates back to 1990 [8].

Considered causes of the anencephaly cluster

The Washington State Anencephaly Advisory Committee's study of the cluster found that both case and control mothers lived in homes served by large public water systems rather than private wells [9]. Nitrate levels in these public water systems were not elevated, ruling out nitrates in drinking water, associated with an increased rate of limb deficiencies, cleft lip and cleft palate [10] as a possible cause.

Fungal contamination of corn masa flour commonly used in Central American cooking was associated with the Brownsville, Texas anencephaly cluster, which predominantly affected Latina women [11,12]. Case and control mothers were equally likely to be Hispanic/Latina or Non-Hispanic white [13], suggesting neither fumonisin nor genetic factors were involved.

Folic acid deficiency was not a contributing factor, as case mothers were more likely than control mothers to have taken folic acid supplements during early pregnancy [14].

Infectious microorganisms, including zoonoses, are a known cause of spatiotemporal disease clusters

Infectious microorganisms were not considered, despite being a common cause of spatiotemporal disease clusters [15,16]. The most common source of microorganisms that

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*Corresponding author:

Ellen S Pierce
Spokane Valley
Washington, USA
Tel: +1 509 922 4562
E-mail: ellenpiercemd@gmail.com

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cause disease in humans is animals [17,18]. Zoonoses are infectious microorganisms that are transmitted from animals to humans and cause disease in humans [19]. Could a particular disease-causing microorganism present in animals in the Yakima Valley result in anencephaly in infected pregnant women?

Yakima Valley dairy factory farms and their manure lagoons

Beginning in 1990 [20], the same year the cluster started, massive dairy concentrated animal feeding operations (CAFOs) known as factory farms moved to the Yakima Valley [21,22], with upwards of over 3700 dairy cows per CAFO [23]. "The Yakima Valley region is one of the largest dairy producing areas in the nation" [24] comprising about 129,000 cows [25]. Residents have long been worried about the environmental impact of the huge amount of manure produced by the CAFOs [26,27]. They have complained about the foul air [26,28,29] and "fly specks and dried feces" [25] covering everything they own. They have brought lawsuits against dairy CAFO operations [30-32].

The hundreds of millions [31] of gallons of manure and manure-contaminated washing water from dairy animals in the massive CAFOs in the Yakima Valley, "equal to the sewage output of the New York City metro area" [33], are stored in manure lagoons the size of football fields and sprayed onto agricultural fields at the rate of 1000 gallons [34] per acre. Living near CAFO's has been associated with significant "neurobehavioral" [35] as well as pulmonary [36] impairment.

The probable zoonosis *Mycobacterium avium* subspecies *paratuberculosis* (MAP)

70% of all dairy herds [37,38] and 100% of dairy farms in the United States with more than two hundred cows [39] have *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in their animals, with within-herd prevalence rates of up to 100% [40]. MAP is the cause of a chronic gastrointestinal disease in domestic livestock called Johne's disease [41] and is a long investigated probable cause of Crohn's disease [42]. A significant segment of the medical community considers MAP to be a zoonotic pathogen [43].

It has occasionally been recognized that MAP, like other zoonotic pathogens [44], may cause a range of diseases in its human hosts that MAP does not cause in its animal host or hosts [45-48]. In addition to Crohn's disease, experimental evidence links MAP to type 1 diabetes mellitus [49-51], Hashimoto's thyroiditis [52-54], multiple sclerosis [55-57], Parkinson's disease [58], rheumatoid arthritis [59,60], Sjogren's syndrome [61,62] and ulcerative colitis [63,64].

MAP organisms are heavily excreted in an infected domestic ruminant's feces or manure

MAP organisms are heavily secreted in an infected animal's feces or manure. Only a few drops, "two thimblefuls" [65] or two milliliters [66] of manure from a dairy cow infected with MAP contain an 'infectious dose' [67] of MAP, i.e., enough MAP organisms to cause infection in a calf. A MAP-infected dairy animal secretes over 25,000 infectious doses of MAP per day in its feces [67], organisms which last almost indefinitely in the manure [68-70], in the soil and environment around dairy farms [71,72] and in soil fertilized with MAP-contaminated manure [73].

Inhalation is a major transmission route of zoonotic pathogens such as MAP from infected animals to humans

MAP organisms easily spread outside of dairy farms and are present in dairy farm dust or "bioaerosols" [74-77]. The inhalation of MAP organisms has been proposed [78] and experimentally documented [79] as a transmission route of MAP infection. It is well known that the inhalation of aerosolized manure that has contaminated soil or water is a common transmission route of zoonotic pathogens from infected animals to humans [29,65,80-88]. In contrast, however, the inhalation of MAP from soil or dirt containing manure excreted by MAP-infected domestic livestock is a rarely recognized source of human infection with MAP [67,77,89-91]. It is also not usually appreciated that the aerosolized manure itself can be inhaled; pathogen-contaminated manure doesn't 'need' to contaminate soil or water before being inhaled. The zoonotic literature refers to this as the "inhalation of animal manure particles" [92].

The mothers lived in the same area as the Yakima Valley dairy factory farms

The mothers of the first four affected babies lived in a single zip code located in the southeast corner of the aggregation of massive dairy farms in the Yakima Valley, "between Sunnyside and Prosser" [93]. (Figure 1) The majority of mothers lived in the same area, in the Yakima River Valley [93]. The mothers also lived "within a quarter mile" of an "agricultural field" or "farm" at the time of conception [94]. They would therefore have potentially been exposed to airborne MAP above and near manure lagoons and agricultural fields sprayed [86,87,95] with MAP-contaminated manure [96].

MAP-infected domestic livestock and other rural anencephaly clusters

Researchers have noted the existence of other rural anencephaly clusters, and that there is an increased rate of anencephaly only in these clusters, not an increased rate of other neural tube defects [97]. They have suggested that a "defect-specific etiologic agent" [97] may cause anencephaly.

The possibility that MAP infection in domestic livestock is the defect-specific etiologic agent of anencephaly is suggested by the existence of other rural anencephaly clusters occurring near MAP-infected domestic livestock. The ongoing epidemic of Crohn's disease in Cardiff in the United Kingdom [89,98,99] is adjacent to a rural area with a historically high rate of anencephaly where the MAP-infected domestic livestock are located [100]. Brownsville, Texas is a rural area with a high concentration of beef cattle [101], also infected with MAP [102], and Brownsville anencephaly case mothers with two or more episodes of periconceptional diarrhea, possibly a result of acute MAP infection, had an 8.4 fold increased risk of anencephaly [12,103]. A study of the relationship between United States beef and dairy CAFOs and infant health found a 7.4% increased infant mortality rate for every 100,000 increase in "animal units" in a county; "the closer a person is to a livestock operation, the more negative the health effect" [104].

There are known infectious causes of animal and human congenital anomalies

There is indirect evidence of a possible association between MAP infection of women in the first four weeks of pregnancy and the development of anencephaly in their fetuses. There are several known infectious causes of bovine congenital anomalies [105]. A sparse literature documents in utero transmission of MAP from ruminant mother to fetus [106-112] .

The viruses and parasite that cause human congenital anomalies such as microcephaly [113-115] do so only if the infection is primary, first occurring during pregnancy. The necessity for an infectious agent to first occur during pregnancy in order to cause congenital malformations in the fetus is the reason persons with probable MAP-associated diseases such as Crohn's disease do not have an increased rate of anencephaly. Since the vast majority of dairy cows are already infected with MAP, primary bovine infection rarely occurs. Primary bovine MAP infection is associated with early fetal loss rather than congenital abnormalities [116]. MAP "may form a close association with the early bovine conceptus" (emphasis added) [106] and unfortunately the human one as well.

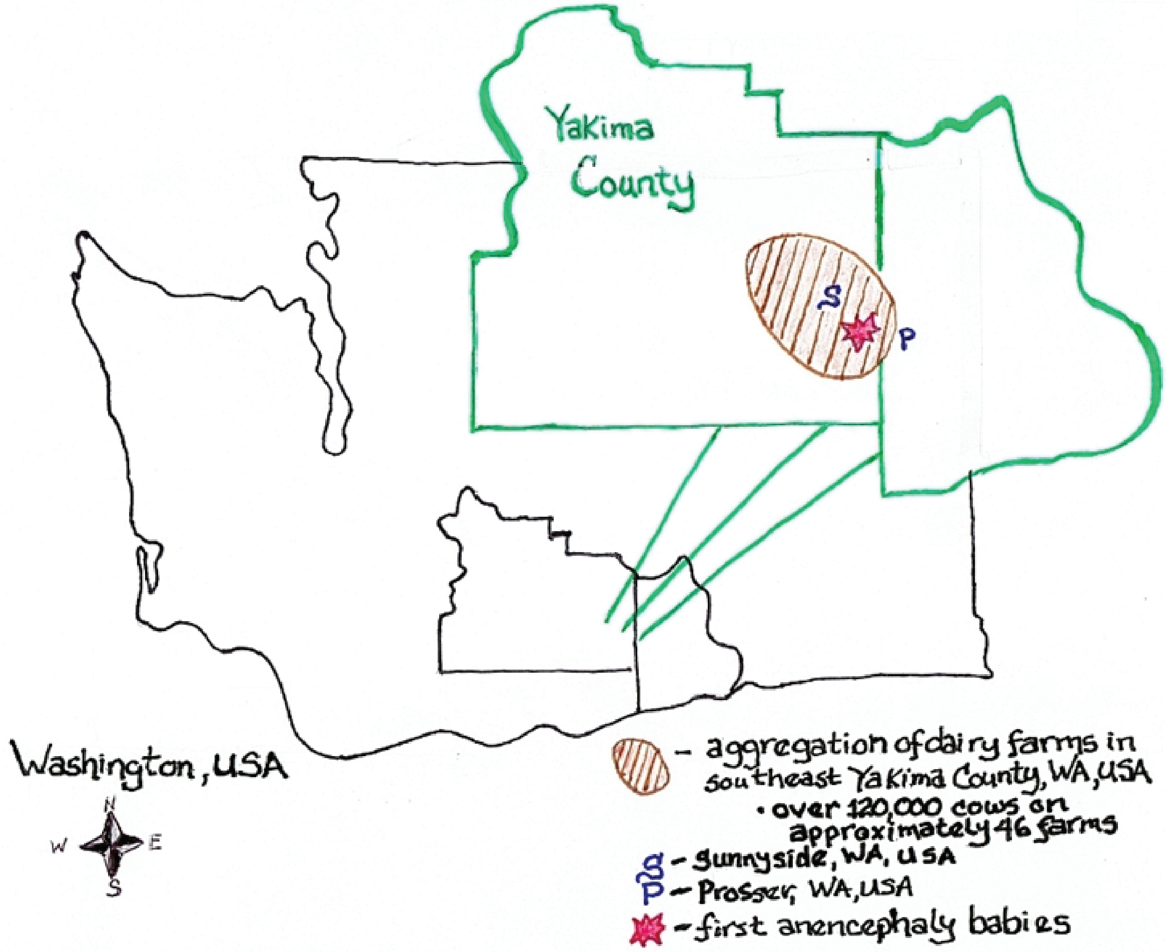

Figure 1: Dairy herds and the Washington State anencephaly cluster. The mothers of the first four babies with anencephaly lived in a single zip code (✸) in Yakima County, between Sunnyside and Prosser, Washington, within the aggregation of massive dairy farms in the Yakima Valley.

How could an infectious agent such as MAP cause anencephaly? The area cerebrovasculosa, an endothelial cell proliferative lesion, replaces the cerebral hemispheres, with subsequent acrania

The two main pathologic features of anencephaly, the area cerebrovasculosa and the acrania, suggest a particular sequence of pathogenetic mechanisms for how MAP or another infectious agent may cause anencephaly. The area cerebrovasculosa is a discrete lesion comprised primarily of a mass of vascular channels lined only by endothelial cells. A 1982 study describes the area cerebrovasculosa as a "bilaterally symmetric cystic mass" filled with "abnormally vascular choroid plexus" [117]. A 1961 study describes the "cephalic masses" of anencephaly as being characterized by "multitudes of vascular channels…regularly lined by endothelium" and "most often devoid of muscular, elastic…or fibrous tissue" [118].

Whether described as a result of abnormal angiogenesis or the proliferation of endothelial cells, the endothelial cell proliferative lesion that is the area cerebrovasculosa is considered by some to be the primary pathologic lesion of anencephaly [118]. MAP infection may cause this endothelial cell proliferative lesion that is the area cerebrovasculosa. The proliferation of endothelial cells has recently been identified as a feature of early bovine MAP infection [119], although this study was of the ileum and not the brain. A 1968 study of remote (non-gastrointestinal) MAP lesions in infected goats describes MAP being present in the endothelial cells of the intermediate sinuses of lymph nodes, and of causing increased glomerular tufting "due to the proliferation of vascular endothelium" [120].

Bacteria including the *Mycobacterium lepromatosis* [121] and the zoonosis *Bartonella henselae* [122,123] are known causes of discrete endothelial cell proliferative lesions. Relevant to this discussion, *Bartonella henselae*, the causative agent of cat-scratch disease, causes endothelial cell proliferation in its human hosts but not in its feline ones [123].

How may the area cerebrovasculosa of anencephaly lead to acrania, the absence of cranial vault bones? The functional matrix hypothesis proposes that bone formation is a result of the functional needs of the adjacent soft tissues and organs [124]. "The cranial cavity represents a classic example of a matrix function, meaning that the cranium grows under the pressure of the brain" [125]. If the non-expanding area cerebrovasculosa has replaced the cerebral hemispheres, the cranial vault bones do not develop because there are no expanding cerebral hemispheres pressing on them to do so.

Folic acid and infectious microorganisms

The fact that case mothers were more likely than control mothers to have taken folic acid in early pregnancy [14] is consistent with the idea that some cases of anencephaly may have an infectious etiology. Folic acid supplementation increases the risk of malaria and other infections [126], and many antibiotics have anti-folate mechanisms of action [127].

Roundup's effect on MAP: a possible contributing factor

The heavy application of Roundup (glyphosate) in the time period of the highest incidence of anencephaly in the Yakima Valley may be a contributing factor, although glyphosate probably does not cause anencephaly on its own as has been proposed

[128]. Glyphosate may instead contribute to the development of anencephaly through its effect on MAP. Glyphosate has no effect on zoonotic pathogens but kills beneficial bacteria [129], and may increase the survival, virulence or pathogenicity of MAP in infected ruminants such as dairy cows [130,131].

Conclusion

Suggestions for future investigations of rural anencephaly clusters

While the existence of rural anencephaly clusters has suggested that such clusters may have an agricultural or environmental cause [97], an infectious agent present in rural environments has not been suspected, although it is well known that there are infectious causes of birth defects and anomalies. The assumption made by the Washington State Department of Health was that, by definition, possible causes of the anencephaly cluster were non-infectious; the investigation of the Yakima Valley anencephaly cluster was led by the Washington State Epidemiologist for Non-Infectious Conditions [132].

No research has tested for the presence of microorganisms in the area cerebrovasculosa or other tissues of babies with anencephaly. Tissues from fetuses with neural tube defects will often be excluded from microbiology studies. Research on the "placental microbiome", for example, has specifically excluded placentas from babies with "fatal fetal anomalies" [133].

No pathologic examination was done of any of the affected babies or their associated placentas, although such studies are recommended by individual pathologists and the College of American Pathologists [134-136].

The Yakima Valley anencephaly cluster was discovered by accident: Nurse Barron only became aware of the cluster when she inquired into the occurrence of anencephaly during a meeting of officials from several area hospitals. Surveillance for neural tube and other birth defects in the United States is state-dependent, and there are three ways a state may identify cases [2]. Active case finding requires a state health department to regularly review hospital and clinic records and collect information on the baby and mother when a birth defect is found. Passive case finding with confirmation means the reporting of newborns with birth defects to a state health department is voluntary, but the state must confirm the case. Washington State has the least aggressive passive case finding system, where both the reporting of cases by health care providers or systems and the follow-up of cases by the state health department is voluntary rather than required. Active rather than passive case finding would therefore help alert state health departments to communities with increased rates of anencephaly.

Maternal infections causing congenital anomalies in the fetus such as microcephaly are diagnosed by serologic and amniotic fluid evaluation of the pregnant woman [113]. Primary CMV infection, for example, is diagnosed by finding IgG antibodies in the mother's blood and amniotic fluid [137]. Like other infectious agents, MAP antibodies and organisms, if present, could be identified in the blood and amniotic fluid of a pregnant woman whose fetus is affected by anencephaly [138-141].

Active case finding, maternal serologic and fetal amniotic fluid correlations, and the pathologic examination of affected fetuses, babies and associated placentas will assist in identifying possible infectious causes of rural anencephaly clusters associated with proximity to domestic livestock. It is the author's hope that publication of this idea will stimulate future investigations of possible infectious causes of rural anencephaly clusters associated with such domestic livestock proximity (Figure 1).

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Conflicts of Interest

The author declares that there are no conflicts of interest.

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